



Detection of additives in food using conducting polymer/CuO modified electrodes

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DECLARATION

I, Onkarabile Gaopalelwe Pooe, hereby state solemnly that the work that is carried out in this dissertation entitled “Detection of additives in food using conducting polymer/CuO modified electrodes”, submitted to the Department of Chemistry, North-West University, Mafikeng campus, in fulfilment of the requirements for the degree Master of Science in Chemistry, compiled under the supervision of Prof. Omolola Esther Fayemi. This dissertation has not been submitted to any other institution within and outside of the North-West University. The sources of information used in the dissertation are referenced accordingly.

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ABSTRACT

This study describes the modification of electrochemical sensors of the copper oxide nanoparticles incorporated with the functionalized multi-walled carbon nanotubes ($\text{CuO}_{\text{ch}}/\text{fMWCNTs}$ and $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$) embedded on the platinum electrode (PtE) towards the detection of capsaicin (CAP). Prior to the fabrication of the PtE, numerous spectroscopic and microscopic techniques were utilized for the characterization of the nanomaterials; these includes the ultraviolet-visible spectroscopy (UV-Vis), fourier transform infrared spectroscopy (FTIR), x-ray diffractogram spectroscopy (XRD), scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDX), and transmission electron microscopy (TEM). For the electrochemical characterization of the Pt-Bare, Pt- CuO_{ch} NPs, Pt- CuO_{gr} NPs, Pt-fMWCNTs, Pt- $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$, and Pt- $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$ electrodes, the cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and square-wave voltametry (SWV) techniques were utilized. Both nanocomposite (Pt- $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$, and Pt- $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$) electrodes demonstrated better electron transfer compared to other electrodes. On the detection of capsaicin, the Pt- $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$ electrode demonstrated a limit of detection (LOD) of $0.0765 \mu\text{M}$ with a linear range of concentration for 0.357 to $2.7 \mu\text{M}$ while the Pt- $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$ electrode displayed the LOD of $0.0881 \mu\text{M}$ with the linear range of concentration for 0.357 to $2.7 \mu\text{M}$. Both proposed electrodes showed good reproducibility and excellent anti-interference capabilities. Real sample analysis was conducted using the sauce and rub, for the sauce, the Pt- $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$ showed the recovery percentages of 98 to 110 % with an average RSD value of 5.77 ($n=3$) and Pt- $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$ gave the recovery percentage of 100-116 % with an average RSD value of 8.24 ($n=3$). While for the rub, the Pt- $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$ showed the recovery percentages of 84-106 % with an average RSD value of 12.18 ($n=3$) and Pt- $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$ gave the recovery percentage of 98 to 105 % with an average RSD value of 3.36 ($n=3$).

Keywords: Capsaicin; electrochemical sensor; nanomaterials; square-wave voltametry; limit of detection.

LIST OF ABBREVIATIONS

AE	Auxiliary electrode
CAP	Capsaicin
CE	Capillary electrode
Ch	Chemical
CV	Cyclic voltammetry
CNTs	Carbon nanotubes
CuO NPs	Copper oxide nanoparticles
EIS	Electrochemical impedance spectroscopy
EDX	Energy dispersive X-ray
FTIR	Fourier transformed infrared spectroscopy
fMWCNTs	Functionalized multi-walled carbon nanotubes
Gr	Green
LOD	Limit of detection
LOQ	Limit of quantification
PtE	Platinum electrode
PBS	Phosphate buffered saline
pH	Potential of hydrogen
RSD	Relative standard deviation
RE	Reference electrode
SEM	Scanning electrode microscopy

SWV	Square wave voltammetry
TEM	Transmission electron microscopy
UV-Vis	Ultraviolet-visible spectroscopy
XRD	X-ray diffraction spectroscopy

LIST OF SYMBOLS

a	Charge transfer coefficient
A	Active surface area
A	Amperes
I_{pa}	Anodic peak current
E_{pa}	Anodic peak potential
I_{pc}	Cathodic peak current
E_{pc}	Cathodic peak potential
E°	Formal potential
F	Faraday's constant
K	Kelvin
μ	Micro
m	Milli
M	Molar
N	Number of electrons
v	Scan rate
R	Universal gas constant
T	Temperature
V	Voltage
λ	Wavelength

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CHAPTER 1 INTRODUCTION AND BACKGROUND

1. Introduction

1.1.1 Additives

Food additives, either synthetic or natural, are materials which are added to food products during production or processing in order to enhance sensory and the physical qualities or extend shelf-life [1]. These additives are either added directly or indirectly. Directly added additives are the ones that are added intentionally to food in controlled levels with their main purpose being to aid in food preservation, processing improvement and food appeal. On the other hand, indirectly added additives are the substances that become part of the food product as a result of packaging or processing, they are usually at low levels [2].

1.1.2 Capsaicin

Although a lot of work has been done to detect capsaicin (CAP), with the use of various electrode sensors modified with carbon nanotubes, conducting polymers, nano-bio-composites and graphite [3-7], there is no published work on the electrochemical detection of capsaicin using copper oxide - multi-walled carbon nanotubes (CuO/fMWCNTs) nanocomposite embedded on the platinum electrode (Pt). This dissertation will therefore report the chemical and green synthesis, characterization of copper oxide nanocomposites (CuO_{ch/gr} NPs), characterization of the purchased functionalized multi-walled carbon nanotubes (fMWCNTs), both the chemical and green (CuO_{ch/gr}/fMWCNTs) nanocomposites, and the construction of the Pt-CuO_{ch/gr}/fMWCNTs nanocomposites as novel sensors for CAP detection in Nando's sauce and Osteoeze rub cream respectively. The proposed sensors have shown remarkable recoveries, low limit of detection values (LOD), enhanced sensitivities, and high selectivity with credit to the outstanding kinetics of the electrons and great surface areas which are the resultant of the synergy between the CuO NPs and fMWCNTs.

1.1.3 Sensors

A sensor is a device that can be utilized to measure an intrinsic property of a particular material, as it emits an electrical impulse with response to the physical stimulus, such as light, heat, pressure, movement or sound, [8]. Sensors are mainly classified as chemical (including electrochemical)

sensors, and biosensors. With the emergence of nanotechnology, many modern sensors that are smaller, have high sensitivity and selectivity, with low production and maintenance costs have been developed [9]. This has resulted in sensors being widely utilized in healthcare, fuel cells and environmental monitoring.

1.1.4 Chemical sensors

A chemical sensor is a device that quantitatively converts information about the presence of a chemical species to an analytically useful signal [10]. Catalytic, gas, and electrochemical sensors are examples of chemical sensors. Compared to biosensors, chemical sensors are highly stable and reproducible [11]. Electrochemical sensors are a class of chemical sensors in which an electrode is used as a transducer element in the presence of an analyte [8]. Material, chemical, surface properties, electrochemical properties and electrode design are the factors to be considered when developing an electrochemical sensor [12]. The ability to analyze samples with less damage to the samples, the high sensitivity and selectivity are the main reasons why pharmaceuticals, industrials and environmental analyses extensively make use of electrochemical sensors [13].

Nanotechnology has greatly impacted the development of biosensors. Since graphene and graphene oxide were discovered there has been a massive improvement in biosensors and other research areas and this is because graphene is a one-atom thick sheet entirely composed of carbon, which is responsible for the chemical and physical attributes of graphene. Graphene has high elasticity, mechanical strength, and thermal conductivity [10].

1.1.5 Nanotechnology

Nanotechnology is technology at a nanoscale, which is a scale at a range of 1 – 100 nm. Nanotechnology is connected with systems and materials, the components and structures of which represent novel, significantly enhanced chemical, physical and biological properties, processes, and phenomena due to their nanoscale size [14]. Smart sensors which are small and energy-efficient have been made possible by nanotechnology, thus leading to the mass production of chemical and biosensors [15]. Nanotechnology has a vast promise in electronics, health and other sectors.

1.1.6 Metal oxide nanoparticles

Nanoparticles (NPs) are discrete nanometer scale (10^{-9} m) assemblies of atoms, having dimensions between the characteristics of ions and of macroscopic materials [16]. Nanoparticles exhibit unique chemical, physical and biological properties at nanoscales compared to their counterparts at higher scales, and this is due to their relatively large surface area to volume, increased reactivity or stability in chemical processes, etc. [17]. Hence, nanoparticles are being adopted across the biomedical, agricultural, industrial, and environmental sectors. Based on the size, morphology and shape of nanoparticles, they can be classified into various types [18]. A variety of synthesis methods have been developed to enhance their properties and reduce the cost of production. Some methods are tuned to achieve specific nanoparticles with the aim to improve their optical, chemical, mechanical and physical properties [17].

Metal oxide nanoparticles are among the functional materials synthesized on the nanoscale. Metal oxides nanoparticles are a diverse class of materials, due to their unique characteristics [19]. Metal oxide nanoparticles are widely attractive due to their applications in catalysis, energy storage and conversion, sensing, optics and electronics [20].

1.1.7 Carbon nanotubes

Carbon nanotubes (CNTs) are graphene sheets rolled-up into cylinders, with diameters as small as one nanometer, and can be considered as single-walled (SW) or multi-walled carbon nanotubes (MWCNTs). Nanotubes have attracted the attention of researchers worldwide, and a significant amount of work has been carried out in the last decade to reveal the structural, electrical, mechanical, electromechanical and chemical properties of carbon nanotubes and to explore the key applications of these novel materials that can be exploited [21]. MWCNTs have been exploited as electrochemical sensors in the fields of medicine, food industry, occupational safety, forensic chemistry and environmental analysis. This is due to MWCNTs possessing properties such as high electronic conductivity, chemical stability, large specific surface area, biocompatibility and ease of modification which enhances the intensity and resolution at which the electroanalytical signals are observed on the working platforms [22].

1.2 Problem description

The detection of capsaicin at trace levels requires the usage of rapid, cost-effective, reliable, highly selective and sensitive analytical techniques. Previously various analytical techniques such as gas

chromatography and high performance liquid chromatography have been used [23, 24]. These techniques gave excellent detection limits, however, they are time-demanding, have complicated sample preparations, require multiple extractions and are not cost-effective.

To overcome these setbacks, a variety of electrochemical methods with electrochemical sensors modified with nanomaterials have been reported. With the exploitation of the properties of CuO NPs and multi-walled carbon nanotubes, this study aims to produce a rapid, cost-effective, highly sensitive and selective, and reliable electrochemical sensor for capsaicin.

1.3 Aim and objectives

1.3.1 Aim

This study's aim is to develop a green and a chemical copper oxide nanoparticle incorporated with multi-walled carbon nanotubes ($\text{CuO}_{\text{ch/gr}}/\text{fMWCNTs}$) nanocomposite modified electrochemical sensors for the detection of additives in food and pharmaceutical products.

1.3.2 Objectives

This study's objectives were to:

1. synthesize copper oxide nanoparticles (CuO NPs) using the chemical and green routes,
2. prepare the green and chemical copper oxide nanoparticles incorporated with multi-walled carbon nanotubes ($\text{CuO}_{\text{ch/gr}}/\text{fMWCNTs}$) nanocomposites,
3. characterize the as-synthesized nanoparticles, functionalized multi-walled carbon nanotubes, and their nanocomposites using the microscopic, spectroscopic and electrochemical techniques,
4. assess the level of sensitivity of the resultant working electrodes pertaining to capsaicin with the use of the square wave voltammetry,
5. detect capsaicin in food and pharmaceutical products using the platinum electrode modified with the green and chemical copper oxide nanoparticles incorporated with multi-walled carbon nanotubes ($\text{CuO}_{\text{ch/gr}}/\text{fMWCNTs}$) nanocomposites with the use of square wave voltammetry.

1.4 Dissertation outline

1. Chapter 1: Introduction and background

On chapter 1, a brief introduction to the study is provided, as well as the aim and objectives on why the study is conducted.

2. Chapter 2: Literature review

On chapter 2, a review on the literature regarding the nanomaterials, the analyte, and analytical instrumentations used in the study was conducted.

3. Chapter 3: Materials and methodology

This chapter deals with the materials that were used in this study, and also the methods that were followed to synthesize the nanomaterials, what instrumentations were used for characterization and how the electrocatalysis and electroanalysis were conducted.

4. Chapter 4: Results and discussions

This chapter focuses on the discussion of the obtained data from the study.

5. Chapter 5: Conclusion and recommendations

Here the conclusion is drawn from the studies, and recommendations are provided on how the study can be further improved.

CHAPTER 2 LITERATURE REVIEW

2. Literature review

This chapter provides the literature review of the analyte (CAP) used in this study, the analytical techniques used to detect the analyte. A broad-view of the applications of the nanomaterials (CuO, fMWCNTs and CuO/fMWCNTs) in analytical chemistry.

2.1 Additives

Additives are substances that are intentionally added to other substances to increase their quality and properties. Additives can be used in the food, pharmaceutical, biomedical and cosmetic industries [25]. In the food industry, additives are used as colorants (turmeric (E100), carmine (E120)), preservatives (sulphur dioxide (E220), acetic acid (E260)) and flavour enhancers (Glutamic acid (E620), calcium salt (E626)) [26, 27]. There are generally three characteristics of food additives: (a) they are substances added to food which cannot be consumed alone as foods, (b) they include natural as well as synthetic substances, and (c) they are added to improve the quality and flavour of food, and to enhance the freshness, processing and preservation of the food [28]. Even though there are many benefits of food additives, the improper or unregulated usage of food additives will cause harm to human health. This then emphasises the importance of analytical methods for determining food additives, to generate guidance on the regulatory management of food additives [29]. In the pharmaceutical industry, additives are used as coating additives, emulgents, taste and smell-enhancers, ointment bases, and antioxidants [30]. Table 2.1 shows the most commonly used food additives and ingredients.

Table 2.1: The most common food additives and ingredients

Name	Symbol
Acetic acid	E260
Ammonia caramel	E150c
Carmine	E120
Guar gum	E412
Sodium benzoate	E211
Sodium nitrate	E250

2.1.1 Capsaicin

Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) is one of the most abundant capsaicinoids found in hot chilli peppers which are responsible for the pungency of the hot peppers. Due to this pungency, the hot peppers have become very desirable for consumers. In addition to capsaicin being used as a food additive, its biological properties have led to increasing interest in the pharmaceutical industry. This includes the potential chemo-preventive and anticarcinogenic effects, analgesic, anti-inflammatory, and antioxidative activities, anti-microbial properties, and the regulation of energy metabolism [31]. Capsaicin has been reported to be beneficial in obesity, cardiovascular and gastrointestinal conditions, various cancers, neurogenic bladder and dermatologic conditions [32]. The molecular structure of capsaicin is depicted on Figure 2.1.

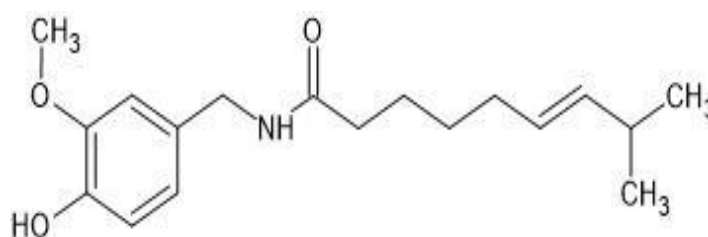


Figure 1.1: The molecular structure of Capsaicin

These properties have led to numerous analytical methods coming into existence for the detection of capsaicin. The Scoville heat unit has predominantly been deployed for the level of heat in peppers. In addition to that, mass spectroscopy, gas chromatography, high-performance liquid chromatography, colorimetric and capillary electrophoresis have been deployed as well. However, these methods have their own limitations. The Scoville test is performed by humans, and this leads to a lack of precision and a degree of subjectivity as it relies on the level of sensitivity to the pungency of the tasters. The analytical methods as well had disadvantages such as high cost, complicated sample preparation, long response time, and the need for a skilled operator [7]. Hence the electrochemical method has gained a lot of attention among the various detection methods, due to its advantages such as cost-effectiveness, rapid response time, high sensitivity, simple sample preparation, and the possibilities of on-field analysis. Researchers have attempted to produce electrochemical sensors for the detection of capsaicin coated with graphene, CNTs, mesoporous silica, and metal oxide nanoparticles as summarized in Table 2.2 [6, 33-35].

