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MUHAMMAD KASHIF

SU92-MSCHW-S23-009

FOS

**Metal Selenide Grafted Carbon Nanotubes As Efficient Electrocatalyst For
Electrochemical Sensing of Uric Acid**



SUPERIOR UNIVERSITY

Thesis Submitted to

The Superior University Lahore

In Partial Fulfillment of the

Requirement for the Degree of

MS Chemistry

By

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DR.Abid Ali:

Assistant Professor:

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Miss Zurwa:

Lecturer:

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This is to certify that the research work presented in this thesis, titled “**Metal Selenide Grafted Carbon Nanotubes As Efficient Electrocatalyst For Electrochemical Sensing Of Uric Acid**” was conducted by “**Muhammad Kashif**” under the supervision of “**Miss zurwa**”

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

With great pride and humility this thesis is dedicated to all of them who were relentlessly present and sacrificed in silence to bring clear light to my scholarly endeavors. And to my dearest parents who have showered me with their unconditional love and endless patience as well as their abiding belief in my capabilities and have ever held the fort of my odyssey. It was their silent support and constant motivation that made me take those hard steps on my way. To my brothers and sisters, whose friendship, jokes and care have helped me to stay in good spirits. They have acted as my refuge in the stress, and I remember that I need some balance and light in my life. I also dedicate this hard work to my deceased grand parents whose blessings and values are inscribed in my conscience. They are no longer here but their memory still motivates me to make a difference in everything I undertake by being excellent and of high integrity. This thesis is dedicated to you, to all the people who silently stood beside me and whose names may not be found in the official list of acknowledgments but whose role in my life has played a significant part. Finally, I am dedicating this research to all those students and dreamers who are still in the quest of knowledge despite lack of resources and hard challenges. Hopefully this can be used as a testimonial of how passion, perseverance and purpose can break any obstacle. The loyalty here is not in honor of one person, but the circle of love, faith, and power that has helped me move along- visible and invisible, actual and unutterable.

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List of Abbreviations

UA	Uric Acid
CVD	Cardiovascular Disease
CHD	Coronary Heart Disease
AMP	Adenosine Monophosphate
IMP	Inosine Monophosphate
ETC	Electron Transport Chain
CuSe	Copper Selenide
CoSe	Cobalt Selenide
NiSe	Nickel Selenide
SWV	Square Wave Volt
XRD	X-ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
Raman	Raman Spectroscopy
GO	Graphene Oxide
RGO	Reduced Graphene Oxide
FTO	Fluorine-doped Tin Oxide GCE – Glassy Carbon Electrode
PBS	Phosphate Buffered Saline
SPE	Screen Printed Electrode
DPV	Differential Pulse Voltammetry
LSV	Linear Sweep Voltammetry
NPs	Nanoparticles
NCs	Nanocomposites
MS	Multiple Sclerosis
PD	Parkinson's Disease
ALS	Amyotrophic Lateral Sclerosis
CKD	Chronic Kidney Disease
BSA	Bovine Serum Albumin
PEDOT	Poly(3,4-ethylenedioxythiophene)
PANI	Polyaniline
HPLC	High Performance Liquid Chromatography
GCMS	Gas Chromatography–Mass Spectrometry
SCE	Saturated Calomel Electrode
NHE	Normal Hydrogen Electrode
EA	Electroactive Area
ROS	Reactive Oxygen Species
SNR	Signal-to-Noise Ratio

ABSTRACT

Uric Acid (UA) is one such significant metabolic biomarker that has association with such diseases as ailments like gout, renal disease, cardiovascular disease and neurological diseases. Although accurate, the traditional detection methods such as spectrophotometry, chromatography and the like are hampered by the fact that they are quite expensive, complicated and also demanding in terms of operation. Conversely, due to the low cost, high-restrictive sensitivity, speed and being suitable to real time and miniature diagnostic applications, electrochemical sensing presents a better alternative. It provides a non-enzymatic carbon nanotube (CNT)/copper selenide (CuSe) grafted sensor that will have the potential to effectively sense the existence of UA. CuSe nanoparticles were conjugated onto carbon nanotube fiber (CNTF) and functionalized carbon nanotube sheets (CNTS), making a (CuSe@CNT) nano-composite. SEM, EDX, UV-Visible and Elementl Mapping used to characterize the material confirmed successful synthesis of CuSe in the form of uniform particle distribution. CNTs were also added to enhance electron transport required in the UA oxidation process, which was conducted in the presence of the catalytic activity of CuSe@CNT. This ink was drop-cast on glassy carbon electrodes (GCE) and dried at 60C. Electrochemical experiments were performed in PBS (pH 7.4) on a three-electrode system (Ag/AgCl reference, Pt counter) with the CV, SWV, and EIS methods. There was a visible oxidation peak of the electrode at +0.25 V and the current response ranged between 0.05 mM to 1.0 mM of UA was directly proportional to the UA concentrations. The sensor exhibited low micromolar limit of detection (LOD), high repeatability and selectivity with minimal interference of common analytes such as uric acid and others. The promise of the platform as a biomedical tool via the demonstrated scalability, low-cost, and high performance of the sensor of uric acid.was proven through the enhanced charge transfer, stability in structure and amplified signal generated by the colligation of the CuSe and CNTs.

CHAPTER.1

INTRODUCTION

1.1. Historical Background

Uric acid exists as a heterocyclic molecule having the molecular formula of $C_5H_4N_4O_3$ (7,9-dihydro-1H-purine-2,6,8(3H)-trione) and a molecular weight of 168Da.

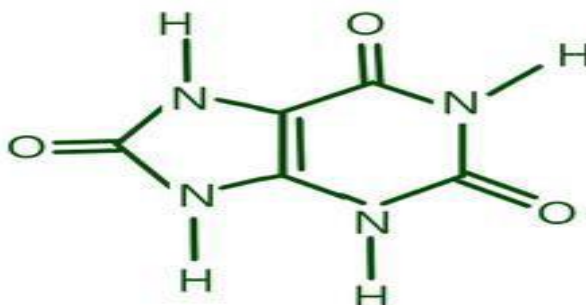


Figure.1 Shows the structural formula of uric acid [1].

Uric acid (UA) crystal composition remained unidentified when Leeuwenhoek first observed them during his work with gouty tophus in 1679 until Stukeley described tophaceous joint observations. Karl Wilhelm Scheele achieved the historic first isolation of UA from urinary calculi as a Swedish scientist in 1776. Since 1798 George Pearson isolated the chemical compound from 200 urinary stones and proposed the term "ouric" or "uric oxide." During his investigation Ludwig Medicus developed the initial chemical structure for UA in 1875. In 1897 H. Emil Fischer implemented his first successful chemical synthesis of UA as he demonstrated the accuracy of Medicus' described structure following his Nobel Prize in Chemistry achievement in 1902. According to Haig and Oxon they published in the British Medical Journal in 1889 that "arery tension shows a direct relation to circulating uric acid when other factors are equally published in 1951 that presented elevated levels of UA in patients with early coronary heart disease signs contrasted with regular population results. Scientific research indicated UA as a potential risk factor for the first time. Research into urea as a potential CVD

risk factor was integrated into the Framingham study protocols for the year 1951. The study presented by Kannel et al. in 1967 utilized the Framingham Study population (n = 5127 individuals) to investigate associations between CHD risk and serum UA concentrations [1].

1.2. Uric Acid Production And Monitoring:

Various enzymes transform purine nucleic acids adenine and guanine into uric acid. Two pathways convert adenosine monophosphate (AMP) to inosine through nucleotidase action or the combined activity of deaminase and nucleotidase. Deaminase breaks down AMP into inosine monophosphate (IMP) which nucleotidase phosphorylates to inosine or nucleotidase directly dephosphorylates AMP into adenosine and then deamination product is inosine. During its transformation process nucleotidase converts GMP into the guanosine molecule. Through its enzymatic action PNP converts both inosine and guanosine nucleosides into final purine bases hypoxanthine and guanine. After hypoxanthine oxidation produces xanthine through XO activity guanine deaminase finishes the process by converting guanine into xanthine. The breakdown of xanthine by xanthine oxidase enzyme leads to uric acid production as the terminal product.

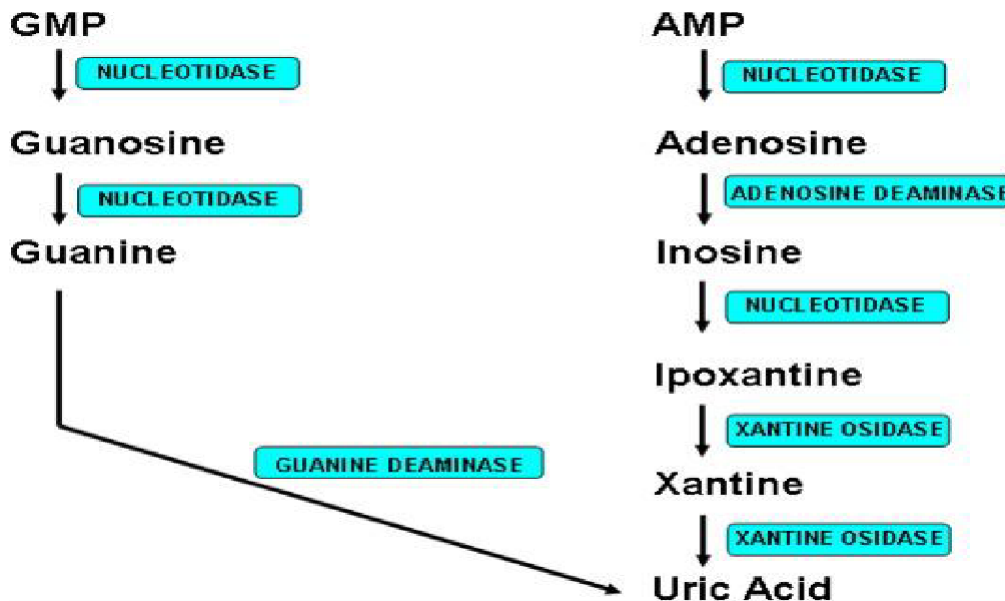


Figure.2 shows the uric acid formation [2].

UA exists as a natural metabolic waste from purine metabolism. The normal uric acid levels of adults are usually within the range of 2.5 to 7.0 mg/dL among men and 1.5 to 6.0 mg/dL in women. The levels of uric acid are usually lower in children than in adults, and they also differ depending on the age. Such as an example is children of 1-10 years of age and the normal level in them is between 2.4 mg/dL and 5.4 mg/dL. The analysis results may change due to several factors including age and gender but represent only two variables [2].

1.2.1 Advantages Of Normal U Level:

Scientists suggest that the survival advantage of UA Uric acid is a naturally occurring antioxidant and it removes free radicals, protects brain cells, bolsters immune activity, balances blood pressure, balances the redox state and balances vitamin C levels, increases neural protection, oversees inflammation, chelates the metal ions, preserves nitric oxide, supports cognitive wellbeing, aids in tissue rebuilding and metabolic end-product of purines and decrease signs of oxidative stress [3].

1.2.2 Disadvantages Of Hyperuricemia And Hypouricemia:

The conditions such as kidney failure, heart attack, stroke, end-stage renal disease, gouty arthritis, uric acid nephropathy, cardiovascular disease, hypertension, atherosclerosis, myocardial infarction, sudden cardiac death, chronic heart failure, nephrotic syndrome as well as renal insufficiency arise from elevated blood plasma UA levels. People with low blood UA measurements are at risk of developing severe neurodegeneration as well as progressive paralysis and respiratory failure, cardiac arrest, sudden death and immunodeficiency disorders, neurological disorders, hemolytic anemia, Parkinson's disease, Alzheimer's disease, multiple sclerosis, Parkinson's disease, Amyotrophic Lateral Sclerosis and cognitive impairment [4].

1.2.2.1 Higher Antioxidant Capacity And Greater Longevity:

Living things face harm from the highly reactive chemical oxygen which generates reactive oxygen species (ROS) while most complex life forms need oxygen to survive. ROS naturally appear inside living cells until their synthesis rate exceeds antioxidant

defense capacity resulting in detrimental effects. This definition implies that oxidative stress describes a condition where pro-oxidants predominate over anti-oxidants in the balance. The defensive mechanism against oxidative damage includes enzymes such as superoxide dismutase and glutathione peroxidase as well as antioxidants and radical [5].

1.2.2.2 Gout:

The process starts when chronic hyperuricemia causes serum urate levels to reach saturation thresholds and leads MSU crystals to form in joint tissues which then produce gout symptoms. A proposed sequence of events now exists which describes the order through which hyperuricemic individuals progress [6]. The development of chronic gouty arthritis or chronic gout usually starts with subcutaneous MSU deposits known as tophi. crystal formation from hyperuricemia conducts persistent inflammatory reactions that lead to structural damage of joints [7].

1.2.2.3.UA And Cardiovascular Disease:

Rises in UA concentration may directly trigger cardiovascular disease or act as a risk factor for this condition or bring about cardiovascular disease through unintentional factors. Renal disease simultaneously enhances SUA levels while activating renin-angiotensin system (RAS) that facilitates development of vascular disease. The combination of elevated blood pressure due to increased SUA level from renal interstitial changes possibly results in vascular harm to both heart tissue and brain structures. The levels of xanthine oxidase along with uric acid concentrations surpass those found in non-atherosclerotic vascular tissues [8].

1.2.2.4 UA And Alzheimer's Disease:

Hypouricemia stands as the main neurodegenerative disorder affecting the brain because it produces dementia alongside memory decline and mental deterioration and creates senile plaques together with neurofibrillary tangles. Epidemiological evidence confirms that lower levels of uric acid in the body lead to increased chances of developing Alzheimer's disease [9].

1.2.2.5. Parkinson Disease

Parkinson is a nervous disorder related to low levels of uric acid as the cellular stress in parts of the brain controlling the movement are aggravated by the reduction of antioxidant response. In case the level of uric acid is not enough, the byproducts are produced to which the integrity of the neurons is impaired. This metabolic imbalance differs with the brain efficiency and it fastens the decline because it mainly affects the network of locomotive coordination. A study suggests that the constant low levels of uric acid could lower defenses leaving individuals more prone to the neurodegenerative condition, which is a hallmark of Parkinson disease [10].

1.2.2.6. Multiple Sclerosis

The low uric acid levels are associated with the autoimmune disease multiple sclerosis (MS) due to its reduced antioxidant activity. Uric acid neutralizes peroxynitrite, reactive nitrogen species that have been involved in the depression of myelination and destruction of the nerves. The central nervous system cannot provide the necessary defense in case of oxidative and nitrosative stress in the event of low levels of uric acid [11].

1.2.2.7. UA And Chronic Kidney Disorder :

Due to hyperuricemia the kidneys maintain body equilibrium through their function of removing blood waste particles and excess fluid and electrolytes. Wasteful substances accumulate when kidney performance declines thus leading to multiple health complications [12].

1.2.2.8. Nephrolithiasis:

The medical condition nephrolithiasis happens when urine contains excessive concentrations of lithogenic substances including calcium, oxalate and uric acid. Nucleation and growth and aggregation of crystals in the renal collecting system create serious pain and bleeding as well as possible urinary system blockage.

1.2.2.9. Preeclampsia And Eclampsia:

Preeclampsia develops during the second or later trimester when women display hypertension symptoms with proteinuria alongside systemic endothelial dysfunction. . The combined effects of glomerular endotheliosis which decreases renal excretion coupled with proximal tubule reabsorption increase and enhanced placental cellular breakdown from oxidative stress lead to increased uric acid production [13].

1.3.Limitations Of Traditional Methods:

Despite of all mentioned aspects about uric acid its compulsory to control its ratio in human body. So there were following traditional techniques that used for its control but due to many limitations we will use the electrochemical sensor for detection of uric acid.

1.3.1. Colorimetric Methods

1.3.2. Spectrophotometric Methods

1.3.3. High-Performance Liquid Chromatography (HPLC)

1.3.4. Gas Chromatography (GC)

1.3.5. Uricase based Assay

1.3.1 Colorimetric Methods

1.3.1.1 Interference From Reducing Substance:Ascorbic acid (Vitamin C) together with free thiol compounds such as glutathione and cysteine adversely affect colorimetric methods used to measure uric acid in biological samples. The lowering agents have multiple effects which impact test accuracy levels [14].

1.3.1.2. Hydrogen Peroxide Depletion:Uric acid produced from uricase reactions with H_2O_2 becomes available for analysis through peroxidase Tests including the Trinder technique. The presence of peroxidase allows hydrogen peroxide to combine with chromogenic substrates resulting in color change that corresponds to the amount of uric acid. Ascorbic acid functions to decrease H_2O_2 rapidly which prevents chromophore generation causing incorrect readings of low uric acid amounts [15].

1.3.1.3. Direct Interaction With Chromogenic Substrates: Color development may be changed by ascorbic acid's direct interactions with chromogenic substrates or assay intermediates. Depending on the particular assay circumstances, this interaction may cause the color signal to be quenched or enhanced, which would compromise the accuracy of the measurement [16].

1.3.1.4. In Vivo Interference Post Vitamin C Ingestion: Consuming vitamin C by mouth will raise serum ascorbic acid concentrations that eventually interrupt the accuracy of urate tests conducted in serum. The Trinder method shows the most prominent effect of such interference

1.3.1.5. Matrix Effects In Colorimetric Assays: During testing biological components in samples create matrix effects that alter the measured results. The measurement of uric acid through colorimetric assays reveals significant effects on the results. This study discusses how hemoglobin and bilirubin, common components in biological fluids, can absorb light at similar wavelengths used in colorimetric assays [17]. The testing process for uric acid in biological materials including plasma, serum and urine experiences several matrix interferences that create measurement errors. The presence of proteins and lipids in biological fluids affects enzyme-based or colorimetric responses that lower assay precision and dependability .

1.3.1.6. Endogenous Metabolites And Lipid Interference: Lipids together with endogenous metabolites affect the optical properties of samples thereby producing matrix effects during testing. A high concentration of lipids in test samples causes light scattering phenomena that reduce the accuracy of colorimetric measurements through absorbance measurements [18].

1.3.1.7 Bilirubin And Hemolysis Effects On Uric Acid Measurement: Explains how hemoglobin and bilirubin can affect colorimetric tests for a variety of analytes, including uric acid. Bilirubin can absorb light with wavelengths comparable to those of test reagents, and hemolysis can release hemoglobin, which can result in inaccurate findings. The detection of uric acid is made more difficult by these matrix effects, particularly when clinical samples exhibit hemolysis or elevated bilirubin levels [19].

1.3.1.8. Sensitivity Limitations:

Because colorimetric assays are very easy and affordable, they are frequently employed to quantify the amounts of uric acid in biological materials. However, when it comes to precisely identifying tiny quantities of uric acid, these tests frequently have sensitivity limits. When tracking individuals with diseases like gout, hyperuricemia, or chronic kidney disease (CKD) that call for accurate assessments of uric acid levels, this restriction may be very troublesome.

1.3.1.8.1. Problems In Identifying Low Uric Acid Levels: Colorimetric tests usually depend on the reaction of uric acid with certain chemicals, which produces a colored product. The amount of uric acid present in the sample directly correlates with the color's intensity. It can be challenging to precisely detect and measure uric acid at low quantities, such as those present in specific clinical situations, since these amounts can produce faint signals [20].

1.3.1.8.2. Dynamic Range And Detection Limitations: Compared to high-performance techniques like HPLC or LC-MS, colorimetric assays usually have a smaller dynamic range, or the range of concentrations across which the test yields accurate and trustworthy findings. Because colorimetric tests have weak sensitivity at the lower end of the measurement scale [21].

1.3.1.8.3. False Negatives Due To Insufficient Sensitivity: The tendency of colorimetric tests to produce incorrect negative results becomes prominent during assessment of small changes in uric acid levels [22].

1.3.2. Spectrophotometric Methods

The main disadvantage is reagent breakdown process affects color development in the assay which produces incorrect uric acid concentration readings [23]. Stability problems in reagents cause tests to become non-repeatable which shortens shelf-life while increasing expenses and requiring constant quality tests .

1.3.2.1. Temperature Sensitivity: The main limitation is reaction kinetics of spectrophotometric tests show dependence on temperature changes. The temperature

variations in either the surrounding environment or during the reaction period affect the color development of test reagents leading to imprecise and inconsistent absorbance results.

1.3.2.2 Calibration: The main disadvantage is periodic calibration variations occur because changes take place in the composition of reagents or the instrument develops sensitivity fluctuations [24]. Standard solution testing must occur on a regular basis to protect both the measurement accuracy and result reliability [25].

1.3.3. High Pressure Liquid Chromatography:

Uric acid measurement in different biological materials including urine, serum and saliva depends heavily on the analytical technique known as High-Performance Liquid Chromatography (HPLC). The implementation of HPLC techniques for uric acid detection faces both cost-related and complexity-related limitations that could impact its use in regular testing practices.

1.3.3.1. Cost Consequences: A complete HPLC setup includes various components that include the chromatograph alongside detectors such as UV and fluorescence and data acquisition tools and different solvents and reagents. Routine maintenance and calibration procedures costs might prove too high for laboratories that operate on limited spending allocations and perform many testing procedures simultaneously [26].

1.3.3.2. Complexity Of Operations: careful approach must be maintained when performing HPLC based analysis of uric acid. Sample preparation utilizes specific techniques to eliminate interfering compounds [27]. The importance of specialized training exceeds any point of exaggeration. Workers require proper training for interpretation of chromatographic data and troubleshooting of complex equipment as well as running complicated systems. Analytical competency requires continuous training and skill advancement resulting in increased complexity regarding its maintenance [28].

1.3.4. Gas Chromatography The analytical technique known as gas chromatography (GC) serves as a common method to sort substances which evaporate without structural

modifications. Uric acid detection using this method is problematic even though it faces several critical obstacles. The debate evaluates why GC fails to analyze uric acid through contrasting the analytical method requirements and uric acid's physicochemical traits alongside operational limitations that arise during the analytical procedure.

1.3.4.1. Uric Acid's Physicochemical Characteristics: Uric acid detection using this method is problematic even though it faces several critical obstacles. Difficulties with Derivatization the GC analysis of uric acid requires derivatization since this method empowers the examination of unstable compounds that are tough to volatilize. The precise detection and measurement of uric acid may be hampered by artifacts or adverse responses brought about by derivatization. All of these elements work together to make GC less useful for uric acid analysis.

1.3.4.2. Issues With Thermal Degradation: Forwarding GC analysis requires high temperatures that create a serious risk of degradation for thermally unstable compounds. Thermal processing stages in sample derivatization combined with vaporization and GC analysis have resulted in the creation of degradation products that may complicate the analysis while possibly leading to inaccurate research outcomes [29].

1.3.4.3. Sample Preparation And Matrix Effects: The various materials contained in blood and urine specimens present analytical difficulties for precise examination of uric acid. Matrix effects can reduce analysis accuracy because they cause either ion suppression or ion amplification effects. Sample preparation for GC-MS analysis often requires complex processes that extend examination times and boost the chance of human errors [30].

1.3.4.4. Limitations Of The Detector: Frequent use of GC detectors includes Organic molecules detection through Flame Ionization Detector (FID). An FID shows minimal or no reaction when detecting oxidized substances along with full unresponsiveness to products like uric acid so it primarily detects hydrocarbons [31].

1.3.5 Uricase Based Assay

The determination of uric acid content in biological materials uses assays that rely on uricase as their primary method. Some chemical and technological conditions cause accuracy and dependability issues in these assays. Appropriate understanding of these interferences becomes crucial in order to ensure proper measurement of uric acid [32].

1.3.5.1. Citric Acid-Containing Anticoagulants' Interference: The presence of citric acid or sodium citrate anticoagulants in blood collection tubes negatively affects measurements that use uricase. The critical component of uricase activity named hydrogen peroxide (H_2O_2) meets its destruction from citric acid [33].

1.3.5.1. Borate Ion Inhibition: Borate ions have the ability to suppress uricase activity and are frequently utilized as bacteriostatic agents in urine samples. This inhibition results in less accurate uric acid readings and a wider range of outcomes.

1.3.5.2. Issues With Automated Systems: Testing uricase with automated systems requires complex modification procedures. The accuracy of measurements suffers due to challenges connected with light-scattering substances present in samples and reagents. The results produced by specific uricase/peroxidase systems perform inferior to alternative assessment methods which presents potential implementation problems when adapting such tests for select analytical equipment [34].

1.4. Electrochemical Sensors

Electrochemical sensors are analytical devices that convert chemical information into an electrical signal through electrochemical reactions. They typically consist of a working electrode, reference electrode, and auxiliary electrode, and operate based on changes in current, potential, or impedance. These sensors are widely used for detecting a variety of analytes, including biological molecules, toxins, gases, and ions. Due to their high sensitivity, low cost, rapid response, and potential for miniaturization, electrochemical sensors are valuable tools in medical diagnostics, environmental monitoring, food safety, and industrial process control. Their performance can be further enhanced using nanomaterials, enzymes, or synthetic recognition elements [35].

1.4.2. Electrochemical Sensors And Redox Reactions:

The sensor functions by using electrochemical reactions between substances. Electrons travel between the species that needs analysis (analytes) and the surface of the electrode as part of an electrochemical redox reaction that creates reducing substances which pick up electrons and oxidizing substances that give up electrons. Electrode surface interaction with the chemical species leads to an oxidation or reduction process which results in electron release or reception. The method delivers continuous analyte measurement service with highly sensitive detection abilities. The combination of electrode materials with their surface dimensions establishes the reaction efficiency since elevated surface areas lead to more active sites alongside faster electronic transfers. The performance of the sensor gets better when nanomaterials such as graphene and carbon nanotubes are used to modify the electrodes' surface properties [36].

1.4.3. Sensors Using Electrochemical Techniques: Electrochemical sensors measure the electrical characteristics changes that occur during electrochemical reactions by employing different detection techniques. The choice of application together with sensor type determines the optimal electrochemical method. The four main electrochemical methods used in sensor applications are voltammetry, amperometry, potentiometry and impedance spectroscopy.

1.4.3.1. Voltammetry The most commonly employed electrochemical detection method is voltammetry because it measures the current output when the working electrode potential changes across multiple values. The current-potential relationship provides essential information about the analyte including its concentration together with its redox properties [37].

1.4.3.2. Amperometry: The working electrode receives a fixed voltage during amperometric measurement which produces current data that varies over time. Analyte concentration in the sample corresponds directly with current output from the system. The assessment of real-time analyte concentrations is possible through amperometric methods [38].

1.4.3.3. Potentiometry: The potential readings in potentiometry must remain stable without any flowing electricity. The clinical diagnostic together with environmental

monitoring sectors constantly employ potentiometric sensors for testing pH and ion concentrations such as sodium, potassium and chloride. One key benefit of using potentiometric detection methods occurs due to the absence of demanding processes needed for sample preparation [39].

1.4.4. Elements That Affect Electrochemical Reactions

The electrode material, surface area, temperature, and analyte concentration are some of the variables that affect how efficient electrochemical processes are. Because it influences the stability of the redox reaction and the rate of electron transfer, the electrode material is a critical factor in determining the electrochemical performance.

electrode material [40].

1.4.4.1. Electrode Material: The choice of electrode material determines its electrical conductivity and stability together with its compatibility with biological environments through materials like gold platinum graphite and carbon-based graphene.

1.4.4.2. Surface Area: The reaction rate becomes faster when the electrode surface area increases because more interaction sites become available for analytes. The electrode surface performance of electrochemical sensors receives enhancement through nanomaterial applications which include graphene and carbon nanotubes and gold nanoparticles [41].

1.4.5. Materials Used In Electrochemical Sensors

Fabrication materials in electrochemical sensors determine their sensitivity together with selectivity and limit of detection (LOD) and response time and stability and reusability. Detectability of target substances in different environmental conditions is directly correlated to material properties which include surface area and electrical conductivity and catalytic activity and porosity and biocompatibility. The optimization of signal transduction pathways becomes possible through modifications of material surface chemistry and morphology and structure [42].

The category of conductive polymers includes three organic materials: PANI, PPy and PEDOT which exhibit electrical conductivity. CPs operate unlike standard polymers

because their backbone includes connected π -electron systems for electrical conduction together with charge distribution. The desirable traits of these materials consist of mechanical flexibility together with simple synthesis capabilities and redox properties as well as adaptable electrical characteristics [43].

1.4.5.1. Nanomaterials Based On Carbon: The extensive research community recognizes carbon-based nanomaterials including graphene and carbon nanotubes (CNTs) and carbon black and carbon quantum dots because of their outstanding electrical and mechanical and thermal properties. Single-walled and multi-walled carbon nanotubes (CNTs) present the best properties for miniature sensor platforms because of their high conductivity and cylindrical nanostructure [44].

1.4.5.2 Metal And Metal Oxide Nanoparticles: Development of electrochemical sensors demonstrates superior capabilities through the implementation of metal and metal oxide nanoparticles including Au, Ag, Pt, ZnO, Fe₂ O₃ and TiO₂ . The quantum effects together with high surface-to-volume ratio caused by their nanoscale dimensions enables metal and metal oxide nanoparticles to enhance catalytic and adsorptive activity [45].

1.4.5.3 Metal-Organic Frameworks (Mofs): MOFs contain crystalline materials with built-in porosity that combines metal ions or clusters attached to organic ligands. The design structure of MOFs enables scientists to manage exact specifications regarding their pore dimensions with their surface area characteristics alongside adjustable chemical properties. MOFs present outstanding characteristics that make them perfect materials for adsorbing particular compounds and concentrating them ahead of detection in sensor devices [46].

1.4.5.4. Ionic Liquids: The state of salts as ionic liquids persists as liquids without freezing at normal room temperature. These compounds are made exclusively from ions which yields low vapor pressure properties while exhibiting broad electrochemical ranges and superior ionic conductance and superior thermal resistance. These characteristics make ionic liquids suitable for electrochemical sensor systems.

1.4.5.6. Biomaterials (Enzymes, Antibodies, Dna): Biosensors contain vital biomaterials made from enzymes in combination with antibodies and DNA aptamers. Bio-recognition elements attached to electrochemical sensors create their selective properties. Electroactive products emerge when enzymes promote specific reactions and these products enable the use of voltammetric or amperometric detection methods [47].

1.5. Fabrication Techniques

1.5.1. Screen-Printing: Experts use screen-printing extensively because it provides both economical and scalable options for fabricating electrochemical sensors [48]. The process involves using patterned meshes to apply conductive inks onto substrates in order to build electrode designs [49].

1.5.2. Drop-Casting: The process of drop-casting includes solution addition onto electrodes followed by drying until a thin sensing material film forms. The straightforward method enables inclusion of different nanomaterials including graphene carbon nanotubes and metal nanoparticles to improve both sensitivity and selectivity in the sensor [50].

1.5.3. Electrodeposition: Through electrodeposition technicians conduct a process of material deposition onto electrodes by utilizing electrical currents. The control provided by this method enables engineers to define both the thickness and growth pattern of the deposited material layer which proves essential for sensor optimization [51].

1.5.4. Chemical Vapor Deposition (CVD): The reaction between gaseous reactants that produces a solid material during substrate deposition is known as CVD for thin film material deposition including graphene and carbon nanotubes. High-performance electrochemical sensors benefit from this technique because it produces highly uniform films with exceptional electrical properties and top-quality results. CVD processes demand both elevated temperature conditions and specialized equipment systems that reduce its practical scaling capabilities [52].

1.5.5. Photolithography: Photolithography exists as a microfabrication method which applies light patterns to substrates to create semiconductor industry designs. Photolithography functions as an essential process to develop sensors that require both high specificity and sensitivity for uses in neural interfaces and lab-on-a-chip devices [53].

1.5.6. Laser-Induced Graphene : The conversion of carbon-rich materials such as polyimide films into porous graphene structures uses a laser as the central process in the LIG technique. The fabrication method provides fast and budget-friendly manufacturing of flexible conductive electrochemical sensor electrodes.

1.5.7. Self-Assembled Monolayers (SAMs): A self-assembled monolayer technology achieves spontaneous molecular organization of surface layers which adapt electrodes by improving their selective features and biological compatibility. Biosensor applications make extensive use of SAMs because these structures enable the development of immunosensors and DNA sensors [54].

1.5.8. Paper-Based Microfluidics: The low cost and easy-to-use operations along with portable features make paper-based microfluidic devices increasingly attractive. The transportation of fluids occurs through paper capillary mechanisms while requiring no external pumping system. The fabrication of these devices happens through three methods including screen printing and wax printing and inkjet printing which enable quick mass manufacturing [55].

1.6. Oxidative Functionalization Of Carbon Nanotubes Enhancing Dispersibility And Surface Reactivity.

1.6.1 Material Required

- Multiwalled or single-walled carbon nanotubes (MWCNTs/SWCNTs)
- Concentrated sulfuric acid (H_2SO_4 , 98%)
- Concentrated nitric acid (HNO_3 , 65–70%)
- Deionized (DI) water
- Centrifuge and centrifuge tube

- Ultrasonic bath or probe sonicator
- Vacuum oven or desiccator
- pH meter or pH indicator strips

1.6.2 Overview: The scientific community started studying carbon nanotubes (CNTs) after Iijima's 1991 discovery due to their exceptional thermal characteristics along with electrical properties and powerful mechanical resistances. Through the process known as surface functionalization and especially oxidative functionalization scientists attach functional groups to CNT surfaces thus improving both reactivity and dispersibility properties [56].

1.6.3 . The Need Of Functionalization By Oxidation: Reactive functional groups include carbonyl ($-C=O$), hydroxyl ($-OH$) and carboxyl ($-COOH$) that oxidative methods attach to carbon nanotubes surfaces. Functionality allows carbon nanotubes to become more compatible with aqueous and polar liquids by increasing their hydrophilic surface characteristics. Chemical changes can occur through reactive sites that surface functionalization provides [57].

1.6.4 Oxidative Functionalization Methods: Acid treatment: CNTs are often treated with a solution of strong sulfuric acid (H_2SO_4) and nitric acid (HNO_3) as part of an acid treatment procedure. By opening the CNTs' ends and adding functional groups, this procedure increases their reactivity [58]. Plasma treatment: oxidation allows researchers to add functional groups on CNT surfaces because it improves the dispersibility and compatibility of the material with polymers.