Table 2.2: Electrochemical sensors for capsaicin detection

Modified electrode	Method	Linear range (μM)	LOD (μM)	R^2	Ref
MIP-B-CD@G electrode	DPV	1.0 – 180	0.007	0.99	[36]
10% CB-SPE	DPV	0.080 – 60	0.028	0.997	[37]
B-CD/CPE	CV	1.44 – 33	0.065	0.991	[38]
MWCNTs – SPE	CV	0.5 – 35	0.45	0.99	[6]
MCFs/CPE	DPV	0.76 – 11.65	0.08	0.999	[39]
N-doped GrNPs-modified ePAD	AsDPV	40.0 – 100.0	0.37	0.999	[40]

2.2 Nanomaterials

2.2.1 Method used for the synthesis of nanomaterials

Nanomaterials are materials that consist of dimensions that are in the nanoscale range (1 - 100 nm); examples of these materials are nanorods, nanoparticles, nanosheets, nanofibers, as well as thin films. These materials consist of magnificent properties such as magnetic, electrical, and optical properties [41]. Hence, they possess a wide range of applications, such as bio-imaging, pharmaceuticals, cosmetics, bioanalysis, bio-sensing, tissue technology, photonics, data storage, biomedical fields, environmental harvesting, and remediation [42-45]. Furthermore, the advancements in electrochemical sensing are brought into fruition by the existence of nanomaterials like metal oxides and semiconductors nanoparticles.

The synthesis of the nanoparticles involves two approaches: the top-down approach and the bottom-up approach. With the top-down approaches, the bulk materials are converted into small particles. With the bottom-up approaches, the small particles are aggregated and form nanoscale range crystals. Figure 2.2 shows the simplified depiction of the top-down and bottom-up approaches.

Top-down approach

This approach involves transforming bulk materials into small nano-sized particles. Although the top-down approaches are simple to use, they produce irregularly shaped and extremely small particles, which makes them ineffective. This is the downside of the top-down approach methods [46]. Methods used in this approach are physical vapor deposition, mechanical milling, electrospinning, etc, [46].

Bottom-up approach

This approach involves the use of atoms or molecules to form nano-sized particles. The produced nanoparticles from this approach are well-defined in shape, size, and chemical composition and are formed through the growth and self-assembly of atoms and molecules as their building blocks [46]. This method is thought to be beneficial due to the fact that the technique is based on the formation of nanocrystals from the atomic-range particles. Sol-gel, chemical, spinning and CVD methods are the types of synthetic methods used in the bottom-up approaches. The two synthetic approaches are summarised in Figure 2.2.

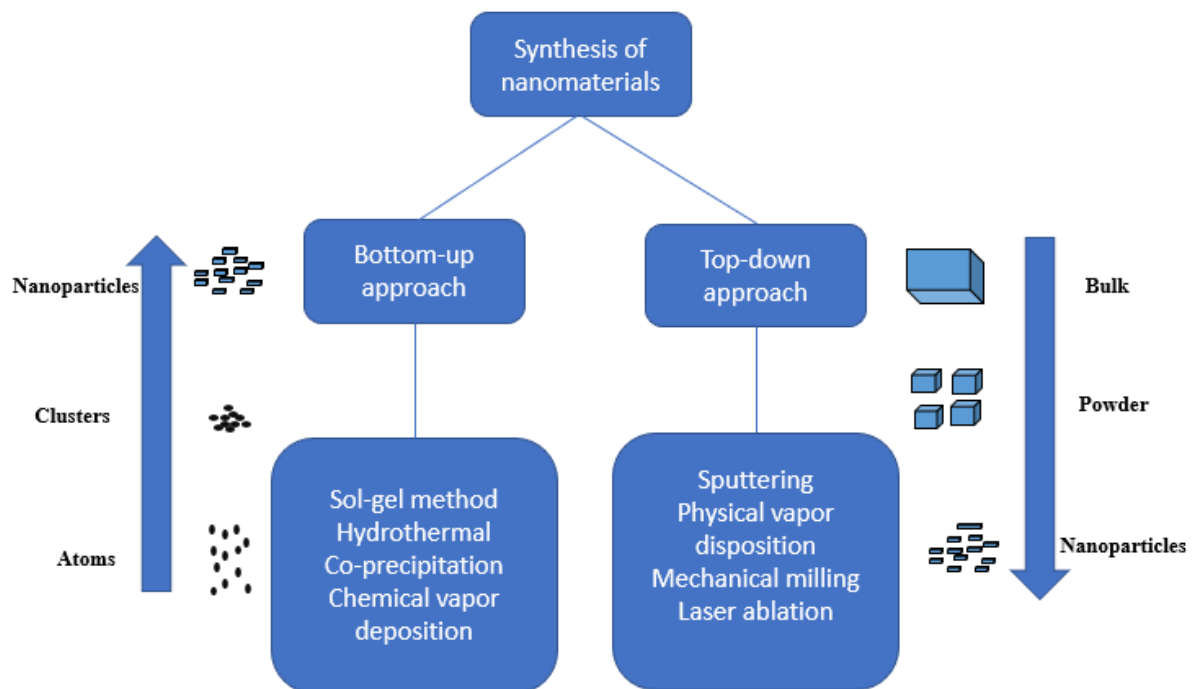


Figure 2.2: Synthesis methods of nanomaterials

2.2.2 Classification and types of nanomaterials

Nanomaterials are classified according to the materials utilized in their synthesis, the origin of the materials and based on their dimensions or structures. Under the material-based nanomaterials we have the carbon-based nanomaterials, inorganic-based nanomaterials, organic-based nanomaterials and composite-based nanomaterials; while under the dimension-based we have the zero dimensional, one dimensional, two dimensional, and three dimensional.

2.2.2.1 Classification based on materials

Carbon-based nanomaterials are a class of nanomaterials with a significant amount of carbon and exhibiting numerous morphologies. They can be hollow tubes or spheres, carbon nanofibers, fullerenes and graphene. The synthesis of these nanomaterials uses different methods such as arc-discharge, chemicals vapor deposition and laser-ablation [47]. Inorganic-based nanomaterials are made up of metals and metal oxides. Metals such as Ag, Au, and Fe can be used in the synthesis and the metal oxides are TiO_2 , ZnO and MnO_2 . Organic-based nanomaterials are composed of organic matter, other than carbon and inorganic materials. These nanomaterials are synthesised by the transformation of organic matter into the desired structures. Composite-based nanomaterials consist of an additional layer of nanomaterials, mixed with additional nanoparticles, bulk materials, or more intricate materials such as metal frame works. Composites are composed of numerous materials such as metal, ceramic, organic, inorganic, carbon-based, or bulk polymers. Depending on the synthesis and materials used for the intended application, they have various morphologies.

2.2.2.2 Classification based on dimensions

These small nanomaterials possess various dimensions being zero, one, two, and three dimensional. The nanomaterials have at least one dimension of less than 100 nm. These nanomaterials exist in single, aggregated, or fused forms with a variety of shapes, including tubular and spherical. Nanofibers, nanotubes, quantum dots, and nanosheets are the most prevalent forms of nanomaterials. Zero-dimensional nanomaterials (0D) are common types of nanomaterials, with all their dimensions existing within the 100 nm scale. The prevalent nanomaterials in this class are hollow spheres, quantum dots, nano lenses, etc [48]. One-dimensional nanomaterials (1D) are a class of nanoparticles which have at least one dimension larger than 100 nm and the other dimension being within the 100 nm scale. Common types of this dimensional nanomaterials are

nanofibers, nanotubes, and nanorods. Two-dimensional nanomaterials (2D) are types of nanoparticles which have two dimensions larger than 100 nm, with the most common examples being nanofilms, nanolayers, and nanocoating. They have plate-like structures. Three-dimensional nanomaterials (3D) are types of nanomaterials having all three dimensions larger than 100 nm, while their components are below 100 nm in size. Three-dimension nanomaterials are formed by the nano range particles coming together. Generally, these materials are nonporous in nature and have a variety of applications. Examples of these materials are nanocomposites, bundles of nanofibers, and multi-nanolayer-type structures. The classifications are shown in Figure 2.3.

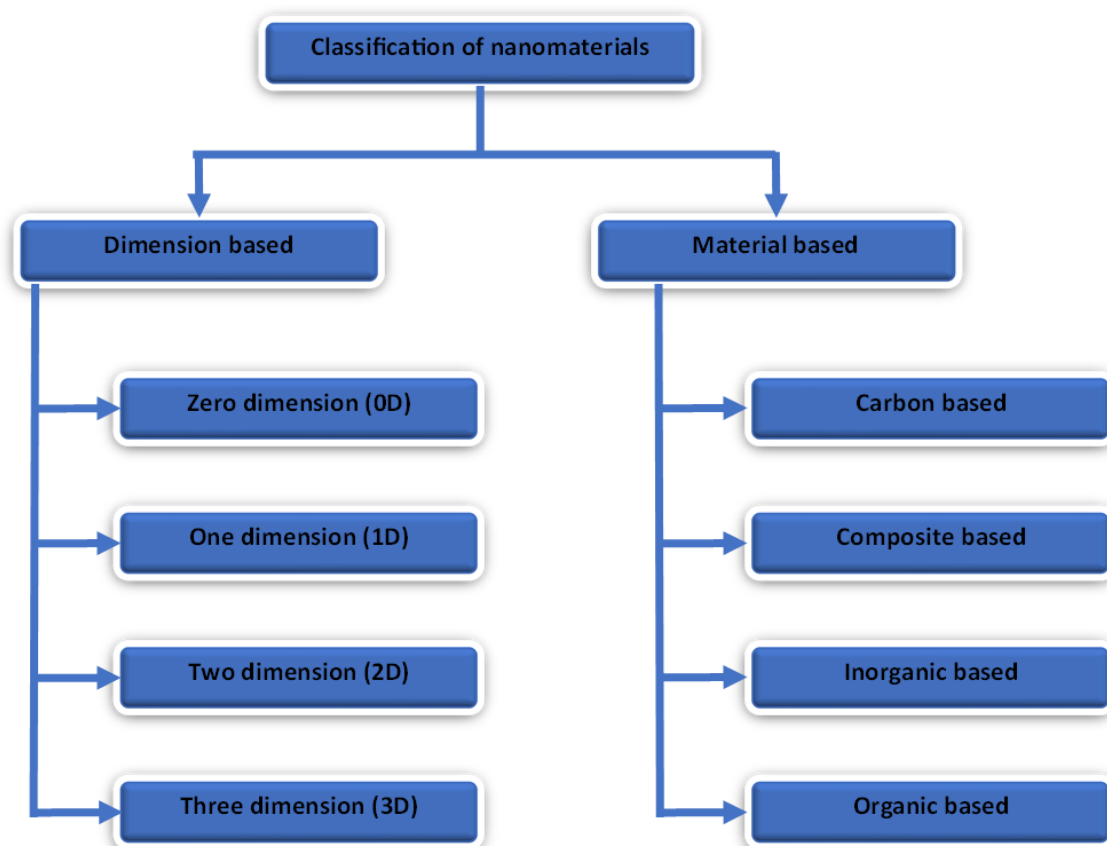


Figure 2.3: Classification of nanomaterials

2.2.3 Metal oxide nanoparticles uses and prospects

Metal oxides are attractive for a large variety of applications which include catalysis, sensor, (opto)electronic materials and environmental remediation. Controlled synthesis of metal oxide nanoparticles is essential for successful application, and solution-phase methods provide more control over the synthesis products.

Copper oxide nanoparticles (CuO NPs) are a p-type semiconductor with a narrow band gap (1.85 eV) [49]. Copper oxide nanoparticles have a natural abundance of starting materials, they have good electrical and optical properties, are non-toxic, and have low production costs [50]. These are reasons why CuO NPs have gained a considerable amount of attention. The copper oxide nanoparticles have gained a tremendous amount of interest, and this is due to the properties which they possess: optical, catalytic, mechanical, and electrical properties. CuO NPs are soluble in dilute acid, solutions like ammonium chloride, ammonium carbonate, and potassium cyanide, and insoluble in water, while they slowly dissolve in alcohols, and ammonia solution [51]. CuO NPs are between 1-100 nm in size. High surface area, superiority of the quantum size effect, volume effect, and microscopic quantum tunnelling effect in magnetic, optical absorption, chemical activity and thermal resistance, catalysis and melting point are a few of the unique chemical and physical characteristics of CuO NPs compared to ordinary copper oxide [51].

The synthesis of CuO NPs has advanced significantly in the last decade, and this is due to their important biomedical and industrial applications. The method that is deployed for the synthesis of the CuO NPs is very important as it determines the size and morphology of the nanoparticles. Various methods of synthesis for CuO NPs have been used, these include: electrochemical method, sonochemical synthesis, sol-gel method, microemulsion system, precipitation synthesis and microwave irradiation [52]. The synthesis of CuO NPs can be grouped into three categories based on the applied techniques, i.e., electrochemical, chemical and biogenic methods [53].

Chemical synthesis of CuO NPs utilizes the bottom-up approach, which is cost-effective and results in uniform-sized and shaped products on a large scale without the requirement of high-throughput equipment. The common chemical synthesis of CuO NPs involves the use of copper salt precursors by organic or inorganic reducing agents. A typical reaction involves the salt precursor being reduced by the reducing agent, and the resulting precipitate is washed and calcined to obtain the nanostructure with different morphology [53]. Reported methods used in chemical synthesis are sol-gel, co-precipitation and sonochemical [54].

Physical synthesis of CuO NPs utilizes the top-down synthetic approach, they produce high quality CuO NPs with uniform and controlled size. The laser ablation technique has gained a lot of attention due to its being eco-friendly, it is simple to operate, and it produces uniform nanoparticles. The commonly used techniques are sputtering, laser ablation, and ball milling [55].

Green synthesis is a derivation of materials using environmentally friendly or green resources with the use of a good solvent, reducing agent and harmless stabilizing material [56]. The green synthesis route is cost-effective, straightforward, sustainable, reproducible and produces more stable compounds. Green synthesis of nanoparticles techniques have been developed to overcome the limitations and hazardous problems of the traditional nanoparticle producing techniques like the chemical and physical synthesis.[52] Green synthesis of CuO NPs using plants such as soybeans, latex of *Calotropis procera* L, *Manihot esculenta*, and *Gloriosa superba* have been reported [57]. The synthetic methods for CuO NPs are summarized on Figure 2.4.