1.6.5.Effects Of Oxidative Functionalization: The incorporation of polar groups transforms carbon nanotubes into more soluble materials for a diverse range of solvents thus enabling easier processing and incorporation into composite materials. Improved

1.6.6 Applications Of Functionalized CNTs: Composites: Better mechanical and electrical properties emerge in CNT-based composites because polymers demonstrate enhanced compatibility with them [59].

Biomedical Applications: Drug administration along with imaging technologies and tissue engineering operations benefit from improved dispersibility and biocompatibility that carbon nanotubes provide. "

1.6.7.The Electrochemical Cell And Electrochemical Reactions:

The basis of all the electrochemical sensors is an electrochemical reaction, the occurrence of which can be fixed upon the connection of the analyte with the electrode surface of the sensor. Under such reactions, the analyte gives electrons to the electrode leading to a voltage or current which is proportional to the concentration of the analyte. The electrochemical sensor consists of a simple electrochemical cell that consists of two electrodes-working electrode and the reference electrode [35]. When applied, a reference electrode creates a constant measurement of a potential of the working electrode levels and counter electrode creates an electrical connection through the working electrode where redox activities take place and detection of the analyte happens. There is a correlation between electrode potential and analyte concentration and this correlation is made possible by the Nernst equation. The potential of the working electrode is directly proportional to the logarithmic expression of the concentration of the analyte to attain accurate real-time determination of the concentrations [60].

Aims And Objective

Objectives of this study are:

- To develop and characterize a novel, electrochemical sensor based on CuSe@CNT modified for the sensitive, selective, and reliable detection of UA.
- Analyze the morphological, structural, and elemental characteristics of the created electrode using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) analysis, UV-Visible & Elemental Mapping.
- Assess the analytical capabilities, such as sensitivity, selectivity, limit of detection, Limit of Quantification and analysis of fabricated sensor

CHAPTER 2

LITERATURE REVIEW

An electrochemical detection system based on combined electrodeposition of reduced graphene oxide and gold nanoparticles became available for measuring uric acid and ascorbic acid.

Testing of sensor performance took place under different pH levels since both uric and ascorbic acid depend on pH for electrochemical oxidation. The test results for uric acid detection reached excellent standards. The sensor revealed detection sensitivity at $0.31 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$ with a detection range from 10 to $500 \mu\text{mol dm}^{-3}$. The sensors were tested for acid detection in both food samples like commercial milk and fruit juice and bodily fluids of urine. The performed measurements strongly agreed with methods adopted in traditional practices to yield exceptional results [3].

The researchers produced a new glassy carbon electrode (SWCNTs array-GCE) by applying simple electrochemistry. The vertically aligned design of the electrodes becomes available for immediate usage through their manufacturer-provided structure. The modified single-walled carbon nanotubes exhibit superior electrode application compared to previous methods due to their enhanced positioning. The SWCNTs array-GCE device manufactured for dopamine (DA) and uric acid (UA) specific detection provides noticeable oxidation potential peak differentiation between these compounds and ascorbic acid (AA) when they exist separately. The acetone pretreatment of SWCNTs array-GCE using atomic force microscopy revealed that it leads to improved electrocatalytic capabilities for the electrode during electrochemical measurements. SWCNTs array-GCE pretreated with acetone reveals a distinct third two-electron oxidation event of 5,6-dihydroxyindole to indole-5,6-quinone thereby demonstrating its outstanding electrocatalytic behavior towards dopamine detection. The research explored SWCNTs array-GCE suitability for detecting DA and UA in genuine human serum and urine solutions. Analysis results indicated promising recovery measures of 94 to 104% thus raising potential as an alternative biosensor substrate for further use [61].

This work presents an electrode strip that functions without enzymes for rapid detection of uric acid in human whole blood. A temporary amperometric sensor strip for uric acid detection was developed by applying a three-electrode setup on polypropylene through basic screen printing (thick-film) methods at minimal cost. The production of working and counter electrodes made use of commercial carbon ink through screen-printing techniques. Screen-printing commercially available silver ink followed by electrochemical pretreatment enabled the integration of pseudo-reference electrode on one substrate. The method was applied to analyze real human whole blood samples resulting in satisfactory results that matched phosphotungstic acid clinical test protocols [62].

A detection technique which combines high sensitivity with good accuracy and rapid response is crucial for simultaneous identification of AA, DA and UA. The detection method used second-order derivative linear sweep voltammetry for the electrochemical measurements. Peak potentials for DA-AA and DA-UA and UA-AA were separated by 212, 130 and 342 mV respectively. PVP-GR/GCE) were 212, 130 and 342 mV respectively. The peak current values rose steadily when researchers decreased the excess potential of AA, DA and UA. When working optimally the proposed sensor provided measurement ranges from 4.0 μM up to 1.0 mM for AA and 0.02 μM to 100 μM for DA and UA simultaneously. The electrochemical sensor achieved detection limits of 0.8 μM for AA together with 0.002 μM for DA and 0.02 μM for UA [63].

Among all essential cell functions of purines the synthesis of monomeric nucleic acid precursors stands as the most consequential task. Some coenzymes contain purines which also serve essential roles in platelet physiology along with providing vital functions to muscles and neurotransmission channels and helping to control energy metabolism and signaling processes.

Medical science has confirmed that excessive uric acid production through purine metabolism becomes an increasing factor in human diseases. Health conditions that result in severe cardiovascular diseases show an inverse correlation to blood uric acid levels. The paper explains through enzyme processes how purines degrade while describing their molecular nature before producing uric acid [2].

Evolutionary development of increased human uric acid levels results from the loss of uricase functionality which occurred due to several gene mutations during the Miocene period. The human body produces UA as its terminal product during purine metabolic processes. The kidneys successfully reclaim 90% of filtered uric acid which shows the physique avoids eliminating this substance. These findings show that evolution and physiology view UA as a beneficial substance because they choose to keep it rather than classify it as hazardous waste [4].

Gout draws enhanced attention throughout academic fields along with clinical practice environments. Various elements might contribute to the increased attention that gout receives in academic and clinical practice contexts. The prevalence of both hyperuricemia and gout has risen in wealthy countries since the 1990s thus leading to an enhanced gout burden. The research focus has grown on gout-hyperuricemia associations with heart conditions along with new opportunities discovered from hyperuricemia treatments. Scientists have proven the ability of imaging techniques to detect preclinical signs of urate deposition while measuring crystal reduction throughout urate-lowering medication treatment. The audience favors targeted therapy as an increasing treatment approach for numerous medical conditions. Medical practitioners increasingly adopt treating to target methods in disease management. The management framework uses diverse diseases as its treatment focus. Therefore, different targets have been. Different targets exist for different burden stages as well as treatment stages. burden of disease and for different stages of treatment. The final strategic target, to which any patient intervention deserves examination as the final therapeutic objective. Healthcare practices need to eliminate completely all urate crystals that exist within tissues. The weight of tissue depositions and therapeutic response to urate reduction therapies should be assessed in specific patient groups. finally that the final strategic objective. The ultimate goal of treating patients with gout in healthcare should be to eliminate the presence of urate crystals. completely resolve urate crystal deposits [7].

Gout exists as an inborn metabolic mistake yet researchers have yet to identify a single culprit for the hyperuricemia which is its underlying cause. 2. An excessive intake of

purines represents the singular cause of hyperuricemia within affected family members. Other specific metabolic disorders result in accelerated purine synthesis according to literature.^{5' 6} The absence of glucose-6-phosphatase which underlies the development of glycogen-storage disease Type I leads to quickened purine synthesis in such patients [64].

The SUA values between black and white populations in the USA are generally similar but the measurements tend to differ significantly among geographic areas and ethnic populations. Populations at risk for hyperuricemia. The patient group includes people with congestive heart failure and those with hypertension along with renal impairment conditions. Research supports the ability of hyperuricemia to make its own predictions regarding hypertension development [7–9]. Nephrosclerosis or reduced renal blood flow caused by hyperuricemia leads to elevation of SUA levels and blood pressure simultaneously. Foot development is impaired by hyperuricemia because the condition stimulates renin production that leads to increased levels of angiotensin II and elevated blood pressure. The National Health and Nutrition Epidemiologic Study 1 (NHANES 1) initiated mortality follow-up during 1971 which qualified it as the initial study employing representative U.S. national population data. The research identified a link between baseline SUA and cardiovascular outcomes among women during its initial period that extended up to 1986. The authors reviewed this same database another time 10 years after it underwent extension to 1992 through their recent accessible year . When researchers examined the data at this point they observed 1593 deaths had happened since the previous analysis [65].

It is known that truncal obesity, hypertriglyceridemia, increased blood pressure, and insulin resistance are signs of metabolic syndrome become a significant risk factor for renal disease and a prevalent characteristic of dialysis patients. Elevated uric acid is one characteristic shared by people with metabolic syndrome. Despite frequently being seen as subordinate to Recent data indicates that uric acid plays a major role in mediating hyperinsulinemia. In particular, fructose elevates uric acid and can quickly develop metabolic syndrome in rats; thus, reducing uric acid in rats given fructose avoids metabolic syndrome symptoms. Additionally, uric acid has been epidemiologically

linked to progressive renal illness in humans and can hasten renal disease in experimental animals. The relationship between uric acid and progressive renal illness in humans became known through epidemiological research and can trigger experimental animals to develop renal disease more quickly. The global epidemic of metabolic syndrome and kidney illness results from foods with high fructose and purines levels that raise uric acid levels in the body [66].

AD stands as the most common neurodegenerative disorder affecting the central nervous system because it causes dementia with cognitive deterioration and memory failure and generates senile plaques and neurofibrillary tangles. The existing geographic prevalence of AD stands at 26.6 million as per estimates but this number will surge to 106.2 million during the year 2050. Research shows that when AD reaches this phase 1 out of 85 people will suffer from the condition placing intense pressure on society [67].

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AD stands as the most common neurodegenerative disorder affecting the central nervous system because it causes dementia with cognitive deterioration and memory failure and generates senile plaques and neurofibrillary tangles [69].

The blood system operates as an environment free from microorganisms in regular individuals. Several sequence-based and ultra-microscopic studies show that blood contamination by gut microbiota becomes possible as intestinal permeability rises. A few

months ago Amar et al. 14–17 carried out extensive research within a big nationwide population to demonstrate how blood microbiome disturbances link directly to cardiovascular events. The device contains 24 test-specific reagents at its disposal and operates through end-point and rate and multipoint assay methods. At the review time the test encountered a limitation since increased bilirubin led to problematic interferences with creatinine phosphorus uric acid and triglyceride levels. The manufacturer continues work to eliminate the interference problem [19].

One strategy for the creation and verification of Scientists have evaluated molecular biomarkers thoroughly for humans by performing research simultaneously in animals while tracking the formation of aflatoxin adducts [70]. An investigation into the Scientific studies show aflatoxin metabolite urine excretion occurs in an area of China where liver cancer cases are particularly common . An immunoaffinity-HPLC system was used to examine individual aflatoxins in twenty-four hours of collected urine samples [20].

The microfluidic paper-based analysis devices (PADs) employ a drying method with bienzyme detection of glucose and uric acid through colorimetric reactions. The design of the 3D PADs channels leads to better end results. The color output acquisition happens with two capturing systems consisting of a standard camera together with Gel Documentation equipment. The implementation of Gel Documentation systems allowed researchers to determine uric acid and glucose with limits of detection at 4.31×10^{-5} M and 3.81×10^{-5} M respectively. These values surpass previously published PAD methods by one order of magnitude. Positive results from human serum samples are found and are consistent with the findings of clinical testing. For the simultaneous detection of glucose and uric acid on the PADs, a colorimetric technique that is inexpensive, easy to use, and quick has been devised with improved sensitivity [71].

The method of calorimetry enables theoretical measurement of heat evolution or absorption during any chemical reaction such as enzymatic reactions. The low sensitivity of calorimetry causes reactions to run for longer durations or employ high-volume samples as a replacement. Restrictions in the analysis of physiological specimen constituents emerge from these two operational obstacles tested unease-based uric acid

measurement as a potential analytical tool for clinical laboratories through experimental research [72].

Current technological methods used for analyzing uric acid in blood and urine either employ chemical or enzymatic reactions to produce allantoin. A chromogen simultaneously reduces while an oxidation reaction proceeds in order to produce a chromophore that can be spectrophotometrically measured. The catalytic reaction utilizes uricase (urate: oxygen oxidoreductase EC 1.7.3.3.) to determine uric acid concentration through direct spectrophotometry at 292 nm absorbance changes and oxygen consumption and produced hydrogen peroxide measurement [25].

An analytical approach must demonstrate precise and speedy methods that establish the presence of unwanted impurities in raw materials because such substances affect both efficacy and safety of the drug product. HPLC represents today's primary pharmaceutical analysis technique since it delivers superb separations with outstanding detection capabilities. The automated and cultured method of Thin Layer Chromatography (TLC) utilizes optimized silica gel 60 particles which possess sizes lower than standard TLC particles. The analytic techniques used for purification include chemical substances, steroids and pesticides and water analysis as well as food dyes and vitamins and pesticide investigation of fruits and vegetables. The current technological advancements help us understand why they have become more popular in today's society [24].

The analytical procedure High-Performance Liquid Chromatography (HPLC) operates as an advanced technique to separate and identify along with quantitative determination of elements within complex mixture compositions. The combination of superior resolution and precision and efficiency makes HPLC an essential tool for service in forensic research. The flexibility of this method for analyzing various chemical compounds decreases alongside its accuracy because it adopts different detection systems such as mass spectrometry, fluorescence and UV-visible. The paper abstract presents the key concepts including the significance of HPLC alongside its practical applications together with a discussion of its limitations [27].

The synthesis process involved creating receptive electrochemical sensors through Au@Pd nanocomplexes supported by reduced graphene oxide which simultaneously detected and isolated sensitivity measurements of uric acid (UA), dopamine (DA) and ascorbic acid (AA) across wide level ranges and minimal detection thresholds. Clear differentiation of oxidation peaks with enhanced peak currents for AA, DA, and UA appeared in the Au@Pd-RGO-modified electrodes because of the exceptional catalytic performance of Au@Pd nanoparticles together with RGO's high conductivity. The annealing process of graphite oxide with melamine resulted in the formation of nitrogen doped GA (NGA). The material mixture displayed robust ability to catalyze the oxidation of all three analytes AA, DA, and UA..In recent years, GA, a two-dimensional graphite monolayer, has garnered a lot of attention because of its large specific surface area, robust mechanical structure, superior electrical conductivity, and high electrocatalytic activity. 17–20 In heterogeneous catalytic processes, graphene oxide (GO) and reduced graphene oxide (RGO), two of its derivatives, are showing promise as materials to support and scatter catalytically active metal nanoparticles. The detection system provided low detection limits together with extended linear detection areas. The nanocomposites using Pd-RGO supported by reduced graphene oxide materials were synthesized. The detection capabilities were outstanding because the sensors displayed both extensive working concentration ranges along with reduced detection barriers compared to Au-RGO-modified electrodes [28].

Thermal methods serve frequently in small molecule and metabolomics analysis for ionization processes and chromatography-based work as well as derivatization and vaporization functions especially within gas chromatography mass spectrometry (GC/MS). Multiple isotope-labelled analytical internal standards consisting of deuterated and ^{13}C -selected molecules have achieved successful application in an optimized derivatization method which enables analysis of diverse metabolite groups in non-automated GC metabolomics (off-line). Chromatographic separation performance and signal suppression decreased at a much lower extent through longer temperature ramps than brief temperature ramps ramp program. Organic acids along with fatty acids and sugars and sugar alcohols showed no changes but amino acids demonstrated

significant differences during the analysis. The analysis benefits from the strength of the GC metabolomics approach but special attention to sampling and heating should be maintained. The analysis process needs thorough attention regarding handling and heating steps for samples both before and during GC evaluations. The experiment requires multiple applications of multi-group internal standards for optimal results. During sample derivatization and vaporization and GC analysis thermal processing leads to the generation of novel substances as well as decomposition products of metabolites [29]. Matrix effects create significant obstacles for the analytical process because they lead to degraded accuracy and sensitivity together with compromised separation procedures. The main objective of this work is to document frequent matrix effect occurrences during the entire analytical journey specifically analyzing preparation processes and detection methods through LC-MS and GC-MS systems. Technical adjustment of ionization methods jointly with improved extraction techniques and optimized chromatographic methods and calibrated approaches helps reduce Through reverse-phase LC (RPLC) researchers use hydrophobic interactions between solute molecules in the sample to achieve analytical separation of components, ligands in the stationary phase and the mobile phase. RPLC has proven itself as a trusted method for complex matrix analysis in combination with MS A standard suppression approach in RPLC method development requires selecting appropriate elution conditions for the wanted analyte [73].

The serious cardiovascular illness known as pulmonary arterial hypertension (PAH) leads to both hypertension and vascular remodeling. PAH stands as an illness that proves almost impossible to detect through clinical evaluation. The medical testing of uric acid provides a method to screen for individuals who have PAH. Multiple investigations have proven that reactive oxygen species (ROS) play a vital role in the formation of PAH. Based on the pathophysiological mechanisms of PAH one must investigate the relationship between UA and ROS. The HPLC-ESI-MS/MS method analyzed UA concentration for PAH monitoring. Researchers utilized both rat models with hypoxia exposure and pulmonary arterial endothelial cells (PAECs) models under monocrotaline (MCT) treatment conditions. The HPLC-ESI-MS/MS method enabled

researchers to determine UA concentrations following H&E staining which served as the validation step. The research introduced thymine as an internal standard of uric acid for the initial time. The UA measurement results showed lower UA amounts in the treatment groups regarding the PAH concentrations ($p < 0.05$) but elevated UA presence was detected in the PAH groups versus normal group amounts. HPLC-ESI-MS/MS proved experimentally that it serves as a time-efficient reliable quantitative approach for PAH detection. The results showed that ROS and UA operate as dual regulatory elements .