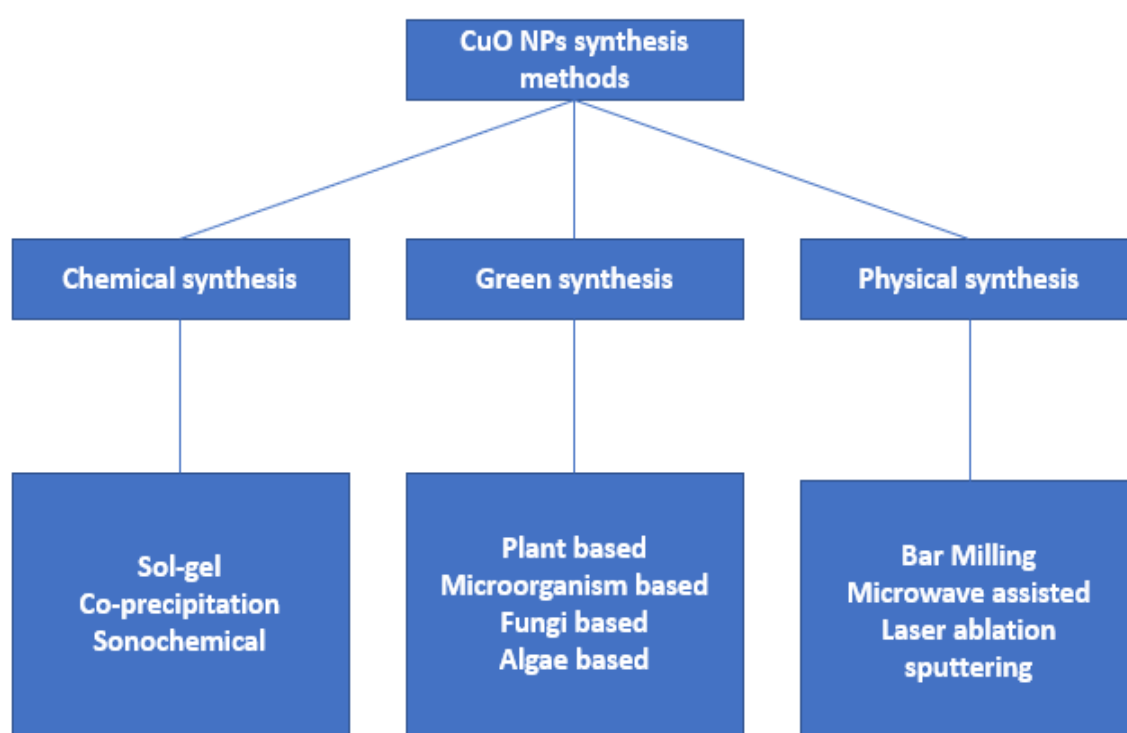


Figure 2.4: Copper oxide nanoparticles synthesis methods

CuO NPs have been applied in biomedical applications as a sensor for glucose, dopamine, hydrogen peroxide, as well as antiparasitic and targeted cancer therapy [53]. In the environmental industry CuO NPs have been reported to be used in environmental remediation as sensors, for adsorption, photodegradation, and as a CO₂ catalytic reducer. The energy sector uses the CuO NPs in the water splitting, fuel cells, lithium-ion batteries, and as supercapacitors [58]. Table 2.3 shows the applications of metal oxide nanoparticles.

Table 2.3: Applications of metal oxide nanoparticles

Sector	Application
Biomedical	Cancer therapy
	Sensors
	Antiparasitic
Environmental	Sensors
	Photodegrading
	CO ₂ catalytic reducer
	Adsorption
Energy	Fuel cells
	Supercapacitors
	Lithium-ion batteries
	Water splitting

2.2.4 Carbon nanotubes and their applications

Since the discovery of carbon nanotubes (CNTs) in 1991 by Iijima, great progress has taken place in their applications. CNTs are seamless cylinders made up of one or more layers of graphene, with open or closed ends. This graphene is normally denoted as single-wall (SWNT) or multiwall (MWNT). SWNTs are typically 0.8 to 2 nm while the MWNTs are 5 to 20 nm in diameter, although the MWNT can exceed 100 nm [59].

The chemical synthesis of CNTs faces a significant challenge as it is difficult to control the diameter, chirality, and wall number. This has led to the three methods which are currently used, arc discharge, laser ablation, and chemical vapor deposition (CVD). With the CVD method, hydrocarbon gases are used as the carbon atom sources and catalytic particles which serve as seeds to nucleate the growth of the CNTs, while the other two methods use solid-state carbon as the precursor, leading to a near-perfect nanotube structure, with the formation of large amounts of by-products [60-62]. Carbon nanotubes synthetic methods are depicted on Figure 2.5.

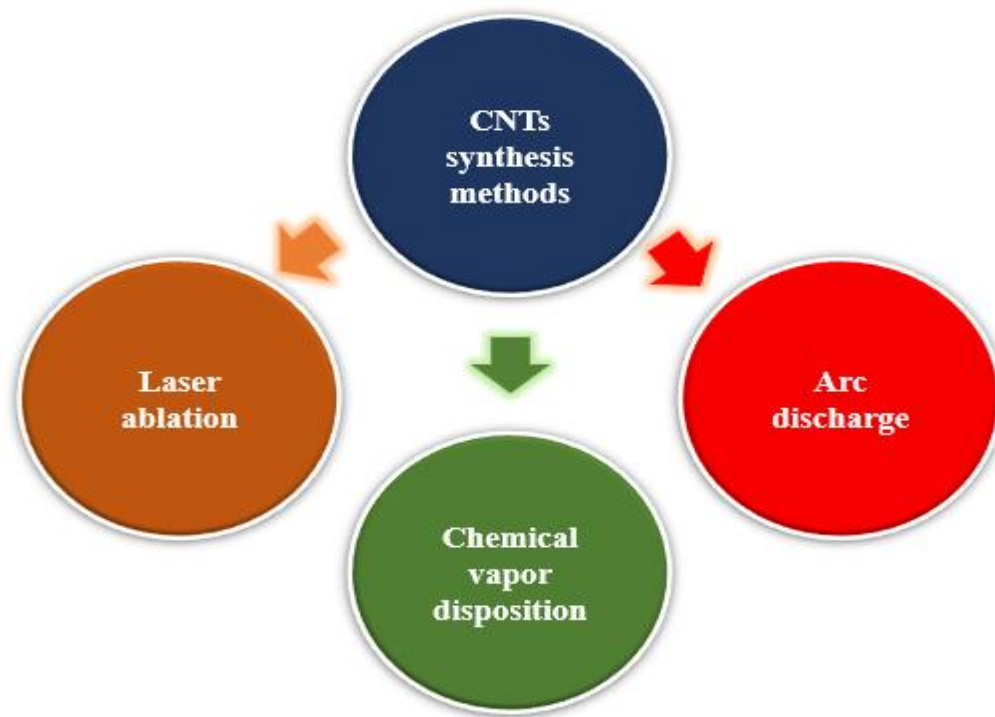


Figure 2.5: Carbon nanotube method of synthesis

CNTs have been reported to possess high surface areas, large aspect ratios, and remarkably high mechanical strength. They have a tensile strength that is 100 times greater than that of steel, and electrical and thermal conductivities that mimic those of copper. These properties make the CNTs to be the quality candidates to be used as fillers in different polymers and ceramics to produce the desirable consumer products [63]. CNTs have been used as nanocomposites recently, this includes CNTs/metal–oxide and CNTs/polymer. These studies were conducted to grasp the super-capacitor behaviours as well as their stability through optimal engineering of the composition, particle size, and other parameters [64]. The electronic property of CNTs that is mostly considered as the important factor is the electron transfer rate. The small diameter and long structure, the electro-activity and high surface are of the CNTs are the key advantages for electrode modification [65].

CNTs possess a wide variety of applications, and this is due to their desirable properties. The size of the CNTs gives them the ability to be used as optical devices. They have been applied as nanoelectrode-based sensors owing to the properties such as high rate of electron transfer and enhanced electrode surface area. Due to the CNTs' high electrical conductivity, small energy spread and substantial chemical stability, they have found application in the field of emission devices. The unique strength and flexibility of CNTs make them suitable for mechanical

applications [66]. Applications of nanomaterials based on their properties are summarized of Table 2.4.

Table 2.4: Applications of nanomaterials based on their properties

Properties	Sector	Applications
Size (nano scale)	Optical devices	Photonic crystals
		Optical antennae
		Solar cells
High electron transfer rate and electrode surface are	Nanoelectrode-based sensors	Hydrogen sensors
		Biosensors
		Gas sensors
Small energy spread, high electrical conductivity and chemical stability	Field emission	Ion sensors
		CNT array emitters
		Lighting
		X-ray generators
Strength and flexibility	Mechanical	Microwave devices
		CNT ropes
		TEM grid

2.2.5 Nanocomposites and their applications

A nanocomposite is a solid material that is comprised of numerous phases with at least one of the phases having one, two, or three dimension(s) in the nanoscale. The development of nanocomposites has given rise to alternative ways to overcome the current limitations of micro-composites and monolithic, thus becoming materials of the future [67]. The high surface to volume ratios, the ability to use small filler size and the space between fillers, improved optical and mechanical properties, high ductility without sacrificing strength, and scratch resistance are some of the advantages that nanocomposites have compared other composite materials. Some of the disadvantages are the lack of understanding between the formulation/property/structure relationship, and the need for simpler particle exfoliation and dispersion [67, 68]. Nanocomposites have been applied in tissue engineering, drug delivery, gene therapy, antimicrobial, bio-renewable, and energy storage [69-74]. The applications of nanocomposites are shown on Figure 2.6.

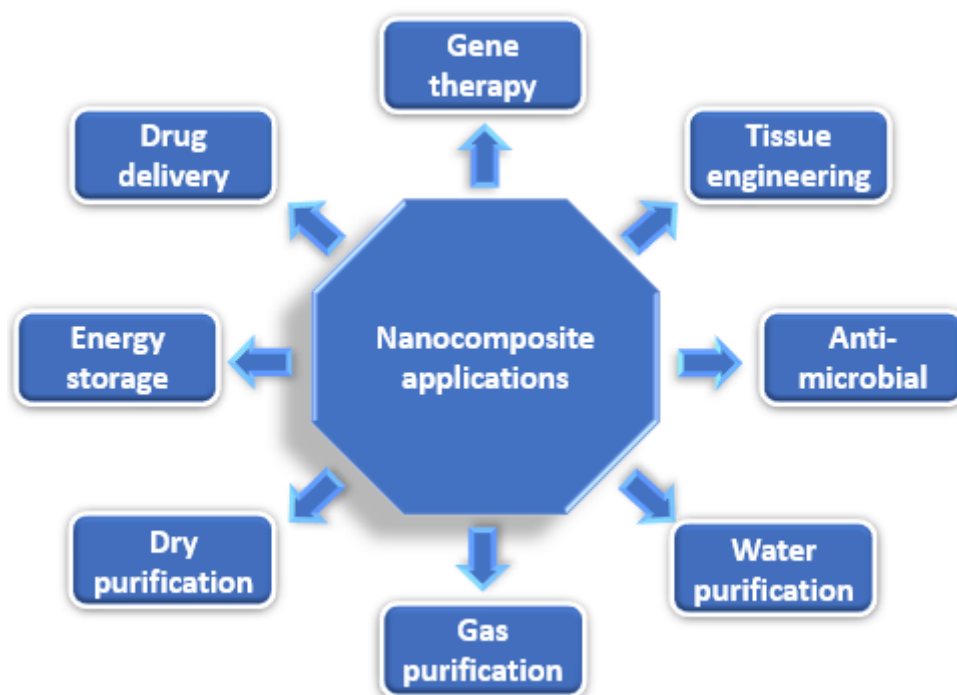


Figure 2.6: Applications of nanocomposites

CuO/MWCNTs nanocomposites have been reported in literature. Shibu et al. used the CuO/MWCNTs nanocomposite in the photodegradation of methyl orange and it yielded good results [75]. CuO/MWCNTs nanocomposite was also used by Felix et al. to produce a highly sensitive enzyme-less electrochemical glucose sensor, the sensor gave excellent limit of detection [76]. Zhang et al used the CuO/MWCNTs nanocomposite for the electrochemical detection of nitrite, and the study showed that the nanocomposite-based electrode had an enhanced selectivity, reliability and stability [77]. On the other hand, Kumar et al used the CuO/MWCNTs for the detection of guanosine, and obtained a highly sensitive, and reliable sensor towards guanosine [78].

2.3 Electrochemical sensors

2.3.1 Platinum electrodes and their applications

Platinum (Pt) is one of the most utilized metal catalysts towards electrochemical reactions. Highly dispersed Pt catalysts with large surface areas are extremely important in the enhancement of electrocatalytic reaction [79]. Pt has been used as a counter-electrode for decades and this is due to its not undergoing electrochemical degradation at high potentials [80].

2.3.2 Electrochemical methods and their applications

Electrochemical analysis is a powerful and versatile analytical method that is able to detect electric currents generated from oxidative or reductive reactions in test compounds [81]. Electrochemical methods are used to evaluate the reaction kinetics, reaction concentration, mechanism, and physical and chemical properties. Electrochemical methods provide quick analysis, high sensitivity and selectivity at a lower cost than other conventional techniques like HPLC, capillary electrophoresis, and liquid chromatography [82].

A typical electrochemical cell is comprised of three important electrodes, namely the reference electrode (RE), working electrode (WE), and auxiliary electrode (AE) as depicted on Figure 2.7 [83]. On the working electrode is where the redox reaction happens, and this reaction can be quasi-reversible, reversible or irreversible and this is determined using cyclic voltammetry. RE is usually made of platinum, gold, and silver as inert materials, it is the electrode of known potential used for the control of potential, and AE is utilized for the connection of the whole circuit. Electrochemical methods have been deployed in various areas such as fuel cells, batteries, electrochemical sensors, environmental applications, and pharmaceuticals [84-87]. A typical three electrode electrochemical electrode set-up is shown on Figure 2.7.

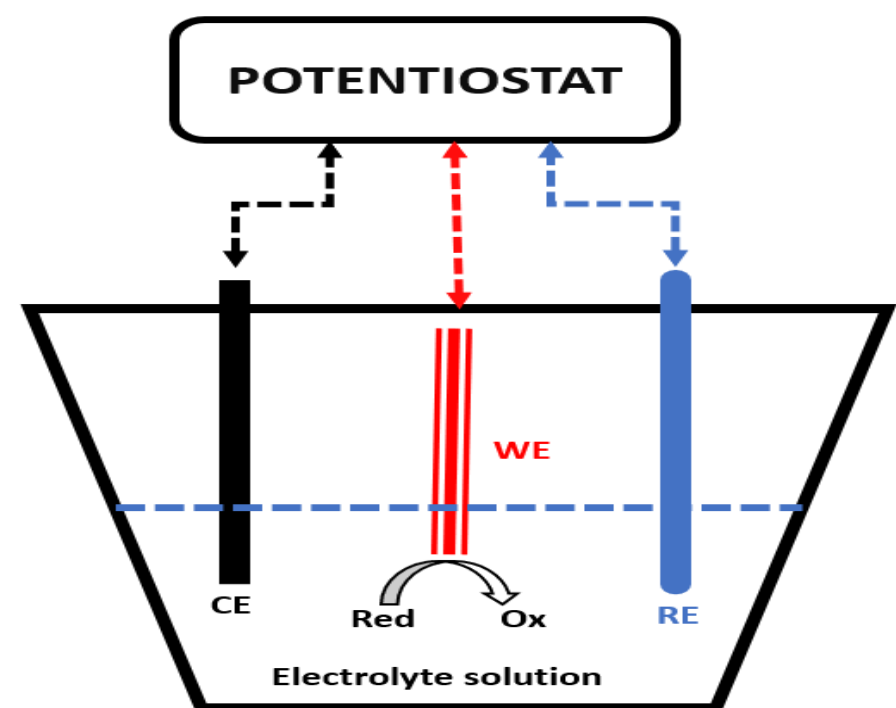


Figure 2.7: A three electrode electrochemical cell set-up

2.3.3 Voltametric techniques

Voltammetry is the study of chemical reactions occurring at an ionic or electronic conductor interface in a solution and this is achieved by measuring the current response after applying an external potential difference across the interface [88]. Examples of voltametric methods include cyclic voltammetry (CV), square wave voltammetry (SWV) differential pulse voltammetry (DPV), linear potential sweep voltammetry (LPSV), etc. Voltametric methods are largely used in different fields due to their low cost, high sensitivity, no pre-treatment required, ability to achieve detection at low concentrations, simple to operate, rapid response and are portable compared to the conventional techniques [89].