Available uric acid levels function as a fundamental diagnostic tool in gout and therapeutic assessment in addition to their value in predicting associated medical conditions. Modern research has primarily ignored the impact of UA collection procedures on laboratory tested results. We analyzed how the substances in blood collection tubes affect UA measurement results because we detected major differences between UA readings in blood samples drawn through different tubes. Our main goals included two parts: (1) Establishing how different blood collection processes alter uricase-based UA test outcomes and (2) Identifying specific variables that create this measurement variability [32].

The UA concentration measured from blood samples kept in ACD tubes remained markedly below those obtained from SST tubes based on statistical significance ($P < 0.01$). A downward pattern emerged from the samples acquired in SC tube . The field of electrochemical detection methods stands as the topic of most intensive research today. Customer satisfaction remains the greatest challenge that science faces today. Materials for two-dimensional (2D) electrodes The combination of graphene with metal-organic frameworks together with MXene and transition metal dichalcogenides enables better control of response time and detection limit and sensitivity and selectivity. These technological advancements accelerated the development of wearable along with poin. Every chemical sensor requires two essential components which include a receptor as well as a physicochemical transducer [33].

The essential features that sensors should meet include rapid responses and accurate measurements, high degrees of selectivity and sensitivity along with repeatability

measures. Glucose oxidase GOx obtained immobilization via direct one-step electropolymerization into a poly v. v. o-phenylenediamine v. POPD film structure. A biosensor based on glucose detection was made through the immobilization of GOx into a POPD film using electropolymerization. The biosensor development led to glucose-based devices with linear response between 8 and 14 mM glucose level and sensitivity from 0.2 to 0.7 mA mM⁻¹ cm² and response times between 4 and 8 seconds and a maximum ascorbate retention of 95% [35]. The PBrPOPD–GOx biosensor functions as a biosensor that permits anodic and cathodic operations at potentials ranging between 0.4 to 0.6 V and 0.2 to 0.2 V versus Ag/AgCl respectively. The combination of an interference-blocking POPD layer with a PB layer achieves low potential functionality to minimize ascorbate interference. Researchers need to make substantial efforts in creating highly selective membranes to prevent interference in biosensors [36]. The exceptional stability combined with higher activity and distinctive electrical and chemical properties of the bimetallic nanostructures pushes their potential application in hydrogenation as well as renewable energy generation and catalysis and sensing fields. The obtained PdAg bimetallic nanoparticles received evaluation through energy dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), and small area electron diffraction (SAED). The electrochemical response of uric acid (UA) at Pd-Ag/CPE served as the main focus when scanning PdAg/CPE under various concentrations, scan rate conditions and pH solutions in phosphate buffer solution with 0.1 M concentration. When detecting UA the modified electrode using PdAg nanocomposite demonstrated the most effective electrocatalytic behavior among all electrodes tested. The PdAg nanocomposite enabled detection of UA at a very low 5.543 nM concentration level (CDL = 36/M) combined with a quantification limit of 16.64 nM (CQL = 106/M). The electrode composed of metal oxides and bimetals and carbon materials and conducting polymers with graphene exhibited unique characteristics and high specific capacitance capabilities [37].

This research reveals how researchers employed a 0.84 mm i.d. capillary tube along with an amperometric needle-type electrochemical glucose sensor for measuring tear

glucose. The new sensor shows glucose sensitivity of 0.032 (0.02 nA/ μ M; n = 6) which enables it to detect low glucose levels at 1.5 (0.4 μ M of glucose; S/N = 3). The established relationship between blood glucose and tears opens possibilities for using tear glucose measurement. Evidence shows that average tear glucose levels have an associated value with blood glucose levels based on a study review by Baca et al. which examines different methods of detecting blood and tear glucose levels⁹. Additional characterization together with support from studies using human and animal subjects will help understand if measuring tear glucose has value for glycemic control. Studies employing high-performance liquid chromatography with pulse amperometric detection (HPLC-PAD) to analyze critically sick patients' tear glucose levels identified no substantial link between blood glucose and tear glucose concentrations [38].

Research shows that the growth of potentiometric sensing continues to be strong. The ability to employ potentiometric sensors in different ways becomes accessible when such technology exists. Scientists are increasingly interested in wearable potentiometric sensors as a specific area of research. The development of disposable and scalable paper-based sensors for potentiometric measurement continues to grow which brings this strategy toward field-level and point-of-care technologies.

Every indicating ion-selective electrode needs a reference electrode for proper functioning. Any inaccuracy in the reference electrode creates an immediate effect on the precision level along with the measurement accuracy of a potentiometric system. The most commonly utilized reliable reference electrodes belong to the second design (Ag/AgCl or Hg/Hg₂Cl₂) when measuring high chloride electrolyte concentrations (for instance 3 M KCl) using no additional bridge electrolyte. The reference electrodes show very stable response patterns [39]. Models of Theory Outlining the ISEs' Reaction. A successful gradual progression of analytical sensor research necessitates precise knowledge and prediction of sensor behavioral patterns. The analysis of EIS data proves difficult since researchers need to identify appropriate ad hoc similar circuits. The DRT method finds its correspondence in equivalent circuits fitting by studying a function that characterizes the time-dependent response of examined systems. The theory behind this optimization remains simple but writers have not articulated the problem correctly so

solutions are impossible to discover through standard least squares minimization. The Bayesian approach demonstrates versatility that allows researchers to incorporate additional informative prior knowledge throughout its application [74].

The scientific implementation of ascorbic acid (AA) and uric acid (UA) expands for medical solution development toward better health solutions. The electrochemical biosensor detection capabilities of molecular imprinted polymers are boosted through cost-effective biological molecule detection using nanoincudes of graphene (GR). MIP sensor devices with nanomaterial-modified electrodes and their electrochemical speed along with economical design and mobility make them the preferred detectors for AA, DA and UA detection. Current electrochemical biosensors based on MIP nanomaterials detect biological substances such as AA and DA and UA.[41]. Exosomes represent little extracellular vesicles that develop from specific cell types. Pathological information about parent cells can be assessed from the biomarkers which include proteins and nucleic acids that exist within exosomes. Cancer patients experience carcinogenesis and metastasis changes due to exosomeWe review methods that use fluorescence and colorimetric as well as chemiluminescent signaling techniques for exosome detection. We additionally present future research paths and therapy possibilities tied to electrochemical exosome analysis methods [42].

The review details PEDT and all its derivatives with an emphasis on their historic development process as well as synthetic methods for hybrid materials along with property scope and current application usage. Electrochemical devices receive various advantages from these polymer solutions through their enhanced redox properties alongside their bandwidth of visible spectrum optical differences that lead to robust conducting states with stable doping conditions .

Biological and medical researchers actively employ gold nanoparticles (GNPs) that have controlled optical and surface chemical and geometrical attributes. A wide range of published investigations includes targeted drug and antigen delivery together with immunoassays and clinical chemistry and photothermolysis of cancer cells and tumors and genomics and biosensorics and optical bioimaging of cells and tissues with modern nanophotonic detection methods. This review analyzes the utilization of GNP

conjugates for delivering target molecules together with photothermal and photodynamic applications and clinical diagnostic methods and analytical systems. The present work includes a summary of GNP immunological traits to differentiate from previously published research [45].

During twenty years metal-organic frameworks (MOFs) have become highly relevant for energy-related applications due to their outstanding functional and structural and chemical diversity. This materials family recently displayed sophisticated electrical properties together with high charge mobility even though most MOFs typically work as electrically non-conducting materials. This paper evaluates the synthetic techniques along with electrical design principles that exist for developing charge-transporting porous frameworks. Researchers have only acquired the ability to create MOFs with high charge mobility and electrical conductivity while maintaining porosity during the past five years [46].

A detailed examination of glucose oxidase (GOD) characteristics exists because this enzyme shows extreme importance in biosensor development. The breakdown process of glucose using alternative enzymes demonstrates weaknesses. Electron acceptors' function in amperometric biosensors. Commercially applicable analytes need enzymes that demonstrate both strong stability and high specificity along with high turnover number. commercially available biosensor achieves its technical enhancements through these features [47]. Fast diagnostics systems have used dipstick and lateral-flow formats as their dominant technology during the previous thirty years. Their portable structure combined with their small dimensions as well as self-interpretable results without additional equipment led to increased consumer market adoption. The technology development of paper-based microfluidics stands at its initial phase while maintaining characteristics of disposability and weightlessness with affordability. This research aims to execute four vital functions: designing paper-based microfluidic devices followed by the optimization of functional microfluidic components and integrating modern detection techniques into paper platforms and analyzing variable readouts with portable devices and mobile camera phones [48].

Microfluidic paper-based electrochemical sensing devices & the combination of photolithography or wax printed patterned paper channels with screen-printed electrodes tool should help electrochemical sensing devices. Electrochemical sensing provides quantitative analysis but colorimetric tests stand as the best option for various applications. 10. The analytical platform discussed in this article provides a basic and fast method of detection for metrological evaluation of aqueous solutions including bodily fluids at a low cost per device of about two cents [49]. This article describes paper-based microfluidic devices (mPADs) which can be made easily through wax screen printing as a fast and affordable process. We examined the relationship between design line width with barrier width dimensions. The resolution together with heat duration and temperature control was established for devices crafted through this method. The combination of glucose oxidase with electrochemistry yielded results alongside colorimetric detection using 1,10-phenanthroline which proved wax screen printing. The research demonstrates that wax screen printing provides a basic budget-friendly method to make mPADs which can benefit underdeveloped countries through their development [50]. Scientific interest grows for placing metal nanoparticles within conducting polymers due to their ability to enhance conductivity together with electrochemical bond development for charge transfer have the capacity to improve the CP's conductivity while simultaneous redox processes. Charge may travel through the polymer chains and reach the scattered metals where the electrocatalytic process takes place because of the polymer's high electrical conductivity. The deposition of Pt Ni Cu and CdS nanoparticles occurred effectively within conductive PEDOT polymer films through implementation of these techniques. SEM images indicate that the nanoparticles distribute evenly inside the films while PEG presence leads to formation of open layer arrangements. The PEDOT:PEG-Ni electrodes have a catalytic effect during the oxidation reaction of formate or formic acid [51].

The remarkable one-dimensional p-electron conjugation mechanical strength and great chemical and thermal stability of Carbon nanotubes (CNTs) makes them highly desirable for many applications. The natural tendency of CNTs exists because of their Van der Waals interactions to form bundles and ropes [75].

The improved characteristics of functionalized carbon nanotubes enable simple production of new nanomaterials and nanodevices. Minnesota-based CNT functionalization techniques create better solubility and chemical compatibility of carbon nanotubes thus enabling diverse characterization methods and subsequent chemical reactivity. The research aims to provide a short summary of CNT functionalization alongside discussing the most recent developments for CNT applications and functionalization techniques [56]. Scientific research reveals that carbon nanotubes (CNTs) gain widespread use because of their beneficial properties in medication delivery applications and additional scientific fields. Both carbon nanotubes with a single wall SWCNTs along with MWCNTs are currently utilized or under research to develop potential applications in composite materials and textiles and medical treatments and energy technologies [57]. The paper delivers an overview of current methods to functionalize carbon nanotubes (CNTs) through covalent bonds and non-covalent attachments as well as through carbon atom replacement processes. The book details functionalized carbon nanotubes applications in biomedicine and electrode materials and electrochemical energy storage and nanoelectronics and composites and sensors [59].

CHAPTER 3

Methodology

3.1. Materials Required:

- The production requires Copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) which serves as the copper precursor.
- The production requires Selenium powder which serves as the Selenium precursor.
- The reducing agent in this reaction system is hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$).
- Carbon nanotubes serve as the supporting material which exists either in original or modified form (CNTs).
- Deionized (DI) water – solvent
- Ethanol – for washing
- Teflon-lined stainless-steel autoclave (50–100 mL capacity)
- Beaker, Patri Dish and Funnel.
- Magnetic stirrer & sonicator
- Centrifuge
- Drying oven or vacuum desiccator

3.2. Preparation Of The Selenium Precursor Solution

Use a scale to measure between 0.2 grams of salted selenium powder. Mix the selenium substance evenly throughout 15 milliliters of deionized (DI) water through constant stirring. The solution requires 1-2 mL drops of hydrazine hydrate to be added one at a time.

3.3. Preparation Of The Copper Precursor Solution

Method: Users should mix 0.3 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with 10 milliliters of DI water until complete dissolution..

Mix the solution thoroughly until all Cu^{2+} ions dissolve from the mixture forming a blue-clear solution.

3.4.Functionalization Of Carbon Nanotubes:

Concentrated sulfuric acid (H_2SO_4) and Water are carefully combined in a volume ratio of 1:1 to create a mixed acid solution. A 100 mL total volume, comprising 50 mL of H_2SO_4 and 50 mL of water used, for example, to treat 50 mg of CNTs. Because the combination is exothermic and releases fumes, adding acids should be done gradually under a fume hood. A delicate stirring method allows the addition of 50 mg pure CNTs to acid mixture over time to avoid clumping formation. Washing by fresh DI water spreads the pelleted CNTs before performing multiple centrifugation steps to reach supernatant pH 7 [56].

3.5.Functionalized Carbon Nanotube Dispersion

Pristine CNTs present difficulty in aqueous solution uniform dispersion because of their strong van der Waals forces that cause aggregation. CNT surface functionalization enables better metal ion interaction and dispersability through the incorporation of hydrophilic surface groups .

3.6 Component Mixing

One of the most important steps in creating the nanocomposite is integrating the copper and selenium precursors with the CNT dispersion. Place the 10 ml solution of CNT dispersion together with 10 ml of copper precursor solution and 15 ml selenium precursor solution inside a beaker. Magnetic stirring of the solution for 30 to 60 minutes is used to ensure proper mixing throughout the mixture. Add 15 mL hydrazine ion drops for production of $\text{Cu}_2 - \text{xSe}$ is enhanced by a slightly alkaline pH through its promotive effect on the reduction Cu^{2+} to Cu^+ followed by selenide ion reactions.

3.7. Treatment With Hydrothermal

Now 50 ml mixture of mixed components along with 40 ml of water is added in 100ml stainless steel autoclave with Teflon coating needs to be filled up to 90% of its capacity to create pressure conditions for the solution combination. A drying oven should heat

the autoclave with its lid sealed for 12 hours at 120°C. The autoclave should reach room temperature before completion of the reaction period. Under hydrothermal reaction conditions the CNT surface enables the formation of $\text{Cu}_2 - \text{xSe}$ nanoparticles which produces a perfectly merged nanocomposite [76].



3.8. Post Processing

The completed hydrothermal synthesis followed by autoclave temperature drop produces a black floating substance in the reaction media. The procedure of centrifugation at 3000 rpm for 10 minutes causes dense $\text{Cu}_2 - \text{xSe}$ -CNT nanocomposite particles to move down to the bottom of the tube while creating a clear to lightly tinted fluid that carries over any unused precursors together with byproducts. The washing process usually repeats three to five times using alternating solutions of ethanol and deionized water and includes centrifugation as a step when material is redissolved. During each cycle the procedure involves centrifugation after using resuspension methods as well as sonication or vortexing. The particle agglomeration reduces through ethanol use while DI water removes Na^+ , SO_4^{2-} , Cu^{2+} and Se^{2-} from solution aside from eliminating muối and metal ions. After washing the pellet gets re-separated via centrifugation before being directed to a sterile container for drying. Vacuum-drying of the sample persists for at least 12 hours at a temperature range between 60 to 80 °C depending on its mass and the equipment used. High vacuum drying techniques protect the $\text{Cu}_2 - \text{xSe}$ nanoparticle structure and function from degradation by preserving both their form and operational capacity. This drying method proves superior to usual air drying. The final production yields dried stable black or dark brown powder which functions for both solid-state use and redispersion applications.

3.9. Electrode Preparation

3.9.1. Deposition Of (CuSe@CNTs) On GC Electrode: The synthesized CuSe@CNT nanomaterial needs precise weighing prior to dispersion in 1:1 v/v ethanol-deionized water solution to reach the desired volatility-efficiency balance. An amount of Nafion around 20–50 μL (5 wt%) is incorporated into the mixture to serve as the proton-

conducting binder which strengthens film structure and enhances charge transfer. The method of choice for creating stable and uniform ink is a process of ultrasonication lasting between 30–60 minutes. The electrochemically active surface of the working electrode requires clean preparation which consists of sonication in ethanol before being treated with deionized water after successive polishing with alumina slurry (0.05 μm). After GCE drying in an oven under nitrogen stream researchers apply 5–10 μL of CuSe_x catalyst ink to the cleaned surface through micropipette measurement. Placing the electrode at controlled temperatures between 50–60 $^{\circ}\text{C}$ inside an oven enables stable film formation by allowing the solvent to evaporate before ending the drying process at room temperature in ambient conditions. After the electrode has dried undergo vacuum the gentle annealing step at 80–100 $^{\circ}\text{C}$ for 1-2 hours helps both improve CuSe@CNT particle-substrate interface contact and reduce selenide phase oxidation before the sensing process begins [77].

3.9.2. Deposition Of (CuSe@CNT) On Nanofibre: Electrochemical sensing of uric acid with carbon nanofiber-based electrodes CuSe@CNT , the first step is the creation or acquisition of a flexible conductor (e.g. carbon nanofiber coated with conductive material). Dissolve the synthesized CuSe@CNT in an ethanol/deionized water combination (1:1) solution containing 5 wt % Nafion binder and ultrasonicate 30-60 minutes in order to form a homogenous catalyst ink. Trim nanofiber mat to the desired size, rinse with ethanol and deionized water and dry. Deposit 5-10mL of the ink on nanofiber and then dry in the air at 50-60 degree centigrade for 1-2 hours. The modified nanofiber was used as a working electrode in a typical three-electrode cell by drying the material in a desiccator, then being rinsed gently using deionized water and then dried in a desiccator and applied in Voltammetric operations to test uric acid in PBS (pH 7-8) with platinum and Ag/AgCl [78].

3.10. Electrochemical Sensing

The three-electrode electrochemical cell firstly contains a prepared CuSe/GCE and later contain CuSe/nanofibre electrode which uses platinum wire as the counter electrode alongside Ag/AgCl as the reference electrode. Electrochemical examinations conducted through SWV, CV, and operate inside PBS using standard physiological pH values (7.0-

8.0) and monitor uric acid concentrations spanning from micromolar to millimolar ranges to providing high performance for sensitive detection at low detection limits **[35]**

CHAPTER 4

RESULTS

4.1.SEM Images Of CuSe@CNT

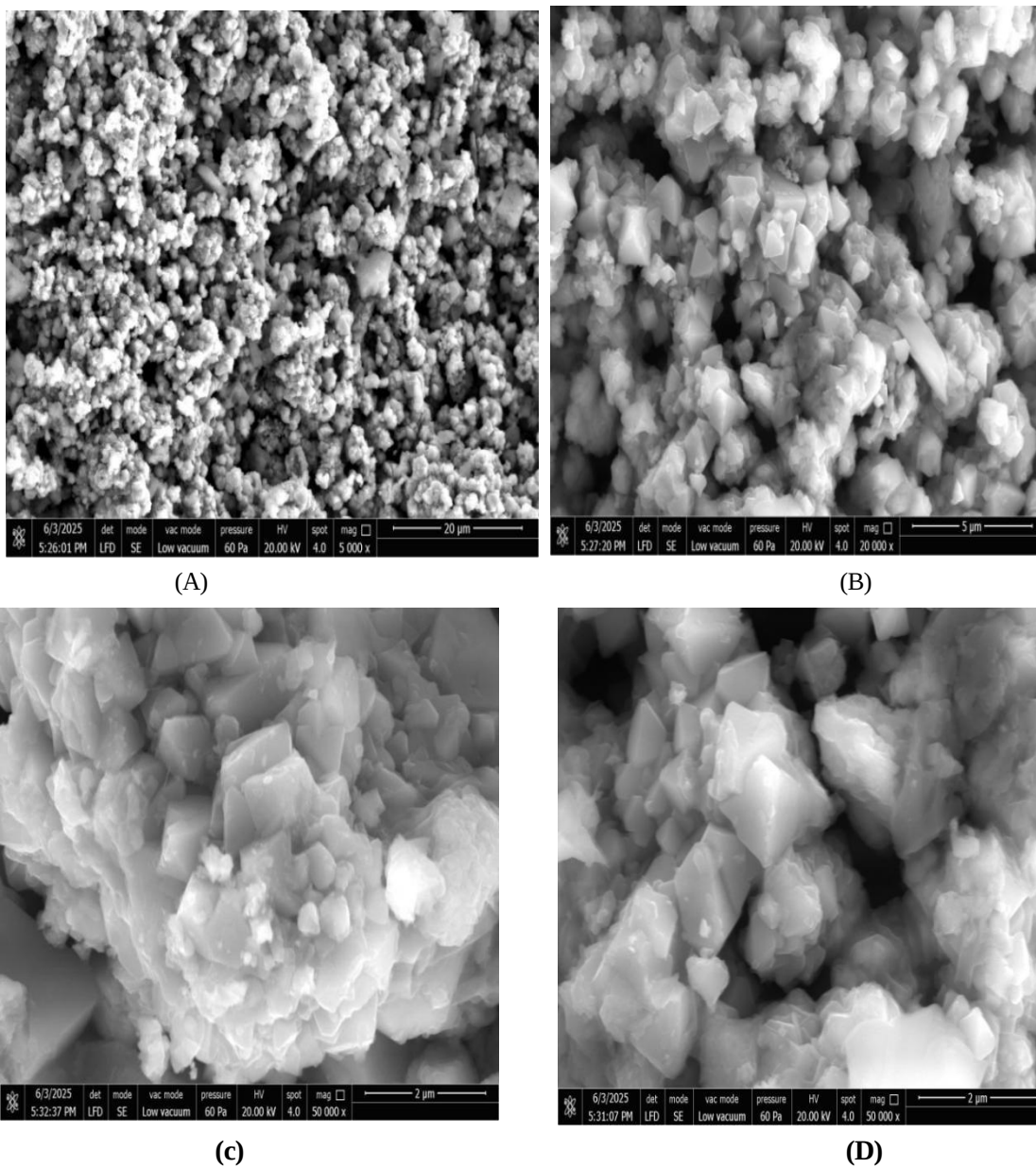
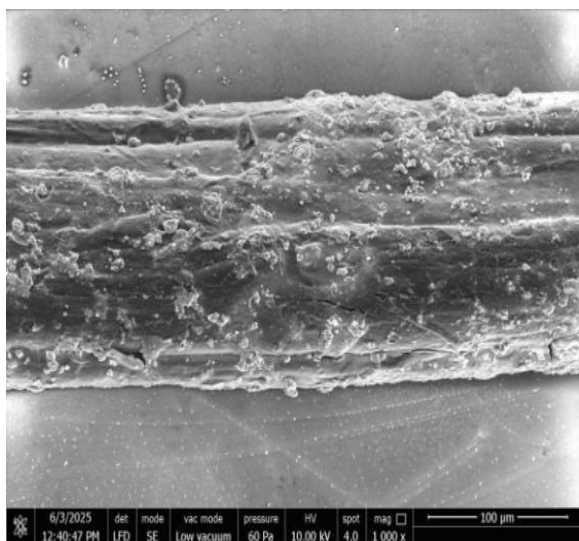
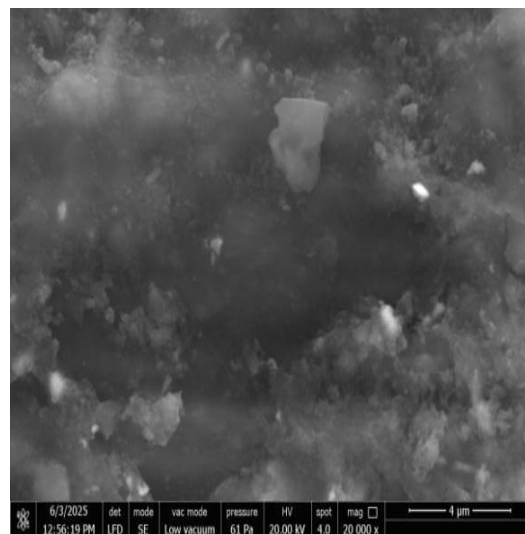


Figure.4.1.A,B C & D Shows the SEM analysis of Powder CuSe@CNTs at different conditions

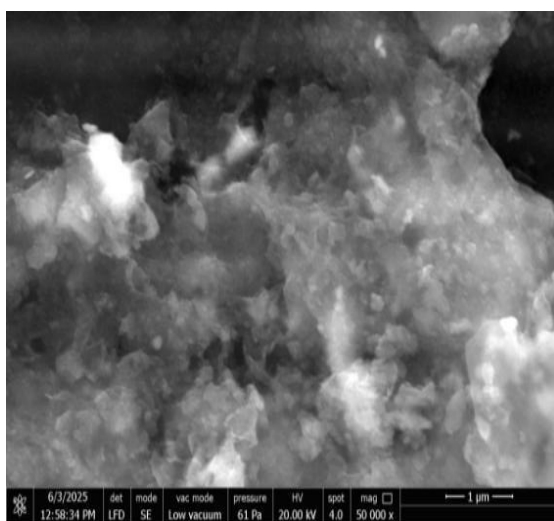
4.1.SEM Images Of CuSe@CNT/CNTF



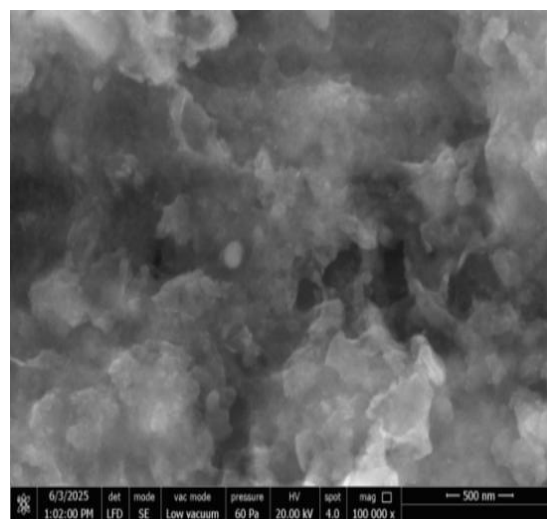
(A)



(B)



(c)



(D)

Figure.4.2. A,B,C,& D Shows the SEM analysis of CuSe@CNT/CNTF at different condition

4.3. Energy Dispersive X-Rays of CuSe@CNT

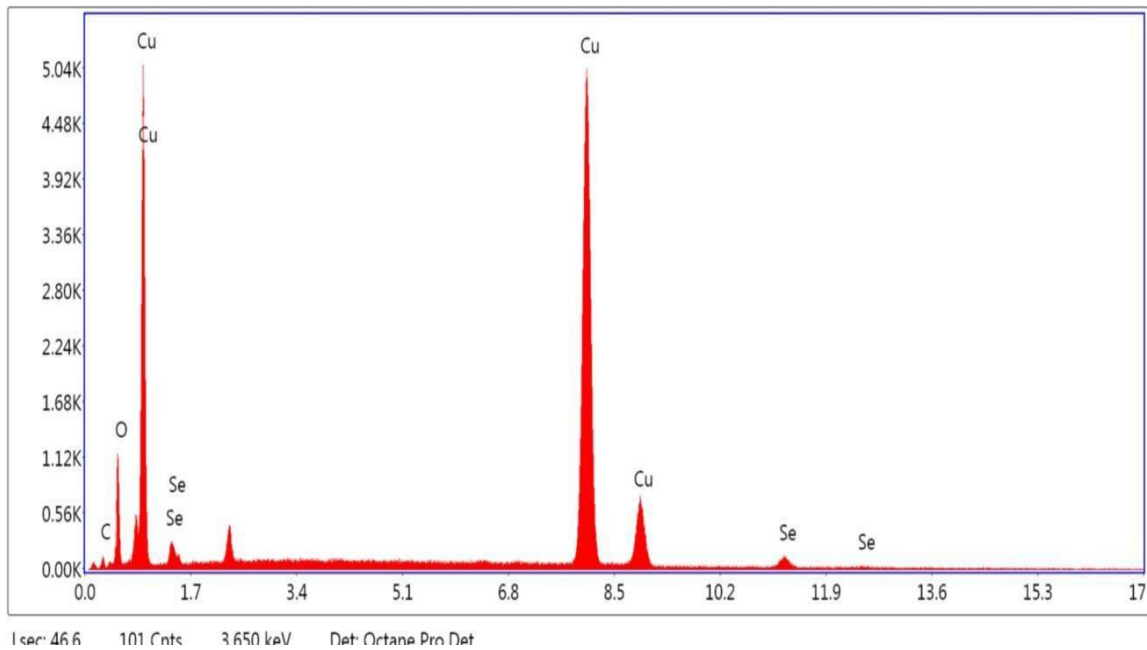


Figure.4.3.Shows the EDX analysis of CuSe@CNT

4.4. Elemental Mapping Of CuSe@CNTs

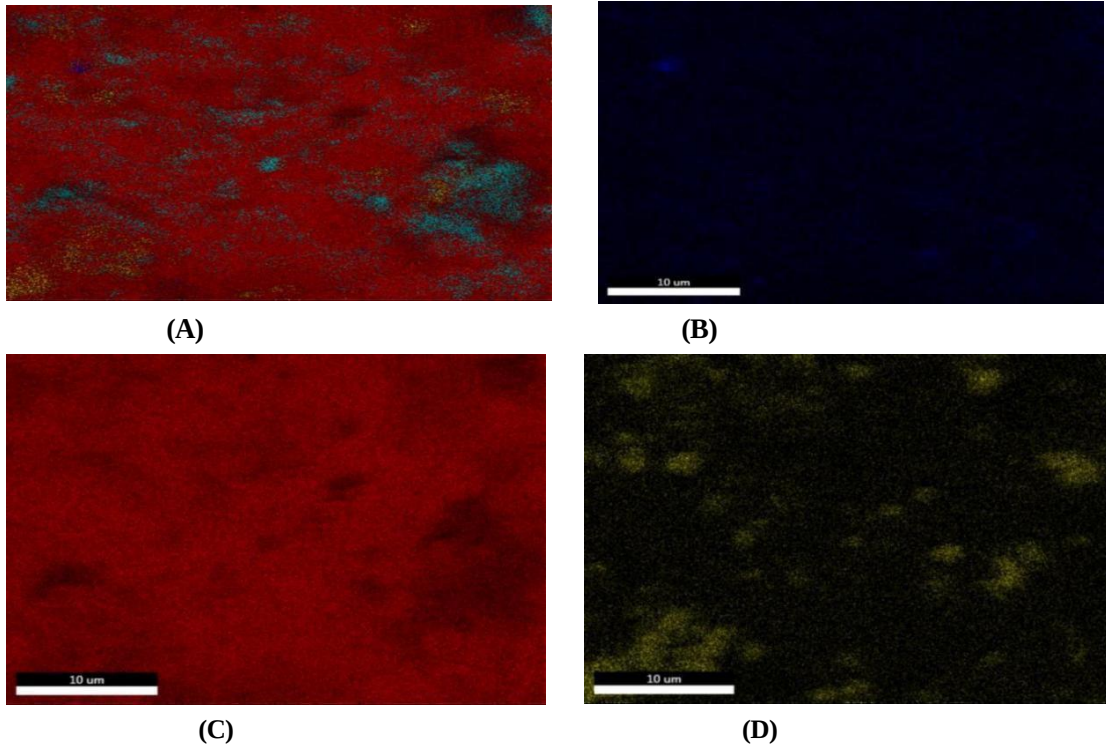


Figure.4.4.(A),(B),(C) and (D) Shows the mapping of CNTs, Copper, Selenium & CuSe_x

4.5. UV-Visible of CuSe @CNTs

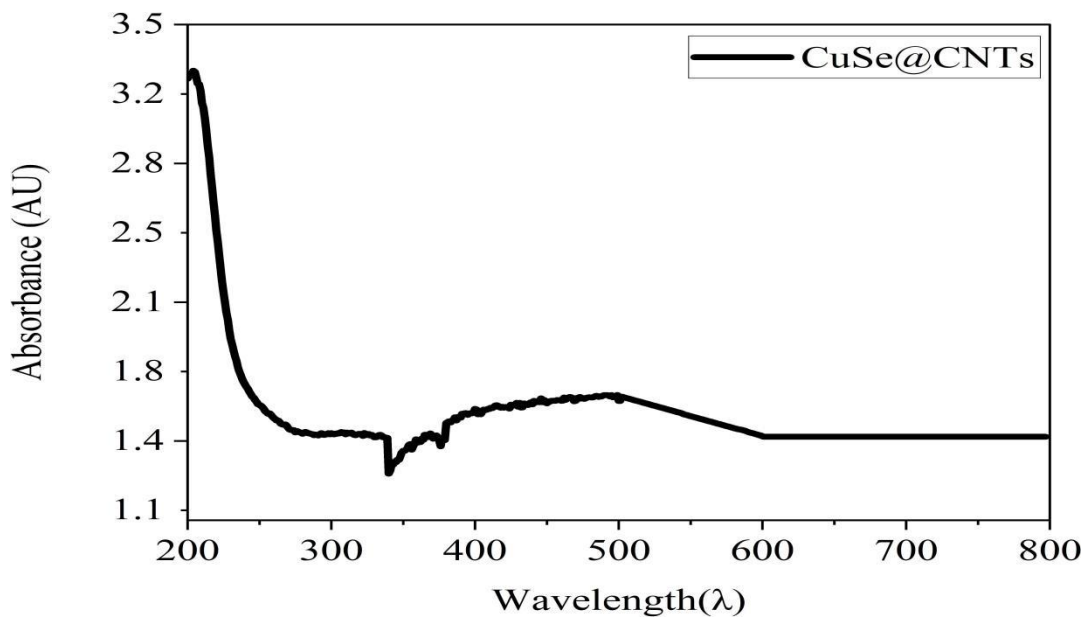


Fig.4.5. Shows the UV-Visible spectrum of CuSe@CNTs

4.6. Cyclic Voltammetry Of Uric Acid By CuSe@CNT/GCE

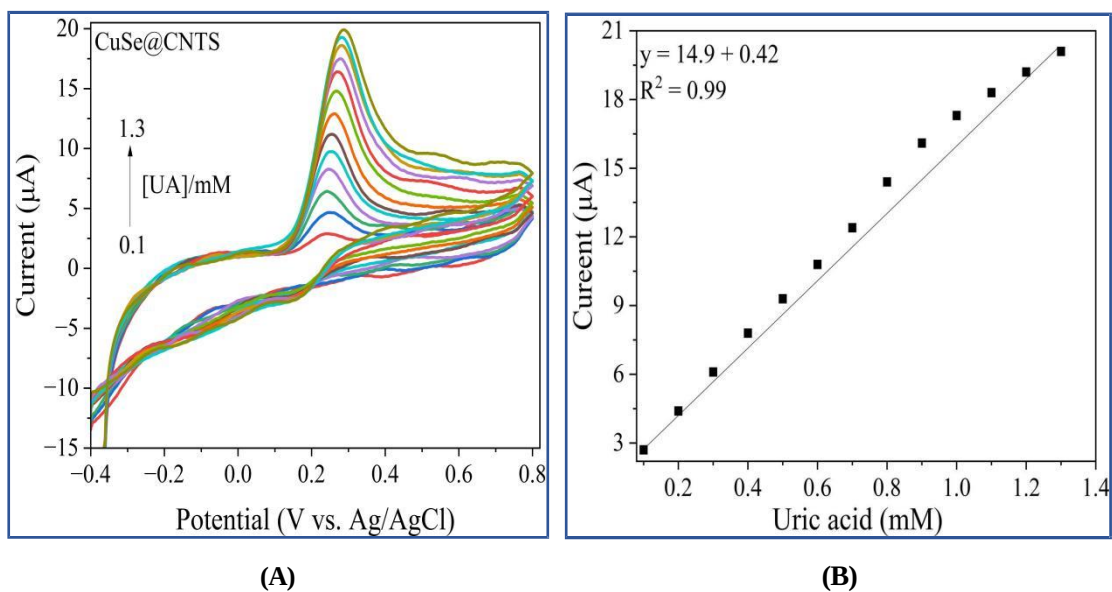


Figure.4.6.(A) shows the cyclic voltammetry of uric acid by CuSe@CNT/GCE while (B) shows the linearity curve of cyclic voltammetry.