2.3.4 Cyclic voltammetry (CV)

Cyclic voltammetry (CV) has become an important and widely used technique in many areas of electroanalytical chemistry. Mainly it is used to study redox reactions and get the much-needed information about the chemical reactions that occur, and it is rarely used for quantitative determination [90]. CV is a rapid voltage scan technique in which the direction of the voltage scan is reversed, while the potential is applied to the working electrode in both the forward and reverse directions, and the resulting current is recorded. Normally the scan rate in the forward and reverse direction is the same. The parameters that are measured in CV are anodic and cathodic peak potential (E_{pa} and E_{pc}), anodic and cathodic peak current (I_{pa} and I_{pc}), and the half peak potentials ($E_{p/2}$) at which the cathodic and anodic currents reach half of their peak value. Figure 2.8 shows a typical cyclic voltammogram [91].

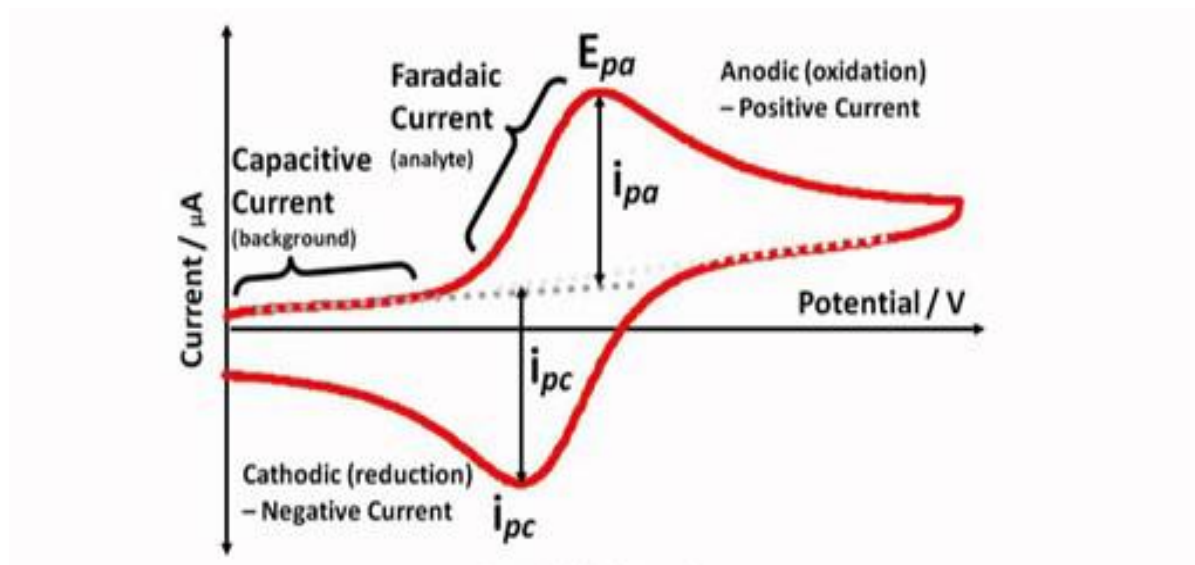


Figure 3.8: A labelled schematic diagram of a cyclic voltammogram [91]

2.3.5 Square wave voltammetry (SWV)

Square-wave voltammetry (SWV) is a powerful electrochemical technique that is well suited for analytical applications, studying the mechanistic processes of the electrode and electrokinetic measurements. SWV is considered as one of the most advanced voltametric techniques, it incorporates the advantages of pulse techniques, cyclic voltammetry, and impedance voltammetry [92]. A typical SWV technique is achieved through applying potential to a stationary electrode, which combines the staircase wave potential and the square wave. The measurement of the current is obtained on the working electrode while the potential between the working electrode with reference electrode is linearly swept over time.

2.3.6 Electrochemical impedance spectroscopy (EIS)

Electrochemical impedance electrochemistry (EIS) is a powerful electrochemical technique used for the characterization of electrochemical systems, it is widely used in various fields like energy, electrocatalysis and medicine[93]. The EIS approach usually consists of applying a sinusoidal current or voltage of a certain frequency and amplitude and measuring the amplitude and phase shift of the output voltage respectively. The procedure is repeated for a number of frequencies, the range is typically in the kHz to mHz, thereby generating a characteristic impedance spectrum [94].

2.4 Spectroscopic methods of characterization

Some spectroscopic techniques include Raman spectroscopy, photoluminescence, ultraviolet-visible spectroscopy (UV-Vis) and Fourier transform infrared spectroscopy (FTIR) [95]. These techniques analyse and provide quantitative and qualitative data of the analyte using light [96]. When light interacts with a material, some of its energy is either absorbed, reflected, or possibly it excites electrons thus leading to photoluminescence. These techniques are employed in many industries, such as the food sector, medical sector, mining sector, and treatment of water. They aid in investigation of the molecular make-up, absorption characteristics and composition of the nanomaterials. For example, electroluminescence can be employed to investigate the surface characteristics of nanoparticles.

2.4.1 Ultraviolet-visible spectroscopy (UV-vis)

UV-visible spectroscopy is a molecular spectroscopy which is based on the Beer-Lambert law for its operation. In the UV-vis analysis, a beam of light is split into two, where 50 % of the beam passes through the reference material while the other 50 % passes through the solution in the transparent cell. Each and every material has its own specific surface plasmon resonance (SPR), which is the specific wavelength at which the analyte absorbs light [97]. This technique is used because it is rapid, accurate, simple and cost-effective when compared to other techniques. It is normally used in various fields like forensics, pharmaceuticals [98].

2.4.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectrophotometer is used for the identification of different functional groups associated with the analytes. At specific resonance frequencies, the incident light from the FTIR machine will cause an absorption when in contact with a vibration frequency of the group or bond corresponding to the same frequency [99]. The FTIR spectra typically lie in the mid-IR region of $2.5 - 15 \mu\text{m}$ between the $4000 - 400 \text{ cm}^{-1}$. A typical FTIR spectra has four regions of types of bonds, a single bond occurs at higher wavenumber region ($4000 - 2500 \text{ cm}^{-1}$), a triple bond occurs at the middle wavenumber region ($2500 - 2000 \text{ cm}^{-1}$), a double bond occurs at the middle wavenumber region ($2000 - 1500 \text{ cm}^{-1}$) and the fingerprint region which occurs at low wavenumber region ($1500 - 400 \text{ cm}^{-1}$) as depicted in Figure 2.9 [100].

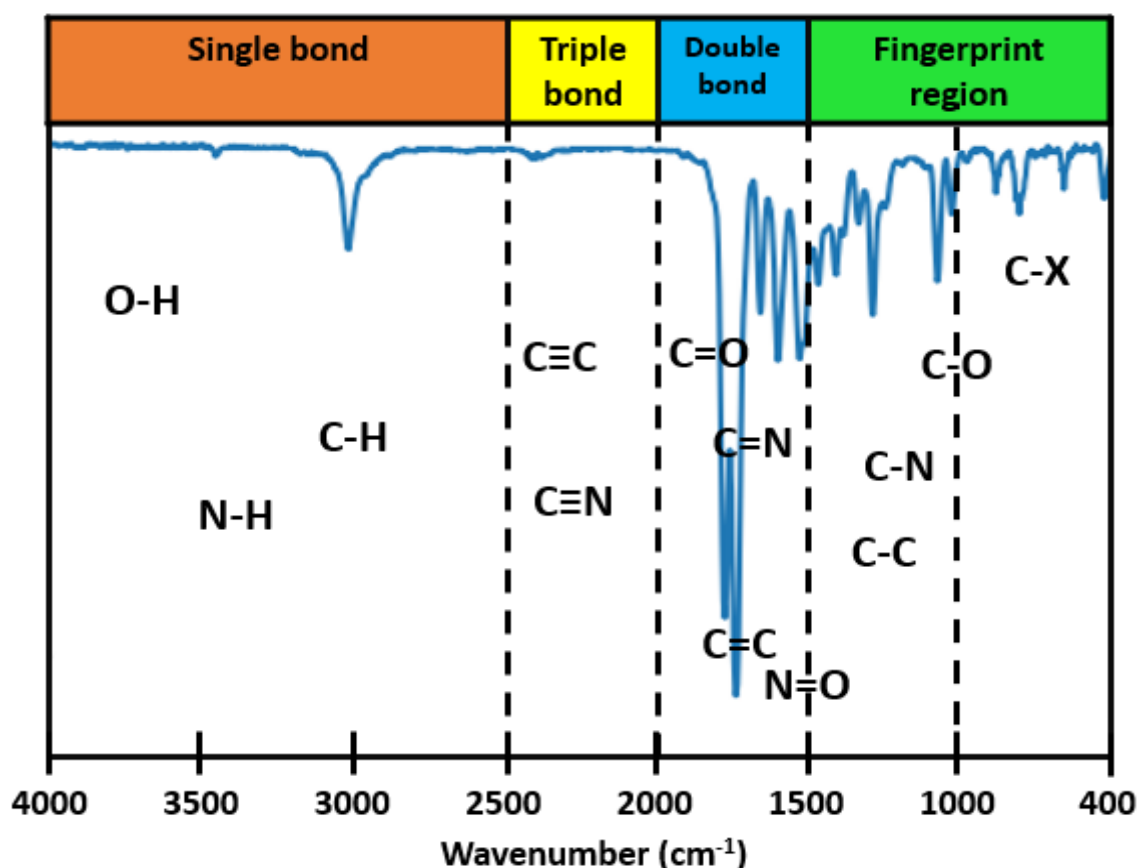


Figure 2.9: A typical FTIR spectra with common types of bond absorptions in the approximate regions

2.5 Surface characterization methods

Examining the surface area of nanomaterials is crucial as it affects their catalytic properties as they are employed as catalysts in electrochemical sensors. When using catalysts, characteristics like texture and chemical surface area are crucial. SEM, XRD, EDX, and TEM are a few of the techniques which are used to for the characterization of the surface of the nanomaterials [101].

2.5.1 X-ray diffractogram (XRD)

Different lattice planes are in diffracting condition, and varying intensities of the diffracted signal according to the crystal structure and space group of the present phase. Each phase produces a characteristic diffraction pattern that allows its identification, when there is a presence of several phases in the system the characteristic patterns of all the phases are superimposed and the intensity of the diffraction peaks are directly proportional to their amounts. The XRD methods are widely used to identify the present phases and the determinations of their respective amounts [102].

2.5.2 Scanning electron microscopy

SEM a technique that uses a focused beam of high-energy electrons to generate a variety of signals at the surface of the solid specimens. The electron-sample interaction gives signals that provide information about the sample, these includes external morphology, chemical composition, crystalline structure, and the orientation of materials making the sample. The SEM instrument consists of the electron column, sample chamber, EDS detector, electronic console and visual display monitors [103].

2.5.3 Transmission electron microscopy

In TEM, a beam of electrons is produced by the electron source and are passed through an extremely fine sample prepared by special processes. During the transmission, an image is formed as a result of the interactions between the sample and electron beams, the image is enlarged and focused and reflected on a screen [104].

CHAPTER 3 MATERIALS AND METHODOLOGY

3. Materials and methodology

This chapter provides a quick summary of the chemicals, equipment and methods of synthesis for the nanoparticles and nanocomposites. Provides a detailed process of the CuO_{ch/gr}/fMWCNTs electrode modification, the preparation of the analyte, the capsaicin sauce and rub preparation, electrochemical and impedance studies, concentration and interference studies.

3.1 Materials

The *Laurus nobilis* (bay leaves) dried leaves (Mafikeng, South Africa) were used for the green mediated synthesis of the CuO. White Copper (II) nitrate trihydrate (Cu(NO₃)₂.H₂O), sodium hydroxide (NaOH), potassium hexacyanoferrate (IV) (K₄ Fe(CN)₆), potassium hexacyanoferrate (III) (K₃ Fe(CN)₆), N,N-dimethylformamide (C₃H₇NO), ethanol (C₂H₆O), functionalized multi-walled carbon nanotubes (MWCNTs), and capsaicin (C₁₈H₂₇NO₃) were purchased from Merck Pty Ltd (Darmstadt, Germany). The sodium phosphate salts (Na₂HPO₄ & NaH₂PO₄) were obtained from LABCHEM and GlassWorld (Johannesburg, South Africa). All reagents were of analytical grade and were used as received without any further purification. 0.1 M Phosphate buffer solution (PBS) was prepared by mixing appropriate quantity of (Na₂HPO₄) and (NaH₂PO₄) and 5 mM [Fe(CN)₆]^{3-/4-} was prepared by mixing appropriate amounts of K₄ Fe(CN)₆, and K₃ Fe(CN)₆ in 0.1 M PBS, pH 7. Deionized (DI) water was used to prepare all solutions in this work.

3.2 Plant extraction

The synthesis of the copper oxide nanoparticles (CuO NPs) follows the proposed method by Velsankar *et al.* with a few alterations. About 10 mg of the dried and finely crushed powder *Laurus nobilis* leaves were boiled at a temperature of 70-80 °C for 60 mins in 100 ml deionised water. The leaf extract was obtained through a filtration process of the mixed solution and stored at 3 °C for the green synthesis [105].

3.3 Chemical synthesis of CuO NPs

For the chemical synthesis, the proposed method by Sabeena *et al.* was adopted with a few modifications. 0.1 M solution of the copper nitrate (Cu (NO₃)₂) was prepared in 100 ml deionized water, transferred into a 250 ml conical flask and was titrated with 0.1 M of the sodium hydroxide (NaOH) solution (dropwise) for 2 h. The solution changed colour from blue to black signifying

the formation of the copper oxide nanoparticles (CuO NPs). The solution was left overnight to precipitate and later filtered using the Whatman paper and dried at 80 °C overnight before being calcined for 4 h at 400 °C [106].

3.4 Green synthesis of CuO NPs

The green synthesis of the CuO NPs was prepared as follows, 10 g of the copper nitrate and 50 ml of the *L. nobilis* plant extract were combined, and the mixture underwent a 2 h stirring period at room temperature. The resultant paste was transferred into a ceramic crucible, dried overnight in the oven at 60 °C and then calcined for 4 h at 400 °C. CuO NPs that were produced were subsequently kept for later use and analysis in an airtight container [105].

3.5 Synthesis of the CuO_{ch}/fMWCNTs and CuO_{gr}/fMWCNTs nanocomposites

Using a glass tube containing *N, N*- dimethylformamide, 10 mg of the synthesized CuO NPs and 10 mg of the purchased fMWCNTs were combined. The mixture underwent a 24 h stirring period at room temperature and a 6 h sonication. This procedure was conducted for both the chemically and green synthesized CuO NPs to create the CuO_{ch}/fMWCNTs and CuO_{gr}/fMWCNTs nanocomposites. The paste of the nanocomposites was kept in storage for later use and analysis.

3.6 Modification of the glassy platinum electrode

A dry cast method was used to fabricate the glassy platinum electrode with the synthesized catalyst (fMWCNTs, CuO_{gr/ch} NPS and CuO_{gr/ch}/fMWCNTs). Prior to each electrode fabrication, the electrode was carefully and thoroughly washed.

3.7 Characterization of the nanomaterials and fabricated electrodes

The synthesized catalysts (fMWCNTs, CuO_{gr/ch} NPS and CuO_{gr/ch}/fMWCNTs) were characterised using the following spectroscopic and microscopic techniques, X-ray diffraction spectroscopy (XRD), Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM), electron dispersion spectroscopy (EDS/EDX), and scanning electron microscopy (SEM). For the fabrication of the platinum electrode, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), as well as square wave voltammetry (SWV) were deployed, whereby the CAP was prepared in 0.1 mM 60:40 PBS-ethanol buffer solution.

3.8 Electrocatalysis

The platinum electrode was used for the electrochemical analysis using the Autolab system. All of the electrochemical experiment was conducted at room temperature with 5 mM phosphate buffered saline being used as the supporting electrolyte at pH 7. The electrocatalytic experiments were conducted using 5 mM $\text{K}_4[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 60:40 PBS: EtOH solution containing 32.7 mM CAP with the scan rate set at 25 mVs^{-1} , with the utilization of the cyclic voltammetry at the potential window of -0.5 to 1.0 V. The selected parameters for the analysis were the anodic and cathodic peak currents and potentials (I_{pa} , I_{pc} & E_{pa} , E_{pc}), formal redox potential (E^0), and the peak separations (ΔE).

3.9 Electroanalysis

The electroanalysis was carried out using the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs modified electrodes using the SWV technique. The SWV technique's operational parameters were established as follows; frequency was set to 10 Hz, E_{amp} set at 0.01 V, E_{step} set at 25 mV, and the potential window was set from 0.2 to 0.8 V. The different concentrations of CAP were detected using the two electrodes with the concentrations ranging from 0.357 – 2.73 μM . The acquired data was utilized to create the calibration plots and compute the calculations for the sensitivity and the lowest detectable concentrations of the modified electrodes.

3.10 Real sample analysis

The analysis of the real samples was conducted on the sauce and rub (Mafikeng, South Africa). 2 mg each of the sauce were measured and added to 25 ml of ethanol. The mixture was stirred for 15 mins, and then filtered using Whatman No 1 filter paper to obtain the capsaicin extract solution. The experiment was conducted using SWV.

CHAPTER 4 RESULTS AND DISCUSSION

4. Results and discussion

This chapter makes provision of the discussion to all the spectroscopic, microscopic, and electrochemical techniques to grasp the behaviour of the nanomaterials ($\text{CuO}_{\text{ch/gr}}$, fMWCNTs and $\text{CuO}_{\text{ch/gr}}/\text{fMWCNTs}$), gives the summary of all the undertaken studies such as interference, concentration and real sample analysis to further grasp knowledge of the properties of the nanomaterials.

4.1 UV-vis characterization

UV-vis spectroscopy was carried out to analyse the synthesized nanomaterials, with the obtained spectra shown in Figure 4.1 for (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$, and (e) $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$. On (a) and (b) the UV spectra of CuO_{ch} and CuO_{gr} NPs exhibit their maximum absorption peaks at 374 and 380 nm respectively, and these peaks are attributed to the shift in surface plasmon resonance (SPR) which is a result of the absorption of metal oxide [107]. On (c) the UV spectrum of fMWCNTs exhibits its maximum absorption peak at 255 nm which is in agreement with the reported results by Yu *et al.* [108]. On (d) and (e) there is an observable change in the intensity of the absorbance of the fMWCNTs and the shift in the maximum absorption peaks (277 and 266 nm respectively) of the nanocomposites, confirming the incorporation of the $\text{CuO}_{\text{ch/gr}}$ NPs with the fMWCNTs. The linear part of the $(ah\nu)$ with respect to $(h\nu)$ was extrapolated to zero, which was used to obtain the direct energy bandgaps of the CuO_{ch} NPs, CuO_{gr} NPs, fMWCNTs, $\text{CuO}_{\text{ch}}/\text{fMWCNTs}$, and $\text{CuO}_{\text{gr}}/\text{fMWCNTs}$, and they were found to be 1.92, 1.82, 3.79, 2.56 and 2.47 eV, respectively. From the obtained results, it can be observed that the incorporation of the chemical and green CuO NPs had an effect on the fMWCNTs' bandgap as the bandgaps of the nanocomposites were lower than the one of the fMWCNTs alone.

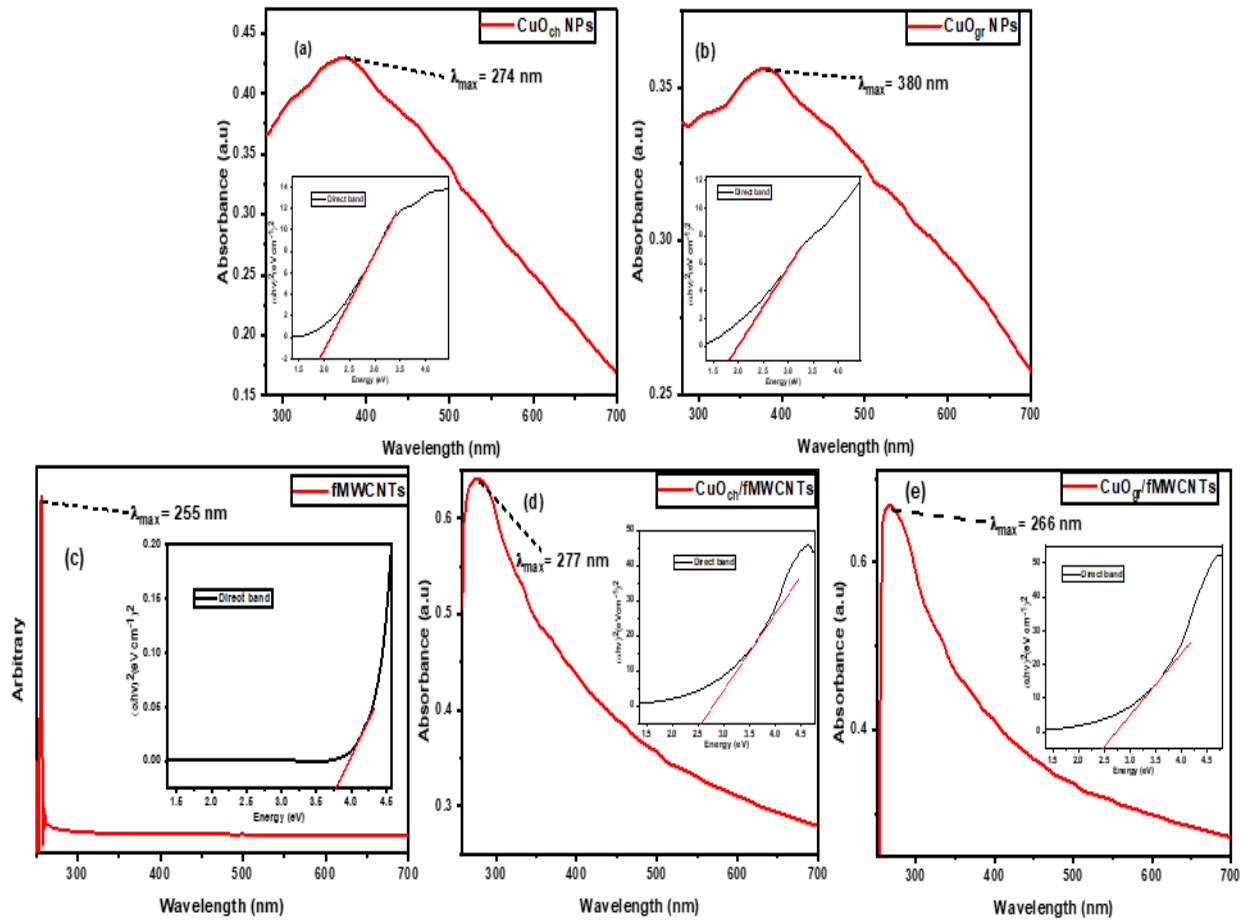


Figure 4.1: UV spectra of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs

4.2 FTIR characterization

The FTIR analyses were performed to identify the functional groups that are present in the synthesized nanomaterials. Figure 4.2 shows the obtained spectra of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs, and (e) CuO_{gr}/fMWCNTs at the spectral range of 4000 to 400 cm^{-1} . On (a) the CuO_{ch} NPs show a strong and broad peak at 3458 cm^{-1} which is attributed to the hydroxyl groups of water molecules absorbed on the surface of the CuO NPs, the peak at the 502 cm^{-1} corresponds to the Cu-O stretching vibration in the monoclinic phase of CuO NPs [109]. On (b) CuO_{gr} NPs the peak at 510 cm^{-1} corresponds with the Cu-O stretch vibrations of the monoclinic phase of CuO NPs. On the region of 1800 – 1000 cm^{-1} there are sharp peaks observed for both (a) and (b). The absorption peaks at 1630 -1609 cm^{-1} are attributed to the aromatic bending of the alkene group C=C, while the sharp peaks at 1372 cm^{-1} are attributed to the deformation vibration of the C-H band of alkene group. The absorption peaks at 1060 – 800

cm^{-1} are attributed to the C-O stretching group of primary and secondary alcohols [110]. On (c) the fMWCNTs spectrum shows strong and broad peaks in the $3000 - 1250 \text{ cm}^{-1}$ region. The absorption bands at $1750 - 1500 \text{ cm}^{-1}$ range are attributed to the C=O groups in different environments (carboxylic acid, ketone) [111]. The absorption peaks at $2400 - 2000 \text{ cm}^{-1}$ are credited to the C=N vibrations on the nitrogen doped carbon nanotubes (N-CNTs) [112]. The peak at $2750 - 2600 \text{ cm}^{-1}$ is credited to the asymmetric stretching vibrations of the C-H [113]. On (d) the CuO_{ch} /fMWCNTs spectrum shows a small absorption peak of $500-450 \text{ cm}^{-1}$ which is credited to the Cu-O stretching vibration, and the broad and intense peaks at the $3000 - 1250 \text{ cm}^{-1}$ are credited to the fMWCNTs. On (e) the CuO_{gr} /fMWCNTs spectrum shows a sharp absorption peak at 483 cm^{-1} which is credited to the Cu-O stretching of the CuO NPs, and the broad and strong peaks at $3700 - 1250 \text{ cm}^{-1}$ are credited to the fMWCNTs. The FTIR spectra for (d) and (e) proved that both the CuO NPs and the fMWCNTs were present in their respective nanocomposites.

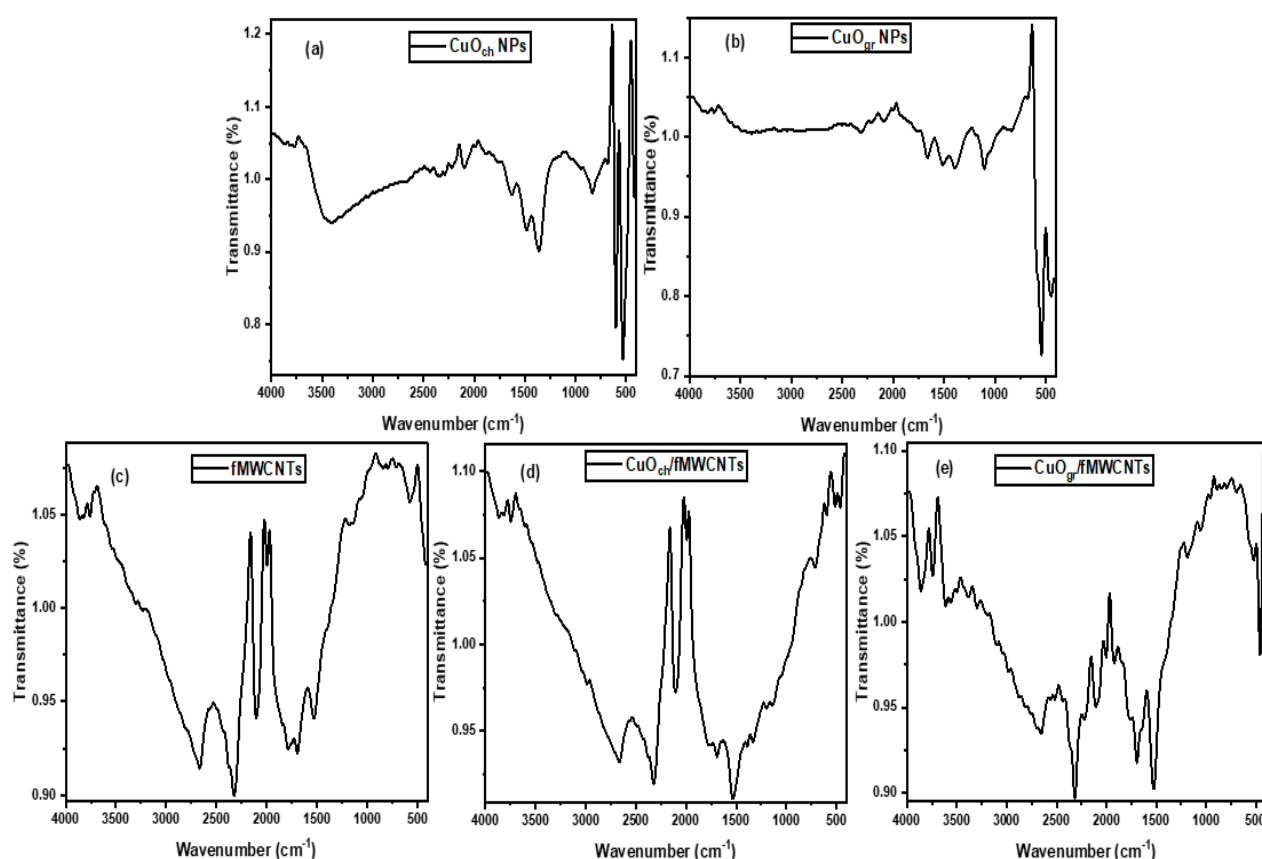


Figure 4.2: FTIR spectra of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch} /fMWCNTs and (e) CuO_{gr} /fMWCNTs

4.3 XRD characterization

To investigate the crystal structures and crystallinity of the nanomaterials, XRD analysis was deployed. Figure 4.3 shows the obtained XRD spectra of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs. Figure 4.3 (a) and (b) showed sharp peaks, thus indicating the high degree of purity and crystallinity of the synthesized CuO NPs [114]. For (a) and (b) the spectra showed identical diffraction peaks, with the differences in the intensities, at $2\theta = 32.8^\circ$ (100), 35.8° (002) (-111), 39.1° (200) (111), 48.7° (020), 53.5° (202), 58.6° (113), 61.9° (022), 66.5° (311), 68.4° (220), 72.7° (113), and 75.4° (400) miller indices of the monoclinic CuO lattice planes, respectively (JCPDS card no. 80-1268) [49]. The spectrum (c) is the diffraction pattern of the fMWCNTs, with the main characteristic peak at $2\theta = 25.92^\circ$ (002), the peak is broad indicating the amorphous nature of the fMWCNTs [115]. The spectra (d) and (e) show the unique diffraction peaks for the CuO_{ch}/fMWCNTs and CuO_{gr}/fMWCNTs nanocomposites, with the diffraction peaks at $2\theta = 32.8^\circ$ (100), 35.8° (002) (-111), 39.1° (200) (111), 48.7° (020), 53.5° (202), 58.6° (113), 61.9° (022), 66.5° (311), 68.4° (220), 72.7° (113), and 75.4° (400) indicating the presences of CuO NPs in the nanocomposite, the diffraction peak at $2\theta = 25.92^\circ$ (002) suggested the presence of the fMWCNTs. With the utilization of the Debye-Scherrer equation 1, the average crystalline sizes of the nanomaterials were calculated to be 14.98, 16.51, 4.79, 13.96 and 15.21 nm for the CuO_{ch} NPs, CuO_{gr} NPs, fMWCNTs, CuO_{ch}/fMWCNTs and CuO_{gr}/fMWCNTs respectively [97]. Given reasons that a single particle is composed of more than one crystalline, there was a difference in particle sizes obtained between the XRD and TEM analysis [116].

$$D = \frac{0.94\lambda}{\beta \cos\theta} \quad (1),$$

Where D is the average crystalline size, λ is the X-ray wavelength of $1.54 \text{ \AA}$, θ is the Bragg diffraction angle and β is the full wavelength half maximum (FWHM) of each peak.