4.7. Scan Rate Of Uric Acid By CuSe@CNT/GCE

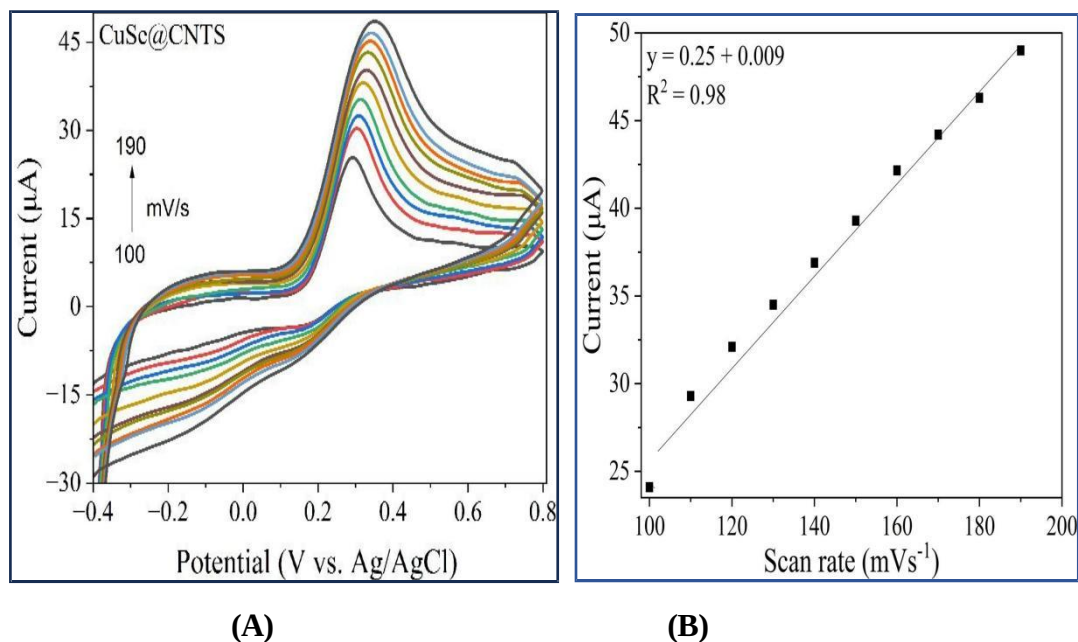


Figure.4.7. shows the response at different scan rate of uric acid by CuSe@CNT/GCE while (B) shows the linearity curve of scan rate.

4.8. Square Wave Voltammetry Of Uric Acid By CuSe@CNT/GCE

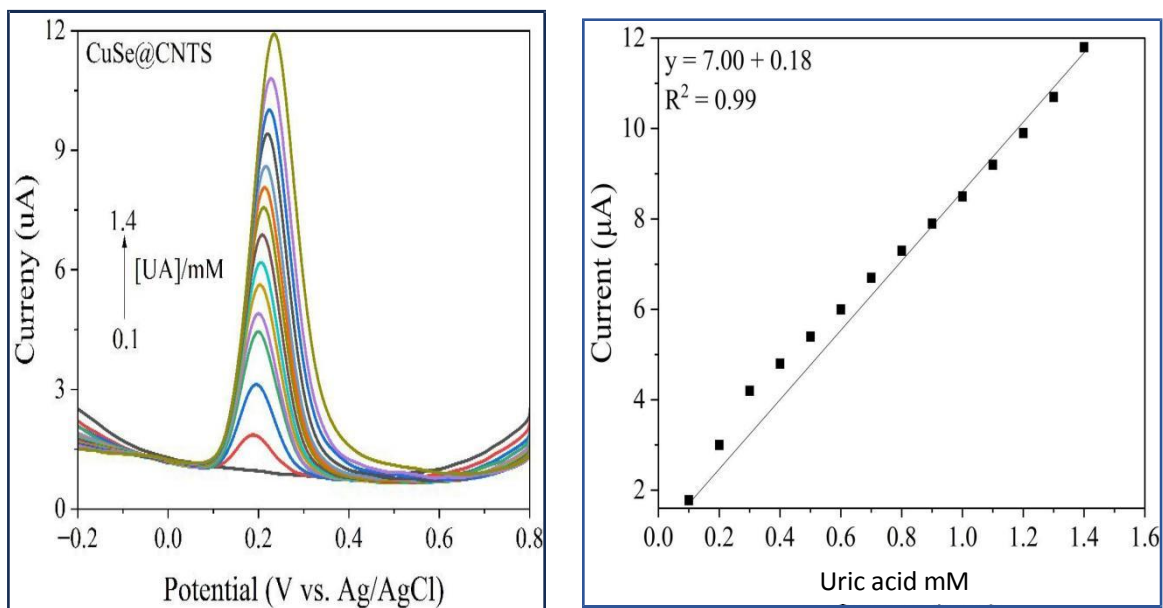


Figure.4.8. shows the response of square wave voltammetry of uric acid by CuSe@CNT/GCE while (B) shows the linearity curve of square wave

4.9. Chronoamperometry Of Uric Acid By CuSe@CNT/GCE

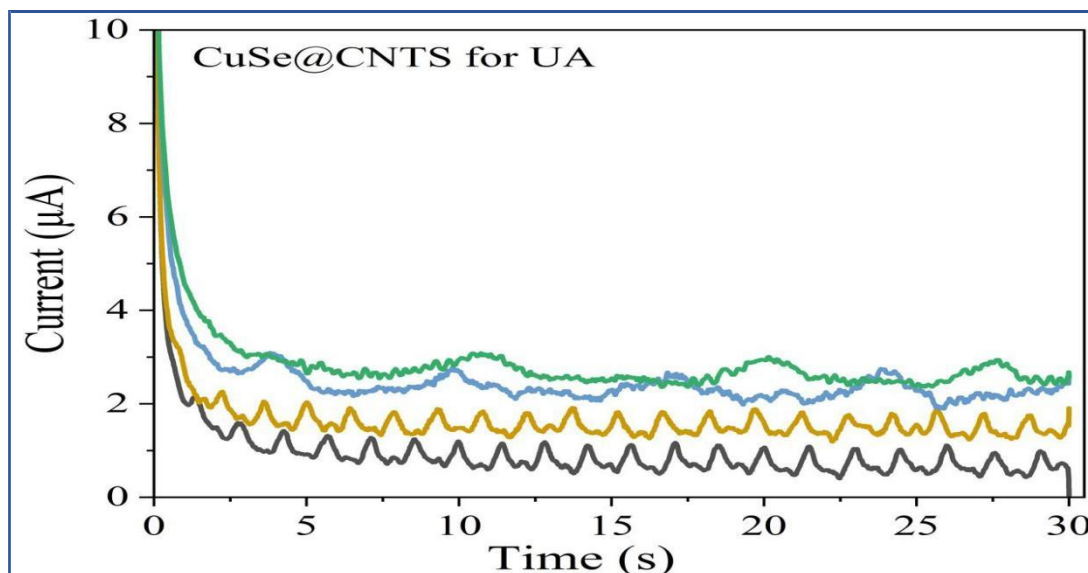


Figure.4.9.Shows the response of chronoamperometry of uric acid by CuSe@CNT/GCE

4.10. Cyclic Voltammetry Of Uric Acid By CuSe@CNT/CNTF

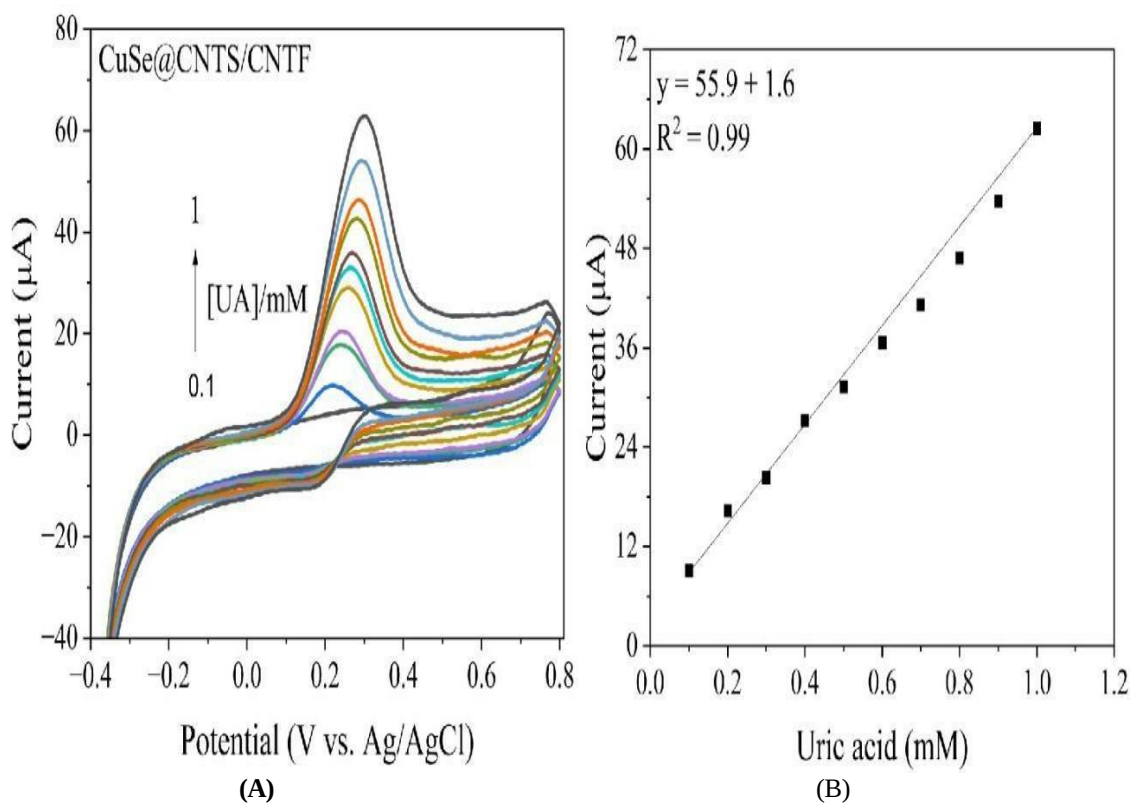


Figure.4.10.(A)shows the cyclic voltammetry of uric acid by CuSe@CNT/CNTF while (B) shows the linearity curve of cyclic voltammetry.

4.11. Square Wave Voltammetry Of Uric Acid By CuSe@CNT/CNTF

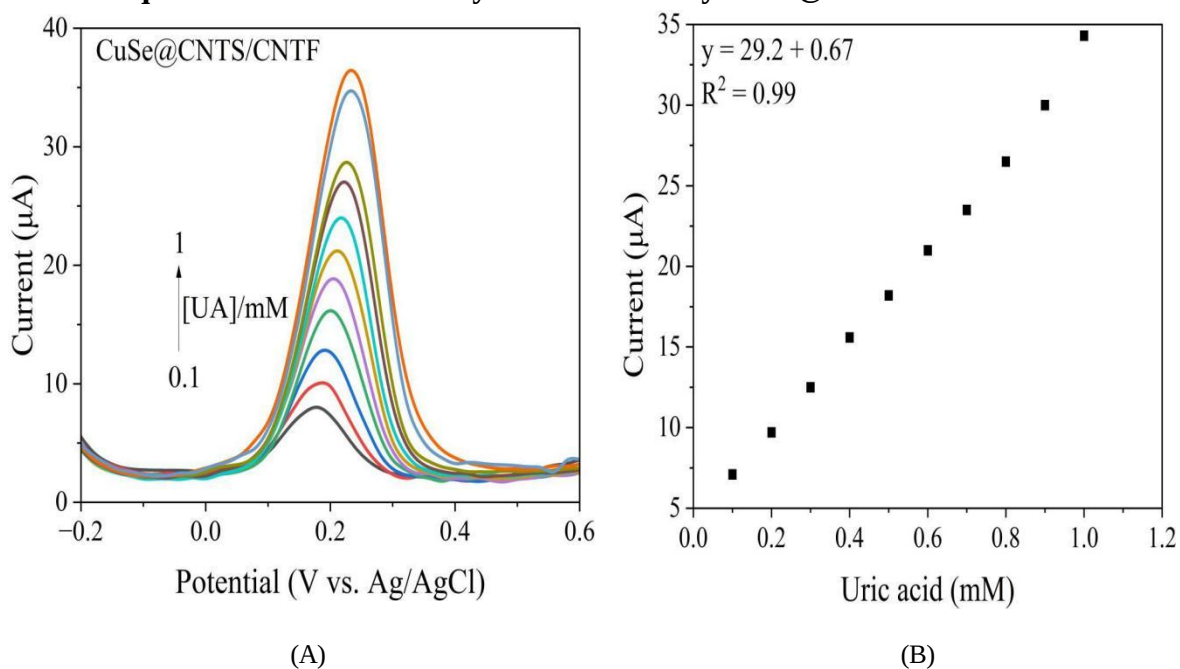


Figure.4.11. shows the response of square wave voltmmetry of uric acid by CuSe@CNT/CNTF while (B) shows the linearity curve of square wave.

4.12. Chronoamperometry Of Uric Acid By CuSe@CNT/CNTF

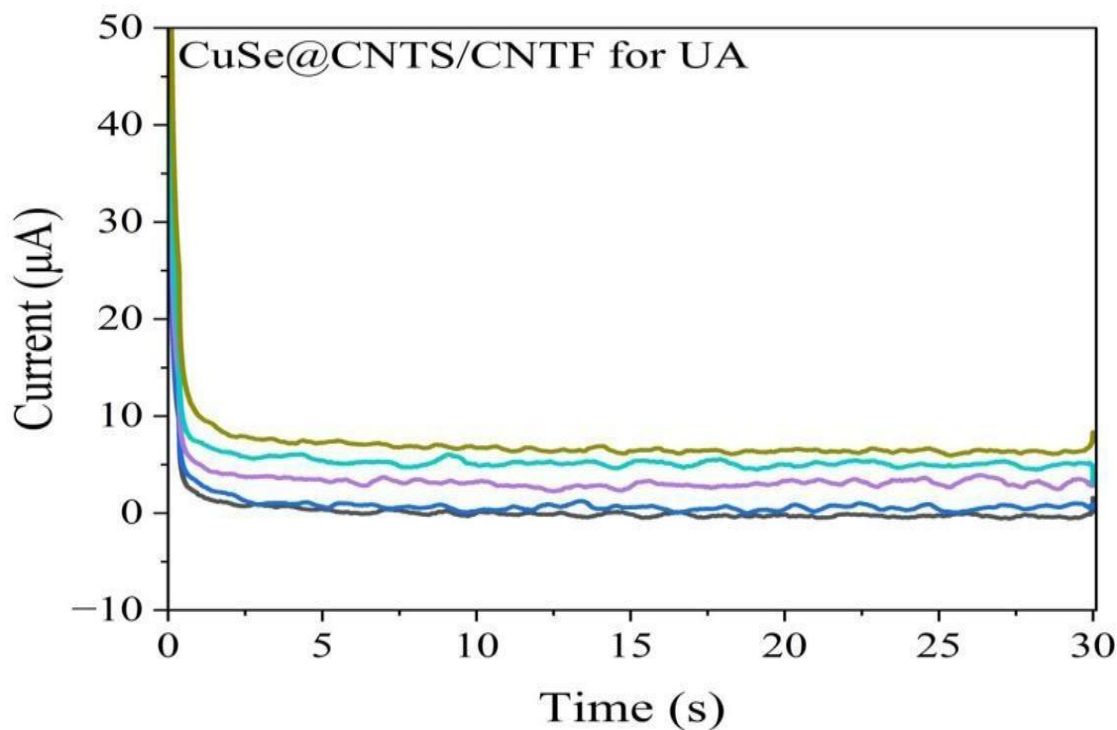


Figure.4.12. shows the response of chronoamperometry of uric acid by CuSe@CNT/CNTF

CHAPTER 5

DISCUSSION

SEM Images Of CuSe@CNTs

Before the main experiment, the SEM of four images was taken with different magnifications (5,000 x, 20,000 x, and 50,000 x) of a nanostructured powder sample in succession, and we can see some similarities in terms of huge surface roughness and complicated morphology.