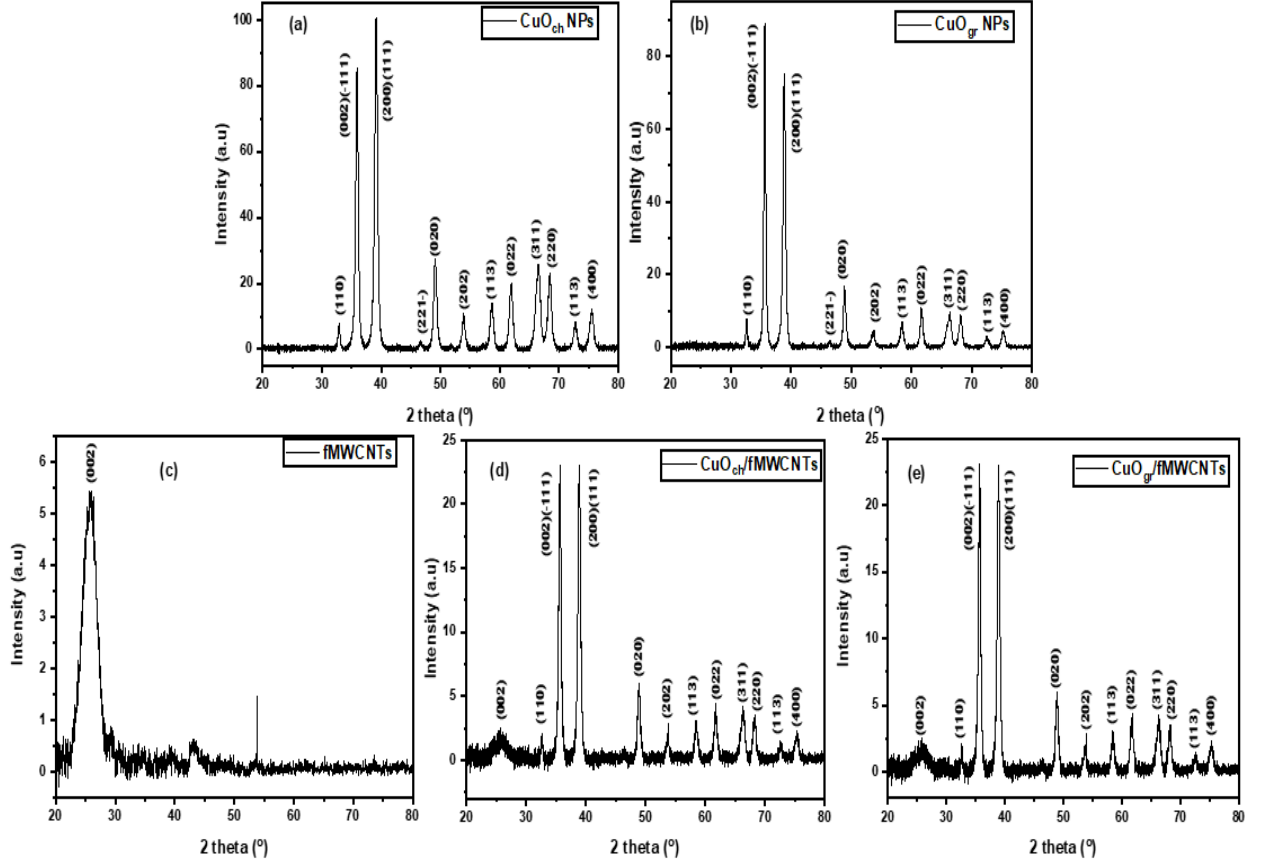


Figure 4.3: XRD spectra of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs

4.4 SEM characterization

The analysis of the surface morphology of the (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs was carried out by the utilization of scanning electron microscopy and is shown in Figure 4.4. On (a) and (b) the nanoparticles have a spherical structure and are agglomerated, which was due to the high surface energy, surface area, and surface reactivity [117]. On (c) the fMWCNTs image shows entangled, agglomerated tubular structures. On (d) and (e) the nanocomposite images show the spherical structures of the CuO NPs being embedded in the agglomerated and entangled tubular structures of the fMWCNTs.

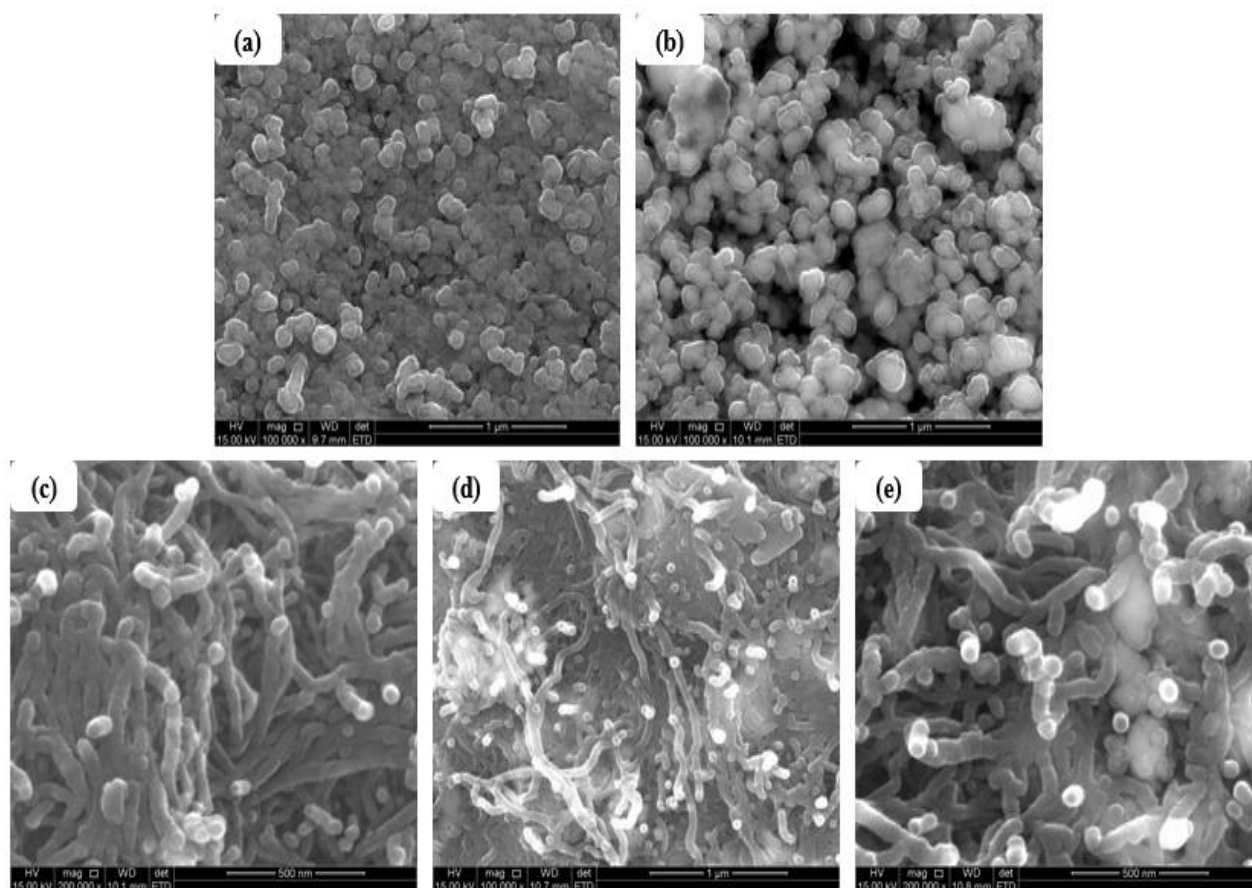


Figure 4.4: SEM images of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs

4.5 EDX characterization

In order to identify the elemental distribution in the nanomaterials, the EDX analysis was performed. Figure 4.6 shows the EDX images as well as the elemental compositions of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs. Image (a) and (b) both show high percentage weights (50 and 56 %) for the copper element, with 21 % of oxygen which confirms the successful formation of the CuO NPs, while the 27 and 22 % for the carbon element can be attributed to the contamination in the synthesis process of the nanoparticles. Image (c) shows the fMWCNTs elemental composition, there is an observation of high levels of carbon, and this is due to the fMWCNTs being mostly composed of carbon atoms, and the low levels of oxygen may be due to the functionalization of the MWCNTs with oxygen. From both images (d) and (e) there is an observable decrease in the copper composition weights, and an

increase in the carbon composition weights and this might be due to the prominence of the carbon in the fMWCNTs plus the carbon contaminants in the CuO NPs.

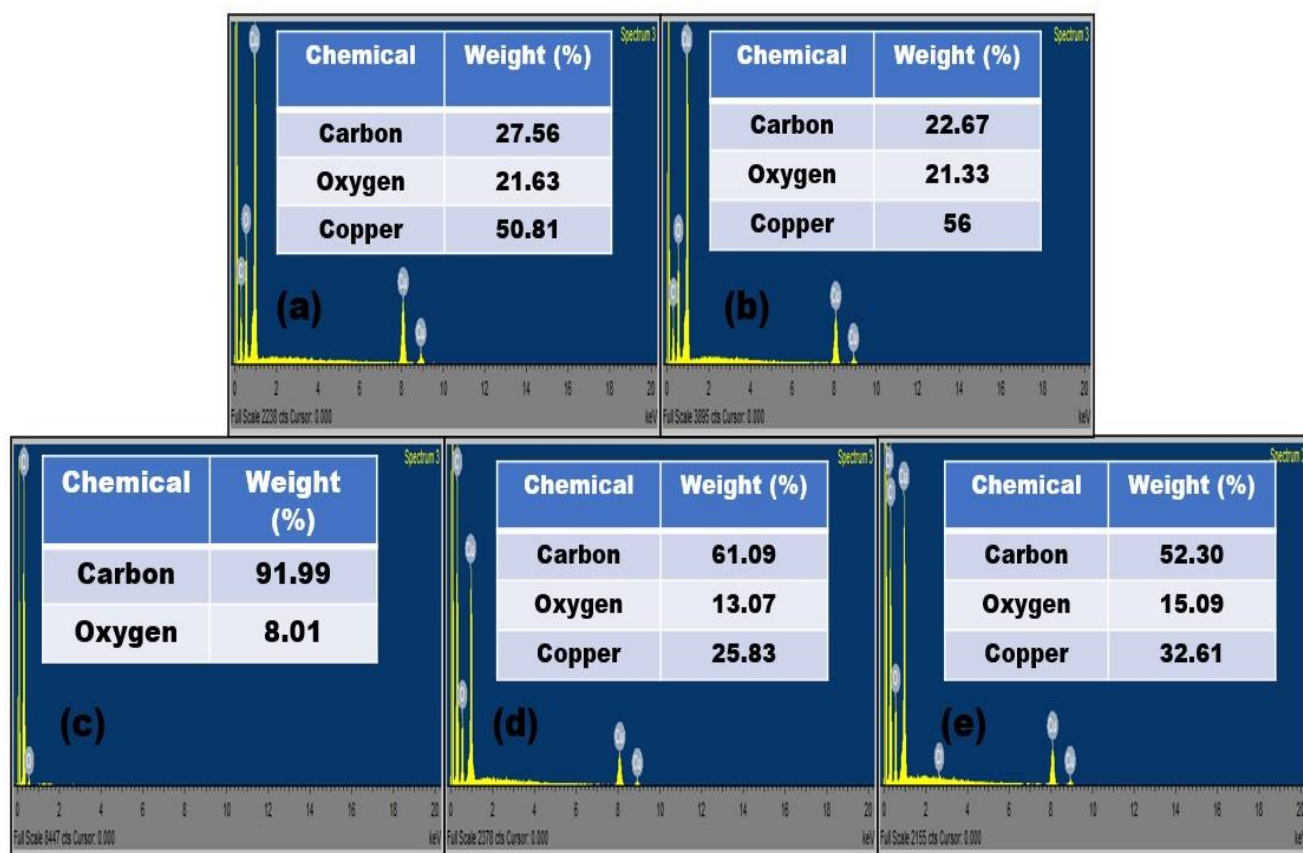


Figure 4.5: EDS characterization of (a) CuO_{ch} NPs, (b) CuO_{gr} NPs, (c) fMWCNTs, (d) CuO_{ch}/fMWCNTs and (e) CuO_{gr}/fMWCNTs

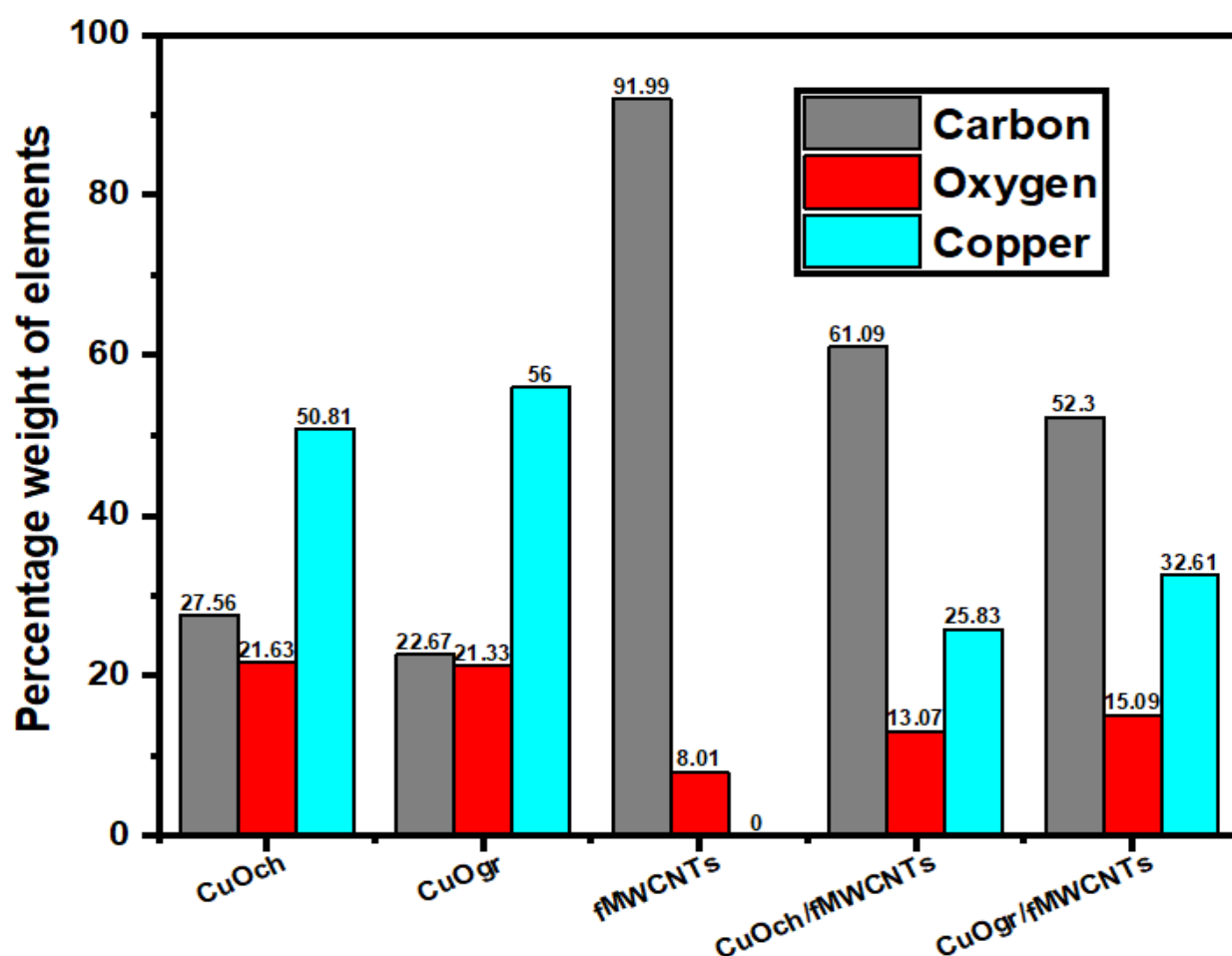


Figure 4.6: Elements present in nanomaterials

4.6 TEM characterization

The shape and size of the nanomaterials were determined by the utilization of TEM. Figures 4.7 (a) and (c) show the TEM images of CuO_{ch} NPs and CuO_{gr} NPs respectively and their corresponding histograms are shown in Figures 4.7 (b) and (d) for CuO_{ch} NPs and CuO_{gr} NPs respectively. The CuO_{ch} NPs exhibited a mixture of spherical and ellipsoid shapes, with the mean particle size of 21.11 nm which was close to the 14.98 nm size calculated from the XRD. The CuO_{gr} NPs exhibited spherical shapes, with the mean particle size of 20.43 nm which was also close to the 16.51 nm size calculated from the XRD using the Debye-Scherrer equation 1. The obtained results were similar to those reported previously by Sharmila et al [118].

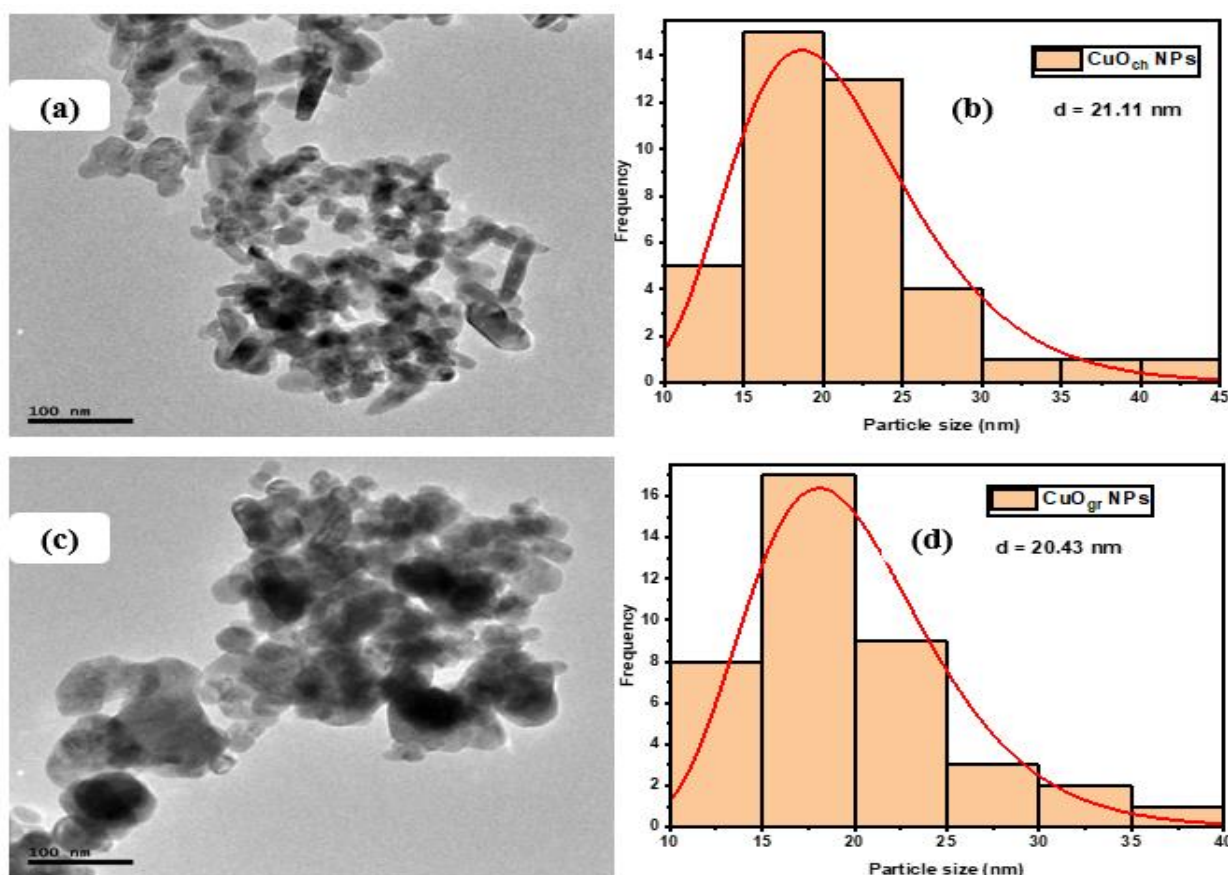


Figure 4.7: TEM images of (a) CuO_{ch} NPs, and (c) CuO_{gr} NPs, and their corresponding histograms (b) and (d)

4.7 Electrochemical characterization in 5 mM K [Fe (CN)₆]^{3-/4-} in 0.1 PBS buffer.

4.7.1 Nanocomposite optimization studies using cyclic voltammetry (CV)

Cyclic voltammetry techniques were used to measure the electrochemical properties of the electrodes. The CV measurements were obtained using 5 mM K [Fe (CN)₆]^{3-/4-} probe prepared in pH 7.4, 0.1M phosphate buffer solution PBS at 25 mV/s scan rate and potential window of -0.5 to 1 V. The optimization CV experiments involved three different ratios being analysed, where the mass of the fMWCNTs was kept constant and only the mass of the CuO NPs was changed, as depicted in Figure 4.8. Figure 4.8 (a) is the representation of the various ratios for the Pt-CuO_{ch}/fMWCNTs modified platinum electrodes, the 2:1 ratio electrode showed the best electron transfer and highest current response of 366.72 μ A, followed by the 1:1 with the current response of 93.77 μ A, and the 3:1 ratio being the lowest with the current response of 40.44 μ A, as depicted on Figure 4.8 (b) of current vs nanocomposite ratios. Figure 4.8 (c) shows the representation of

the various ratios for the Pt-CuO_{gr}/fMWCNTs modified platinum electrodes, where the ratio of 1:1 had the highest current response of 155.62 μ A, followed by the 1:3 ratio with the current response of 140.65 μ A, and the 2:1 being the lowest with the current response of 7.88 μ A, as depicted in Figure 4.8 (d) of the current vs nanocomposite ratios.

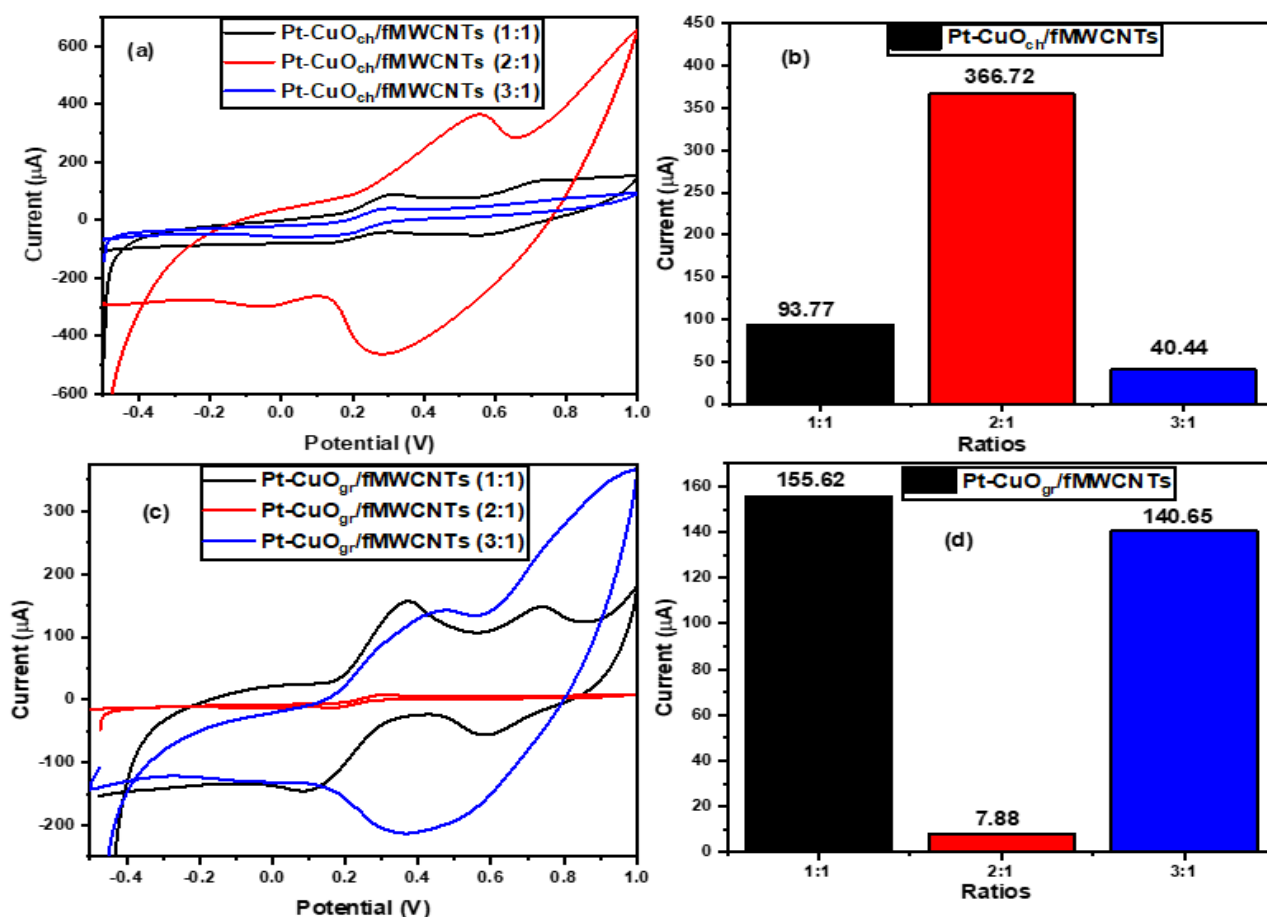


Figure 4.8: CV spectra for the ratio optimization of (a) Pt-CuO_{ch}/fMWCNTs and (c) Pt-CuO_{gr}/fMWCNTs, and their current vs ratio plots (b) and (d)

4.7.2 Electrochemical comparative studies of the unmodified and modified platinum electrodes with the utilization of cyclic voltammetry (CV)

The CV in 5 mM K [Fe (CN)₆]^{3-/4-} in 0.1 PBS at scan rate of 25 mV/s in a pH of 7.4 with the potential window of -0.5 to 1 V, was utilized to investigate the comparative current response and charge transfer of Pt-Bare, Pt-CuO_{ch} NPs, Pt-CuO_{gr} NPs, Pt-fMWCNTs, Pt-CuO_{ch}/fMWCNTs, and Pt-CuO_{gr}/fMWCNTs as depicted in Figure 4.9 (a). Figure 4.9 (b) represents the peak current response of all the modified platinum electrodes. The I_{pa}/I_{pc} values of the reaction processes for

the Pt-Bare, Pt-CuO_{ch}, and Pt-CuO_{gr} electrodes suggest that they are quasi-reversible as they are close to 1, while those of Pt-fMWCNTs, Pt-CuO_{ch}/fMWCNTs, and Pt-CuO_{gr}/fMWCNTs are reversible as their values are 1. The anodic peak current response decreased in the following trend Pt-CuO_{gr}/fMWCNTs ($I_{pa} = 156.86 \mu\text{A}$; $E_{pa} = 0.38$) > Pt-fMWCNTs ($I_{pa} = 123.32 \mu\text{A}$; $E_{pa} = 0.31$) > Pt-CuO_{ch}/fMWCNTs ($I_{pa} = 89.01 \mu\text{A}$; $E_{pa} = 0.34$) > Pt-Bare ($I_{pa} = 15.77 \mu\text{A}$; $E_{pa} = 0.27$) > Pt-CuO_{gr} NPs ($I_{pa} = 14.31 \mu\text{A}$; $E_{pa} = 0.26$) > Pt-CuO_{ch} NPs ($I_{pa} = 11.92 \mu\text{A}$; $E_{pa} = 0.25$). The high current response on the nanocomposites electrode as compared to the bare and nanoparticles electrodes is ascribed to the electrocatalytic property of the fMWCNTs. The ΔE_p values for all the electrodes were larger than 0.059 V for a fast one electron transport, suggesting that the electrocatalytic activity was enhanced on the bare and modified electrodes with the nanocomposites and the fMWCNTs electrodes having a rapid electron transport process as a result of the heightened surface-to-volume ratio. With the use of the Randles-Sevcik equation 2, the electroactive surface area for each of the electrodes was computed [119].

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

Where i_p is peak current (A), n is electron transfer, A is electroactive surface area (cm^2), D is diffusion coefficient (cm^2/s), C is the concentration of $\text{K}_3\text{Fe}(\text{CN})_6$ (mol/cm^3), and v is scan rate (V/s). The EASA values were found to be $0.643 < 0.772 < 0.851 < 4.801 < 6.651 < 8.460 \text{ cm}^2$ for Pt-CuO_{ch} NPs, Pt-CuO_{gr} NPs, Pt-bare, Pt-CuO_{ch}/fMWCNTs, Pt-fMWCNTs and Pt-CuO_{gr}/fMWCNTs electrodes, respectively. The Pt-CuO_{gr}/fMWCNTs EASA value was 9.94, 13.16, and 10.96 times larger than the Pt-bare, Pt-CuO_{ch} NPs, and Pt-CuO_{gr} NPs, respectively. The summarized parameters from the comparative studies are shown in Table 4.1.