The sample in the first image (5,000 magnification) is seen as a heavily packed mass of agglomerated particles with a broad size distribution; the sample is seen as having a porous network structure and many fine particles agglomerated together indicating a high value of particle-particle interaction or possible sintering of particles. Moving on to the second image (20,000x magnification), we can see more finer features: some of the particles are of faceted geometries indicating some degree of crystallinity while we get clearer edges and smooth faces amongst the agglomerates. Many of the particles give a glimpse of cubic or tetragonal symmetry which indicates the signs of well-developed crystal habits. Individual crystallites become highly resolved in the third image (50,000x magnification) with sharp, clearly defined polygonal shapes, flat faces, and sharp edges. These crystallites also include nano-scale irregularities in their surface as well as some smaller particulates adhered, suggestive of multi-scale texture and un-complete crystal growth or secondary nucleation. The scanning electron microscopy (SEM) in forth image presented taken at 50000X under low vacuum settings (60 Pa) with a secondary electron (SE) detector at an accelerating voltage of 20.00 kV. The resulting surface morphology is clean and smooth with a limited amount of amorphous content or surface contamination pointing to a successful synthesis with a high degree of purity..

SEM Images Of CuSe@CNTs/CNTF

The image at the low magnification of 1000x does produce a fiber like structure which has a continuous coating of the same width and visible particulate matter is observed along the surface. The surface roughness as well as the existence of micro-sized deposits

are a sign of successful surface modification. This topographical profile would be very useful since more tangledness would directly lead to a higher surface area, which is critical in increasing the availability of active sites, developing mechanical interlocking, and enhancing material-to-material interactions as seen in reinforced composites, filtration membranes, or energy devices.

The micrograph acquired at 50,000x magnification shows that the nanoscale particulate aggregates the formation of which is uniform and results in a porous and interconnected network. This porosity at the nanoscale level is important in increasing the active surface area which is very desirable in catalytic reactions, adsorption process and energy storing devices like batteries and super capacitors.

The image (scale bar 100 nm) at the highest magnification of 100,000x shows a clear representation of the nanoporous network wherein the interconnected spongelike structure can be highlighted, and formed of closely packed nanoscale particle like structure can be highlighted, and formed of closely packed nanoscale particle. This nanostructured porous morphology also allows the transport charges to move quicker, ion diffusion to be improved as well as to increase material reactivity, which is why the material is perfect and suitable in high-performance electrochemical and environmental applications.

A maximized surface-to-volume ratio is very beneficial to the processes that depend on the surface interaction, such as sensing, catalysis, and energy storage due to the high degree of porosity and structural homogeneity at this scale.

In forth image morphology of the material can be seen in high-magnification SEM imaging at 100,000x to give detail of a nanostructured surface. The figure exhibits the evident growth of a pores, connective network created on the basis of agglomerated nanoparticles that is highly advantageous in promoting material performance in numerous enhanced applications. The porous morphology can be seen as the visible vulnerabilities and interconnected pores between the particles, and it has much more-than-enough surface area. .The homogeneity and continuity of the nanoparticle pattern is

observed and indicates a uniform way of fabrication, so it provides reliable production of the same material with similar surface properties.

Energy Dispersive X-Rays

Energy Dispersive X-ray Spectroscopy (EDS or EDX) spectrum that reflects the elemental composition of the synthesized material which is probably a copper selenide based carbon nanotube (CuSe@CNT) composite. Based on the intensity of its peaks, the results are ascribed to Copper (Cu) at 1.0 keV, 8.0 keV and 8.9 keV and Selenium (Se) 1.4 keV, 11.2 keV and 12.5 keV, indicating the effective integration of both Cu and Se in the nanostructure.

Also, carbon (C) and oxygen (O) peaks at approximately 0.3 keV and 0.5 keV indicate that there are carbon nanotubes and slight bonded layer oxidation of the surface present which may have been caused by exposure to the environment or by the leftover oxygenated functional groups in the case of CNT functionalization. The lack of any surprise hills also confirms the purity of the material synthesized. The formation of CuSe nanoparticles embedded with CNTs is important to quaid their status as an effective electrocatalyst in the detection of uric acid.

Elemental Mapping Images Of CuSe@CNTs

These four elemental mapping images (A, B, C and D) give essential information on how a cobalt selenide-based carbon nanotube (CuSe CNT) composite, nanomaterial in its own right and designed to operate in fields of advanced energy storage, catalysis or sensing, is actually put together, and how the elements are distributed within it.

Image A shows Carbon C distribution. The carbon element is most probably present as Image A, since it is a part of the carbon nanotube backbone. The image demonstrates a very homogeneous and smooth (uninterrupted) distribution of the signal on the field of view. It means that CNTs are uniformly distributed in the sample and create a networked system.

Image B -shows the distribution of Copper (Cu). The image B shows a spotty and localized signal, an indication that there is the presence of Coppert in the places that are

appearing constantly. This result means that copper is not evenly distributed, but deposited in the form of nanoparticle or clusters.

.Image C indicates a wider and diffused distribution as in comparison to copper. A larger of the image is captured by the selenium signal, which insinuates either better dispersion or greater reactivity to the surrounding matrix. Such uniformity explains either Se has been co-deposited or has diffused on the CNT surface. The mixing of Se and Cu clearly depicts the successful creation of CuSe 1 & 2 phases, which are essential to functional intactness of the composite. Image D shows CuSe ell Composite (Cu Se Overlay). Probably image D is a composite or overlay map, where signals of copper and selenium are summed. The white, agglomerated areas indicate the areas with both Cu and Se -i.e. nano-particles of copper selenide held together on CNTs. Bright intensity and focal clusters indicate that CuSe is formed and exists in the isolated dense segments. These are the composite active sites. This photo serves as the direct proof of the success of this material in terms of such aspect as nanoscale of hybridization between CuSe and CNTs.

UV-Visible Images Of CuSe@CNTs

UV-Visible absorption spectroscopy of the CuSe based CNT composite shows the characteristic absorption peaks which revealed that there was successful implementation of the copper selenide nanoparticles coated on the carbon nanotube matrix. A strong absorption band at 250-350 nm is eminent and attributed to the $\pi \rightarrow \pi^*$ transitions of the CNTs and possible charge-transfer interactions between Cu and Se ions. An extended absorption tail into visible (400-700 nm) range denotes the semiconductive nature of CuSe and a prospect of visible light capture that is important in photoelectrochemical applications. The red shifted position of the peaks relative to that of pristine CNTs indicates the more closely coupled electronic states of CuSe and CNTs, improving the optically active phase of the CNTs. It is the presence of the bandgap variations that signifies synergistic relations in the hybrid structure to enhance charge carrier separation and mobility. On the whole, UV-Vis results aid to conclude on the synthesis of a stable UV-Vis hybrid of CuSe@CNT with improved optoelectronic characteristics that may be utilized as electrochemical sensors.

Cyclic Voltammetry Of Uric Acid By CuSe@CNT/GCE

Cyclic voltammetry (CV) was employed to investigate the electrochemical behavior of CV allowed to determine the electrochemical properties of uric acid (UA) when applied to a synthesized sensor electrode using the redox behavior evaluation method. A substantial technique which examines electron transfer speeds while determining analyte electrochemical characteristics exists.

The present study demonstrated the CV analysis produced an anodic peak with a potential e.g., + 0.25 V vs. Ag/AgCl that indicated uric acid oxidation. The absence of an opposite cathodic signal throughout the reverse scan indicates that uric acid oxidation occurs irreversibly in agreement with other recorded data. The peak current measurement and peak definition improved substantially when the modified electrode was compared against the bare electrode. The electrocatalytic performance improved due to the selected modification method which added such as CuSe@CNT for its combination of high surface area with superior electrical conductivity and efficient electron transfer channels. The study examined the redox process through observations of Concentration. The findings present in that peak current increased proportionally to the concentration variation and the peak current displayed a linear correlation with the concentration thus validating the mechanism follows diffusion-controlled kinetic behavior. The sensor demonstrated ability for quantitative detection through its linear calibration behavior within designated concentration spans. The slope of the line provided the sensitivity measurement and the LOD determination followed the 3σ criterion for statistical precision. The developed electrode offers better analytical for peak potential as well as linear response range detection limit and sensitivity thus making it a reliable platform for electrochemical uric acid detection with prospective clinical and biomedical applications.

Scan Rate Of Uric Acid By CuSe@CNT/GCE

Visualizing uric acid cyclic voltammetry under different scan rate conditions develops strong understanding about the electrochemical speed and performance of the modified electrode system. The increase in scan rate simultaneously leads to greater anodic peak

currents along with positive shifts of peak potential which proves that electron transfer acts irreversibly and follows diffusion-controlled oxidation kinetics. Electronic mobility and mass transport efficiency grow steadily within scan rates because the electrode material provides both a high surface area and electrically connected framework. The electrode performs electrochemically stable signal analysis across all scan rates because its internal charge propagation operates efficiently without affecting analytical measurement quality.

The scan rate-dependent activity of the sensor matches reported mechanisms from literature making the device suitable for sensitive uric acid detection in real time while ensuring diagnostic accuracy.

Square Wave Voltammetry Of Uric Acid By CuSe@CNT/GCE

A modified electrode made from CuSe@CNT detected uric acid (UA) using the highly sensitive electroanalytical method Square Wave Voltammetry (SWV). SWV records the clean symmetric anodic peak of UA electrochemical oxidation that appears at +0.20 V V referenced to Ag/AgCl. SwV graphs display a potential scale from -0.2 V to +0.6 V represented by the X-axis together with a current scale from 0 μ A to 12 μ A shown on the Y-axis. Excelling sensitivity performance of the sensor produces distinct peaks when detecting 0.1 mM UA concentrations while causing a current output of approximately 12 μ A. Structural and conductive collaboration in CuSe@CNT enables better electroactive surface area performance together with faster electron transfer kinetics to enhance sensitivity. The selection process produced SWV parameters that achieved peak symmetry through background capacitive current suppression while operating with pulse amplitude of 25 mV, frequency of 15 Hz and step potential of 4 mV. A linear relationship was found between anodic peak current and UA concentrations from 0.1 mM to 1.0 mM based on the high calibration curve slope alongside a significant value of $R^2 > 0.99$. The sensor was able to detect trace concentrations of UA because the calculated limit of detection (LOD) reached 0.05 mM using the 3σ criteria. The SWV measurement technique offered superior performance than CV for both narrow peak definition and precise quantification. Research findings demonstrate that CuSe@CNT sensor platforms stand ready as effective portable diagnostic tools for uric acid detection

Chronoamperometry Of Uric Acid By CuSe@CNT/GCE

A potential step during chronoamperometry leads to an instant current spike because the electroactive species undergoes rapid oxidation or reduction at the electrode surface. When analyte molecules spread from bulk solution to reach the electrode surface the current pattern starts to decrease.

The current-time relationship from this phase shows characteristics of a diffusion-controlled reaction and agrees with Cottrell equation standards. The current reaches a stable state when electron transfer rates match the analytical species' diffusion rates. Native electrotransfer rates at electrodes depend heavily on both electrode surface dimensions and their materials components for determining current intensity. Accurate current measurements require steady baselines along with reduced outside interferences.

Cyclic Voltammetry Of Uric Acid By CuSe@CNT/CNTF

The cyclic voltammetry (CV) of Uric acid (UA) on a modified electrode with CuSe@CNT Fibre. CV was performed within the potential window of -0.4 V to +0.8 V vs Ag/AgCl, with current responses recorded in example of the potential window of -0.4 V to +0.8 V vs Ag/AgCl. A clear oxidation of UA anodic peak at +0.25 V. Current value at the anodic peaks increases proportionally depending on the concentration of UA between 0.1 and 1.0 mM, which shows sensitive electron transfer is consistent with a high sensitivity.

This action proves the electrocatalytic characteristic of the CuSe@CNTS Fibre construction with the high changeable conductivity guaranteed by CNTS and active changeable locations by actual redox sites ensured by CuSe. The low and constant single scan background current provides high signal-to-noise ratios, which means that electrodes are stable. This broad potential window (-0.4 V to +0.8 V) excludes interference and decomposition of the solvent. The high peak at a relatively low potential (+0.25 V) signifies less overpotential and better selectivity, means more suppressed interference of species that are oxidized at higher voltages. Steep critical rise in current and sharp profiles indicate high rate of electron transfer. The hybrid and

structured CuSe@CNTS/CNT fibre have a large surface area, good charge transfer, and catalytic activity

Square Wave Voltammetry Of Uric Acid By CuSe@CNT/CNTF

This figure (A) has displayed a voltammetric profile that ascertains the analytical result of analyzing uric acid through the use of a CuSe-@ CNTS/CNTF based electrode system. In the abscissa, electrical input will run negative to positive potential, generally in a scale of -0.2 and 0.6 volts referenced Ag/AgCl, though it can go substantially higher in practice, and the ordinate captures the strength of electrical current in microampere units, a scale that can also go much higher. On this diagram, there will be a series of multi-colored waves that are related to ascending levels of concentrations of uric acid at various levels beginning with 0.1 millimolar at the low end of the range and rising to 1 millimolar, both as indicated by the vertical scale of concentrations with the directional arrow. The voltammetric curves reports a linear and sensitive correlation confirm that the present response is proportional with the level of uric acid being raised in agreement with the unique peak increment that is observed throughout the DPV graph, indicating precise sensitivity of detection.

Chronoamperometry Of Uric Acid By CuSe@CNT/CNTF

The above chronoamperometry graph shows the (microamperes) current response Vs time (s) at room temperature when CuSe@CNTS/CNTF was used to detect UA. The current at the electrode surface shoots up at time zero in result of the instantaneous electrochemical oxidation or reduction of UA as the potential is applied. Once this temporary phase is accomplished, the current does not show changes but remains at the steady-state value over the remaining time up to 30 seconds thus indicating that the rate of diffusion of UA molecules is in balance with the turnover reaction of the UA molecules.

The steady current indicates good electrode behaviour, low levels of noise and stability in electrocatalytic activity. The graph shows different curves at different levels of UA concentrations or conditions and these are reflected by slight differences in the steady state current levels. The consistency of the current upon the post decay authenticates the

effectiveness and replicability of the sensor in detecting UA under the CuSe@CNTS/CNT fibr framework.

The detailed characterization and electrochemical study of the prepared CuSe@CNT nanocomposite proved that it has high prospects of electrocatalyst to detect uric acid (UA) with very low nanomolar limits. SEM morphological information satisfied the surface deposition of CuSe nanostructures over the surface of CNT and forming interconnected nanoflake collections. This architecture offered a good surface-volume ratio, which was necessary in promoting a stronger adsorption of UA molecules and larger numbers of electroactive sites. The uniform elemental distribution of Cu, Se and C was also confirmed by EDX spectra as a guarantee of the composite homogeneity and analysis showed clearly the presence of different crystalline peaks of CuSe and remaining CNT peaks showing the structural incorporation of two materials together without distorting any of the phases. Electrochemical analysis was started with cyclic voltammetry (CV) proving an outstanding oxidation peak at +0.34 V (vs. Ag/AgCl) of UA on the CuSe@CNT-decorated and gastrocnemius-modified electrode, which was much greater than both the unmodified and modified CNT electrodes in terms of current. The role of this protuberance was because synergistic interactions between conductive CNT scaffold and redox-active CuSe nanoparticles enabled the enhanced electron transfer rate and surface reactivity. The scan rate dependence of the CV experiments revealed that the oxidation current varied linearly and proportional to the square root of the scan rate, which procreates that an electrochemical process is controlled by the diffusion. This conduct gave credence to efficient migration of UA molecules toward an electrode interface and the applicability of the CuSe@CNT composite in analytical sensing usage. Square wave voltammetry (SWV) provided absolute quantitative analysis which is sharp and symmetric yielding, and is proportional to the current intensities of UA concentration. The calibration curve depicted excellent linearity within a concentration independent of 0.1 to 1000 qpm and very low detection limit that proved the high sensitivity and usability of the composite in on trace level detection of UA in clinical matrices. Real-time sensing response was evaluated by chronoamperometric measurements. With each increment of UA, the current followed a stepwise increase

that is rather rapid, with the response stabilizing in a few seconds, and thus the reduction reaction has a fast electron transfer rate. High sensitivity together with the fast steady-state response confirms that the material can be used in practical and continuous monitoring processes. Selectivity tests indicated that the UA resistance to the potentially interfering species was still negligible, which means very high specificity of the CuSe@CNT electrode to UA. This is due to the selective behavior that is attributable to the good binding interactions and oxidation potency variations between UA and interfering compounds. Ample reproducibility of the fabricated sensor was also observed where the relative standard deviation between independently prepared electrodes was minimal. Repeatability experiments demonstrated that there was stability throughout the repeated cycles of the experiment, and long-term stability experiments demonstrated that the sensor had an outstanding stability with more than 90 percent of the sensor still supporting its response even after several days of storage. In brief, the affiliation of CuSe with CNTs not merely added the conductivity and surface activity but localized the ability to detect uric acid efficiently, stably, selectively. Both structural and electrochemical properties make this composite prospective non-enzymatic biosensing platform in bioanalytical fields.

CHAPTER 6

CONCLUSION

The study was able to validate the usefulness of the metal selenide-grafted carbon nanotubes (CNTs) as a great and stable material in the electrochemical detection of uric acid. The high conductivity and large surface area of CNTs was combined with superior redox activity and catalytic behavior of metal selenides to form a synergistic nanocomposite that provides superior sensing capability.

The customized nanostructure demonstrated better electrochemical performance with high sensitivity, low detection limit, extended linear response range and good selectivity. This is because of the higher active sites, high rate of transferring electrons and structural arrangement of the composite that was stable.

Furthermore, the synthesized sensor also showed exceedingly good performance in terms of stability, repeatability, and reproducibility thus making the use of the sensor extremely feasible. Besides achieving the objective of developing an efficient non-enzymatic sensing system of uric acid, the work here also adds value to the general advancement of hybrid nanomaterials in biosensing system.

To sum up, the metal selenide grafted CNTs are one of the potentially successful material classes of the next-generation electrochemical sensors which can give the base of detecting other biomolecules and developing portable and low-priced diagnostic devices.

REFERENCES

1. Ndrepepa, G., *Uric acid and cardiovascular disease*. Clinica chimica acta, 2018. **484**: p. 150-163.
2. Maiuolo, J., et al., *Regulation of uric acid metabolism and excretion*. International journal of cardiology, 2016. **213**: p. 8-14.
3. Mazzara, F., et al., *Electrochemical detection of uric acid and ascorbic acid using r-GO/NPs based sensors*. Electrochimica Acta, 2021. **388**: p. 138652.
4. Álvarez-Lario, B. and J. Macarrón-Vicente, *Uric acid and evolution*. Rheumatology, 2010. **49**(11): p. 2010-2015.
5. Watanabe, S., et al., *Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity*. Hypertension, 2002. **40**(3): p. 355-360.
6. Rock, K.L., H. Kataoka, and J.-J. Lai, *Uric acid as a danger signal in gout and its comorbidities*. Nature Reviews Rheumatology, 2013. **9**(1): p. 13-23.
7. Perez-Ruiz, F., N. Dalbeth, and T. Bardin, *A review of uric acid, crystal deposition disease, and gout*. Advances in therapy, 2015. **32**: p. 31-41.
8. Hisatome, I., et al., *Uric acid as a risk factor for chronic kidney Disease and Cardiovascular Disease—Japanese Guideline on the management of Asymptomatic Hyperuricemia—*. Circulation Journal, 2021. **85**(2): p. 130-138.
9. Spitsin, S. and H. Koprowski, *Role of uric acid in Alzheimer's disease*. Journal of Alzheimer's Disease, 2010. **19**(4): p. 1337-1338.
10. Sampat, R., et al., *Potential mechanisms for low uric acid in Parkinson disease*. Journal of Neural Transmission, 2016. **123**: p. 365-370.
11. Sotgiu, S., et al., *Serum uric acid and multiple sclerosis*. Neurological Sciences, 2002. **23**: p. 183-188.
12. Gaitonde, D.Y., D.L. Cook, and I.M. Rivera, *Chronic kidney disease: detection and evaluation*. American family physician, 2017. **96**(12): p. 776-783.
13. Bellomo, G., *Serum uric acid and pre-eclampsia: an update*. Expert review of cardiovascular therapy, 2012. **10**(6): p. 701-705.
14. Kroll, M.H. and R.J. Elin, *Interference with clinical laboratory analyses*. Clinical chemistry, 1994. **40**(11): p. 1996-2005.

15. Oliveira, R., et al., *Development of a flow microsensor for selective detection of nitric oxide in the presence of hydrogen peroxide*. *Electrochimica Acta*, 2018. **286**: p. 365-373.
16. Meng, Y., et al., *Enhanced sensitivity and precision in an enzyme-linked immunosorbent assay with fluorogenic substrates compared with commonly used chromogenic substrates*. *Analytical biochemistry*, 2005. **345**(2): p. 227-236.
17. Tempst, P., *Mass-encoded, synthetic biomarkers and multiplexed urinary monitoring: new frontiers in disease monitoring*. *Clinical Chemistry*, 2013. **59**(12): p. 1694-1695.
18. Qiu, J., et al., *Association between blood microbiome and type 2 diabetes mellitus: A nested case-control study*. *Journal of clinical laboratory analysis*, 2019. **33**(4): p. e22842.
19. Ambus, T., et al., *Evaluation of the Beckman Synchron CX4 clinical chemistry analyzer in a hospital laboratory*. *Journal of Clinical Laboratory Analysis*, 1990. **4**(2): p. 120-125.
20. Sulem, P., et al., *Identification of low-frequency variants associated with gout and serum uric acid levels*. *Nature genetics*, 2011. **43**(11): p. 1127-1130.
21. Desideri, G., et al., *Is it time to revise the normal range of serum uric acid levels?* *European Review for Medical & Pharmacological Sciences*, 2014. **18**(9).
22. Basketter, D.A. and I. Kimber, *Information derived from sensitization test methods: test sensitivity, false positives and false negatives*. *Contact Dermatitis*, 2007. **56**(1): p. 1-4.
23. Kamal, A.H., S.F. El-Malla, and S.F. Hammad, *A review on UV spectrophotometric methods for simultaneous multicomponent analysis*. *Eur J Pharm Med Res*, 2016. **3**(2): p. 348-60.
24. Norazmi, N., et al. *Uric acid detection using uv-vis spectrometer*. in *IOP Conference Series: Materials Science and Engineering*. 2017. IOP Publishing.
25. Watts, R., *Determination of uric acid in blood and in urine*. *Annals of Clinical Biochemistry*, 1974. **11**(1-6): p. 103-111.
26. Gupta, M.K., et al., *A comparative review on high-performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC) &*

- high-performance thin layer chromatography (HPTLC) with current updates. Current Issues in Pharmacy and Medical Sciences, 2022. 35(4): p. 224-228.*
27. Ahmed, R., *High-Performance Liquid Chromatography (HPLC): Principles, Applications, Versatility, Efficiency, Innovation and Comparative Analysis in Modern Analytical Chemistry and In Pharmaceutical Sciences.* Clinical Investigation, 2024. **14(9)**: p. 524-535.
 28. Jiang, J. and X. Du, *Sensitive electrochemical sensors for simultaneous determination of ascorbic acid, dopamine, and uric acid based on Au@ Pd-reduced graphene oxide nanocomposites.* Nanoscale, 2014. **6(19)**: p. 11303-11309.
 29. Tsochatzis, E.D., et al., *Thermal degradation of metabolites in urine using multiple isotope-labelled internal standards for off-line GC metabolomics-effects of injector and oven temperatures.* Journal of Chromatography B, 2021. **1181**: p. 122902.
 30. Williams, M.L., et al., *Matrix effects demystified: Strategies for resolving challenges in analytical separations of complex samples.* Journal of Separation Science, 2023. **46(23)**: p. 2300571.
 31. Kirchner, M., et al., *Possibilities and limitations of quadrupole mass spectrometric detector in fast gas chromatography.* Journal of Chromatography A, 2005. **1090(1-2)**: p. 126-132.
 32. Taira, S., et al., *Comparative Analysis of Derivatization Reagents for Catecholamines and Amino Acids.* Applied Sciences, 2021. **11(13)**: p. 6217.
 33. Ryan, E.M., et al., *Antioxidant properties of citric acid interfere with the uricase-based measurement of circulating uric acid.* Journal of Pharmaceutical and Biomedical Analysis, 2019. **164**: p. 460-466.
 34. Sifakis, J., *System design automation: Challenges and limitations.* Proceedings of the IEEE, 2015. **103(11)**: p. 2093-2103.
 35. Singh, R., et al., *A review on recent trends and future developments in electrochemical sensing.* ACS omega, 2024. **9(7)**: p. 7336-7356.

36. Garjonyte, R. and A. Malinauskas, *Amperometric glucose biosensor based on glucose oxidase immobilized in poly (o-phenylenediamine) layer*. Sensors and Actuators B: Chemical, 1999. **56**(1-2): p. 85-92.
37. Mallikarjuna, K., et al., *Simple synthesis of biogenic PdAg bimetallic nanostructures for an ultra-sensitive electrochemical sensor for sensitive determination of uric acid*. Journal of Electroanalytical Chemistry, 2018. **822**: p. 163-170.
38. Yan, Q., et al., *Measurement of tear glucose levels with amperometric glucose biosensor/capillary tube configuration*. Analytical chemistry, 2011. **83**(21): p. 8341-8346.
39. Zdrachek, E. and E. Bakker, *Potentiometric sensing*. Analytical chemistry, 2020. **93**(1): p. 72-102.
40. Pan, Y., et al., *Preparation of electrochemical sensor based on zinc oxide nanoparticles for simultaneous determination of AA, DA, and UA*. Frontiers in chemistry, 2020. **8**: p. 592538.
41. Geleta, G.S., *Recent advances in electrochemical sensors based on molecularly imprinted polymers and nanomaterials for detection of ascorbic acid, dopamine, and uric acid: a review*. Sensing and Bio-Sensing Research, 2024. **43**: p. 100610.
42. Bagheri Hashkavayi, A., et al., *Advances in exosome analysis methods with an emphasis on electrochemistry*. Analytical chemistry, 2020. **92**(19): p. 12733-12740.
43. Groenendaal, L., et al., *Poly (3, 4-ethylenedioxythiophene) and its derivatives: past, present, and future*. Advanced materials, 2000. **12**(7): p. 481-494.
44. Novoselov, K.S., et al., *Electric field effect in atomically thin carbon films*. science, 2004. **306**(5696): p. 666-669.
45. Dykman, L. and N. Khlebtsov, *Gold nanoparticles in biomedical applications*. 2017: CRC Press.
46. Sun, L., M.G. Campbell, and M. Dincă, *Electrically conductive porous metal–organic frameworks*. Angewandte Chemie International Edition, 2016. **55**(11): p. 3566-3579.

47. Wilson, R. and A. Turner, *Glucose oxidase: an ideal enzyme*. Biosensors and bioelectronics, 1992. **7**(3): p. 165-185.
48. Yetisen, A.K., M.S. Akram, and C.R. Lowe, *based microfluidic point-of-care diagnostic devices*. Lab on a Chip, 2013. **13**(12): p. 2210-2251.
49. Nie, Z., et al., *Electrochemical sensing in paper-based microfluidic devices*. Lab on a Chip, 2010. **10**(4): p. 477-483.
50. Ma, M., et al., *Highly sensitive and reproducible non-enzymatic glucose sensor fabricated by drop-casting novel nanocomposite with 3D architecture and tailorable properties prepared in controllable way*. Talanta, 2018. **180**: p. 133-143.
51. Armel, V., et al., *Designed electrodeposition of nanoparticles inside conducting polymers*. Journal of Materials Chemistry, 2012. **22**(37): p. 19767-19773.
52. Wang, X., et al., *CVD graphene as an electrochemical sensing platform for simultaneous detection of biomolecules*. Scientific reports, 2017. **7**(1): p. 7044.
53. Girault, P., et al., *Porous silicon micro-resonator implemented by standard photolithography process for sensing application*. Optical Materials, 2017. **72**: p. 596-601.
54. Ozoemena, K. and T. Nyokong, *Voltammetric characterization of the self-assembled monolayer (SAM) of octabutylthiophthalocyaninatoiron (II): a potential electrochemical sensor*. Electrochimica acta, 2002. **47**(25): p. 4035-4043.
55. Shen, L.L., G.R. Zhang, and B.J. Etzold, *Paper-based microfluidics for electrochemical applications*. ChemElectroChem, 2020. **7**(1): p. 10-30.
56. Mallakpour, S. and S. Soltanian, *Surface functionalization of carbon nanotubes: fabrication and applications*. RSC advances, 2016. **6**(111): p. 109916-109935.
57. Nagaraju, K., R. Reddy, and N. Reddy, *A review on protein functionalized carbon nanotubes*. Journal of applied biomaterials & functional materials, 2015. **13**(4): p. 301-312.
58. Kharlamova, M.V., M. Paukov, and M.G. Burdanova, *Nanotube functionalization: Investigation, methods and demonstrated applications*. Materials, 2022. **15**(15): p. 5386.

59. Dubey, R., et al., *Functionalized carbon nanotubes: Synthesis, properties and applications in water purification, drug delivery, and material and biomedical sciences*. Nanoscale Advances, 2021. **3**(20): p. 5722-5744.
60. Ogude, N. and J. Bradley, *Electrode processes and aspects relating to cell EMF, current, and cell components in operating electrochemical cells*. Journal of chemical education, 1996. **73**(12): p. 1145-1149.
61. Yang, Y., M. Li, and Z. Zhu, *A novel electrochemical sensor based on carbon nanotubes array for selective detection of dopamine or uric acid*. Talanta, 2019. **201**: p. 295-300.
62. Chen, J.-C., et al., *A disposable single-use electrochemical sensor for the detection of uric acid in human whole blood*. Sensors and Actuators B: Chemical, 2005. **110**(2): p. 364-369.
63. Wu, Y., et al., *Simultaneous and sensitive determination of ascorbic acid, dopamine and uric acid via an electrochemical sensor based on PVP-graphene composite*. Journal of Nanobiotechnology, 2020. **18**: p. 1-13.
64. Kelley, W.N., et al., *A specific enzyme defect in gout associated with overproduction of uric acid*. Proceedings of the National Academy of Sciences, 1967. **57**(6): p. 1735-1739.
65. Feig, D.I., D.-H. Kang, and R.J. Johnson, *Uric acid and cardiovascular risk*. New England journal of medicine, 2008. **359**(17): p. 1811-1821.
66. Cirillo, P., et al., *Uric acid, the metabolic syndrome, and renal disease*. Journal of the American Society of Nephrology, 2006. **17**(12_suppl_3): p. S165-S168.
67. Yuan, M., L. Xu, and X. Yin, *Uric acid and the risk of Alzheimer's disease: is there an association?* American Journal of Alzheimer's Disease & Other Dementias®, 2016. **31**(3): p. 294-295.
68. Martinello, F. and E.L. da Silva, *Mechanism of ascorbic acid interference in biochemical tests that use peroxide and peroxidase to generate chromophore*. Clinica chimica acta, 2006. **373**(1-2): p. 108-116.
69. Martinello, F. and E.L. da Silva, *Ascorbic acid interference in the measurement of serum biochemical parameters: in vivo and in vitro studies*. Clinical biochemistry, 2006. **39**(4): p. 396-403.

70. Groopman, J.D. and T.W. Kensler, *Molecular biomarkers for human chemical carcinogen exposures*. Chemical research in toxicology, 1993. **6**(6): p. 764-770.
71. Chen, X., et al., *Determination of glucose and uric acid with bienzyme colorimetry on microfluidic paper-based analysis devices*. Biosensors and Bioelectronics, 2012. **35**(1): p. 363-368.
72. Rehak, N.N., G. Janes, and D.S. Young, *Calorimetric enzymic measurement of uric acid in serum*. Clinical chemistry, 1977. **23**(2): p. 195-199.
73. Li, Q., et al., *Determination of uric acid in biological samples by high performance liquid chromatography-electrospray ionization-tandem mass spectrometry and study on pathogenesis of pulmonary arterial hypertension in pulmonary artery endothelium cells*. RSC advances, 2018. **8**(45): p. 25808-25814.
74. Ciucci, F. and C. Chen, *Analysis of electrochemical impedance spectroscopy data using the distribution of relaxation times: A Bayesian and hierarchical Bayesian approach*. Electrochimica Acta, 2015. **167**: p. 439-454.
75. Ulman, A., *Formation and structure of self-assembled monolayers*. Chemical reviews, 1996. **96**(4): p. 1533-1554.
76. Liu, X., et al., *Hydrothermal synthesis of copper selenides with controllable phases and morphologies from an ionic liquid precursor*. Nanoscale, 2011. **3**(12): p. 5090-5095.
77. Kumar, A.K.S., et al., *A mini-review: How reliable is the drop casting technique?* Electrochemistry Communications, 2020. **121**: p. 106867.
78. Kaewket, K., et al., *Anti-fouling effects of carbon nanofiber in electrochemical sensing of phenolic compounds*. Journal of The Electrochemical Society, 2021. **168**(6): p. 067501.