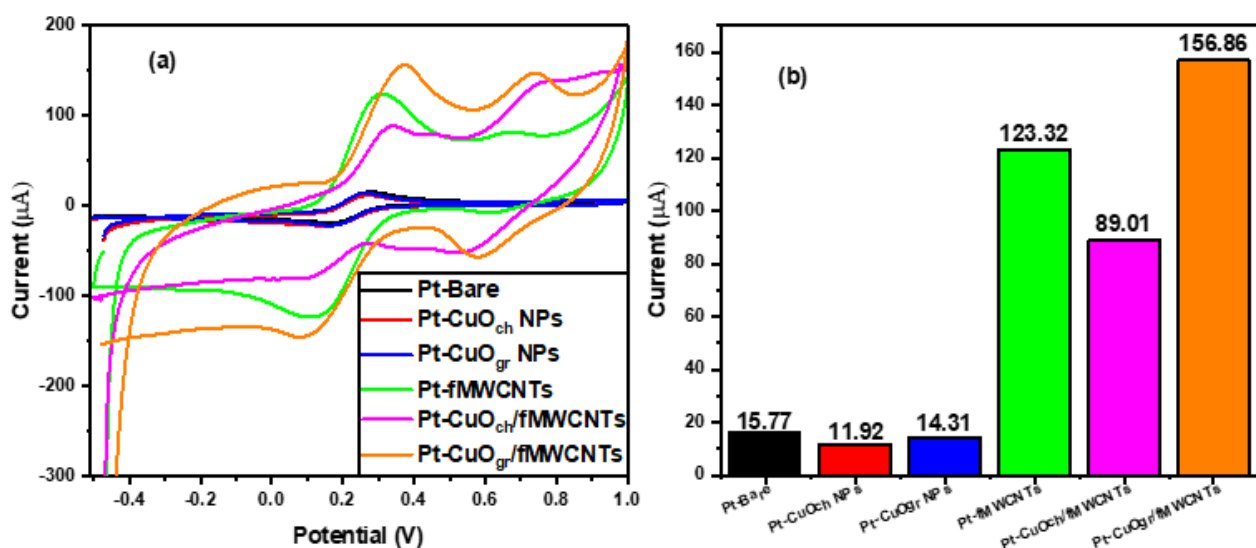


Figure 4.9: CV spectra of the comparative studies of the fabricated platinum electrodes

Table 4.1: Summarized parameters of the comparative studies in 5 mM K [Fe (CN)₆]^{3-/4-} in 0.1 PBS.

Electrodes	I _{pa} (μA)	I _{pc} (μA)	I _{pa} /I _{pc}	E _{pa} (V)	E _{pc} (V)	E ^{1/2} (V)	ΔE (V)
Pt-Bare	15.77	-18.53	-0.85	0.27	0.17	0.22	0.10
Pt-CuO _{ch} NPs	11.92	-21.89	-0.52	0.25	0.15	0.20	0.10
Pt-CuO _{gr} NPs	14.31	-21.31	-0.67	0.26	0.16	0.21	0.10
Pt-fMWCNTs	123.32	-123.34	-1.00	0.31	0.11	0.21	0.20
Pt-CuO _{ch} /fMWCNTs	89.01	-79.76	-1.12	0.34	0.09	0.22	0.25
Pt-CuO _{gr} /fMWCNTs	156.86	-145.84	-1.08	0.38	0.08	0.23	0.30

4.7.3 The effect of scan rate variations on the nanocomposite modified platinum electrodes using cyclic voltammetry (CV)

4.7.3.1 Scan rate effect at the Pt-CuO_{ch}/fMWCNTs in 5 mM K [Fe (CN)₆]^{3-/4-}.

The effect of the scan rate ranging from 25 – 350 mVs⁻¹ on the anodic and cathodic peak current response of the Pt-CuO_{ch}/fMWCNTs modified electrode towards 5 mM K [Fe (CN)₆]^{3-/4-} prepared

in 0.1 M PBS buffer at pH 7.4 were conducted with the utilization of CV. From the obtained scan rate voltammograms, the linear plots correlating to the square root of scan rate ($V^{1/2}$) vs anodic (I_{pa}) and cathodic (I_{pc}) current were obtained as depicted in Figure 4.10. With the linear regression equations 3 and 4 of

$$I_{pa} = 21.5643 v^{1/2} - 24.96 (R^2 = 0.9988) \quad (3),$$

and

$$I_{pc} = -21.9994 v^{1/2} + 25.10 (R^2 = 0.9994) \quad (4)$$

Laviron's equations 5 a,b were utilized to investigate the number of electrons transferred and charge coefficient, where the number of electrons transferred (n) was found to be 0.5151, while the charge transfer coefficient (α) was 0.535.

$$E_{pa} = \frac{E^0 + 2.303RT}{(1-\alpha)nF \log v} \quad (5a),$$

$$E_{pc} = \frac{E^0 - 2.303RT}{\alpha nF \log v} \quad (5b).$$

Where R is the gas constant ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), T is the temperature (298 K), and F is the faraday constant ($96.500 \text{ C mol}^{-1}$), E^0 is the formal potential, n is the number of electrons transferred, α is the electron transfer coefficient.

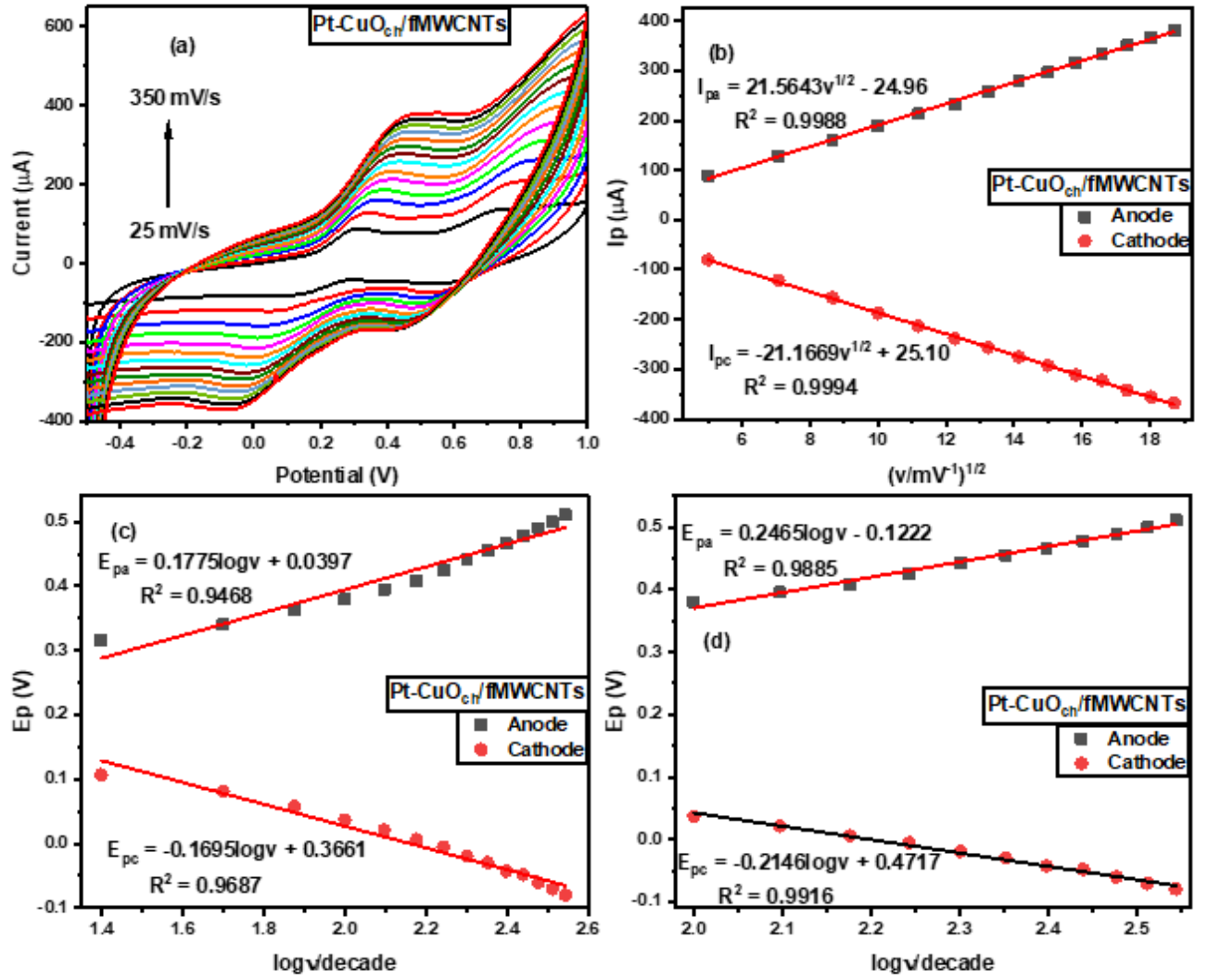


Figure 4.10: CV spectra for the effect of scan rate at Pt-CuO_{ch}/fMWCNTs electrode and its regression plots

From the measurements on the CV Figure 4.10a, the linear plot of the anodic and cathodic peak potentials (E_{pa} & E_{pc}) vs the log of scan rate Figure 4.10d was constructed and the Tafel value (b) of the Pt-CuO_{ch}/fMWCNTs was computed to be 0.355 mVdec⁻¹ using the equation 6,

$$E_p = \left(\frac{b}{2}\right) \log v + \text{constant} \quad (6).$$

The obtained Tafel value was high when compared to the standard value (0.118 mVdec⁻¹), suggesting that an adsorption-controlled process takes place on the electrode surface by the reactants.

4.7.3.2 Scan rate effect at the Pt-CuO_{gr}/fMWCNTs in 5 mM K [Fe (CN)₆]^{3-/4-}.

The effect of the scan rate ranging from 25 – 350 mVs⁻¹ on the anodic and cathodic peak current response of the Pt-CuO_{gr}/fMWCNTs modified electrode towards 5 mM K [Fe (CN)₆]^{3-/4-} prepared in 0.1 M PBS buffer at pH 7.4 were conducted with the utilization of CV. From the obtained scan rate voltammograms, the linear plots correlating to the square root of scan rate (V^{1/2}) vs anodic (I_{pa}) and cathodic (I_{pc}) current were obtained as depicted in Figure 4.11. With the linear regression equations 7 and 8 of

$$I_{pa} = 53.449 v^{1/2} - 143.07 (R^2 = 0.9937) \quad (7),$$

and

$$I_{pc} = -40.962 v^{1/2} + 72.20 (R^2 = 0.9990) \quad (8).$$

Laviron's equations 5 a,b were utilized to investigate the number of electrons transferred and charge coefficient, where the number of electrons transferred (n) was found to be 0.330, while the charge transfer coefficient (a) was 0.555

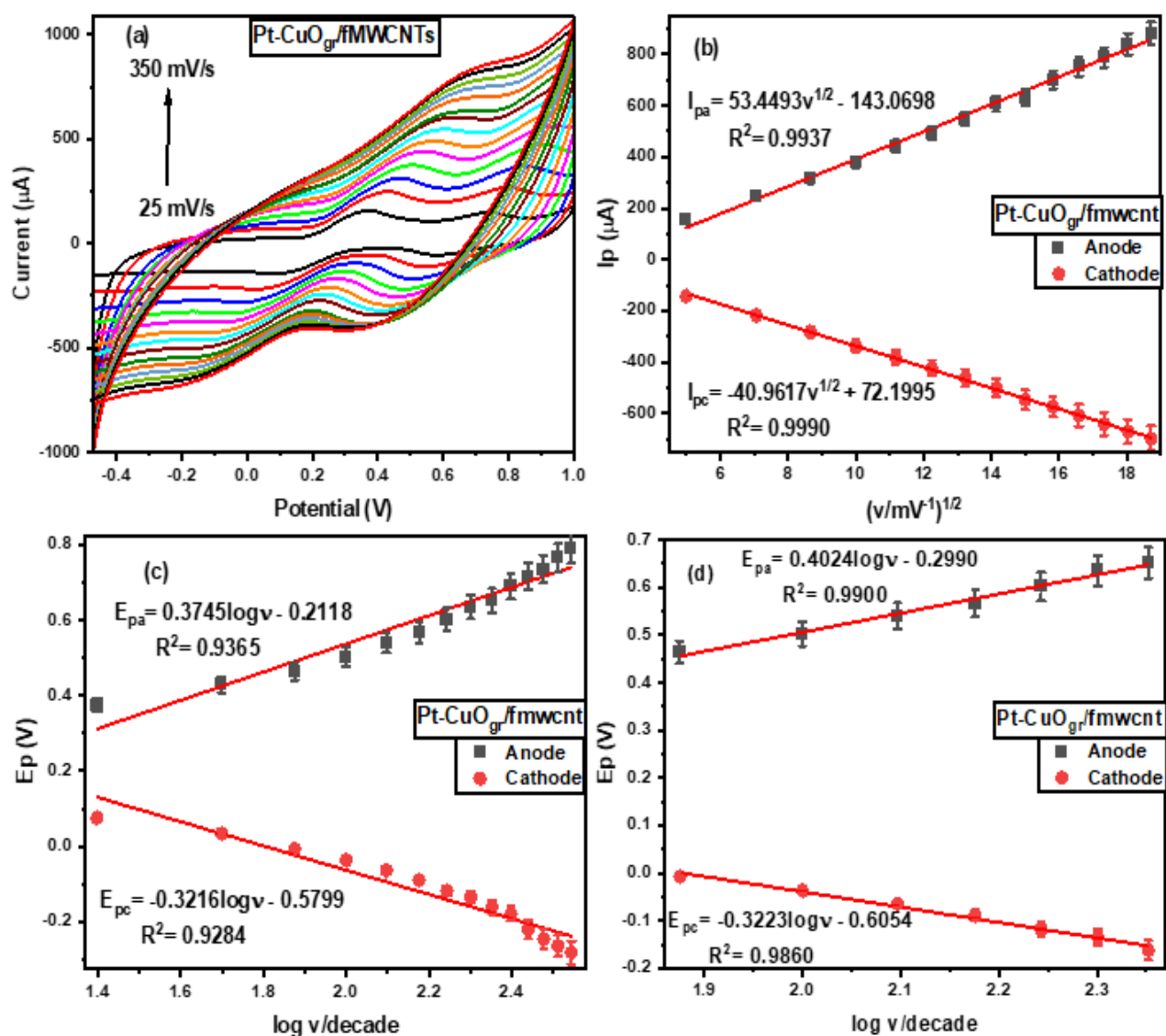


Figure 4.11: CV spectra for the effect of scan rate at Pt-CuO_{gr}/fMWCNTs electrode and its regression plots

Equation 6 was used to calculate the Tafel value of the Pt-CuO_{gr}/fMWCNTs electrode and was found to be 0.805 mVdec⁻¹. This value is high when compared to the standard value, suggesting that an adsorption-controlled process takes place on the electrode surface by the reactants.

4.8 Electrocatalysis OF CAP.

4.8.1 pH studies on the modified nanocomposites electrodes utilizing cyclic voltammetry in CAP probe.

The pH studies were conducted to find the pH at which there is a better anodic current response towards capsaicin. The studies were conducted with the utilization of CV at 25 mVs^{-1} in 60:40 PBS: EtOH solution containing 32.7 mM CAP at various pH (3, 5, and 7). The buffer was chosen with relation to one of the reported studies on capsaicin detection, with a slight modification [120]. Figure 4.12 demonstrates how various pH buffer solutions affect the detection of capsaicin. For both proposed sensors there was a higher current response at the pH 3, hence this pH was used for the rest of the studies.

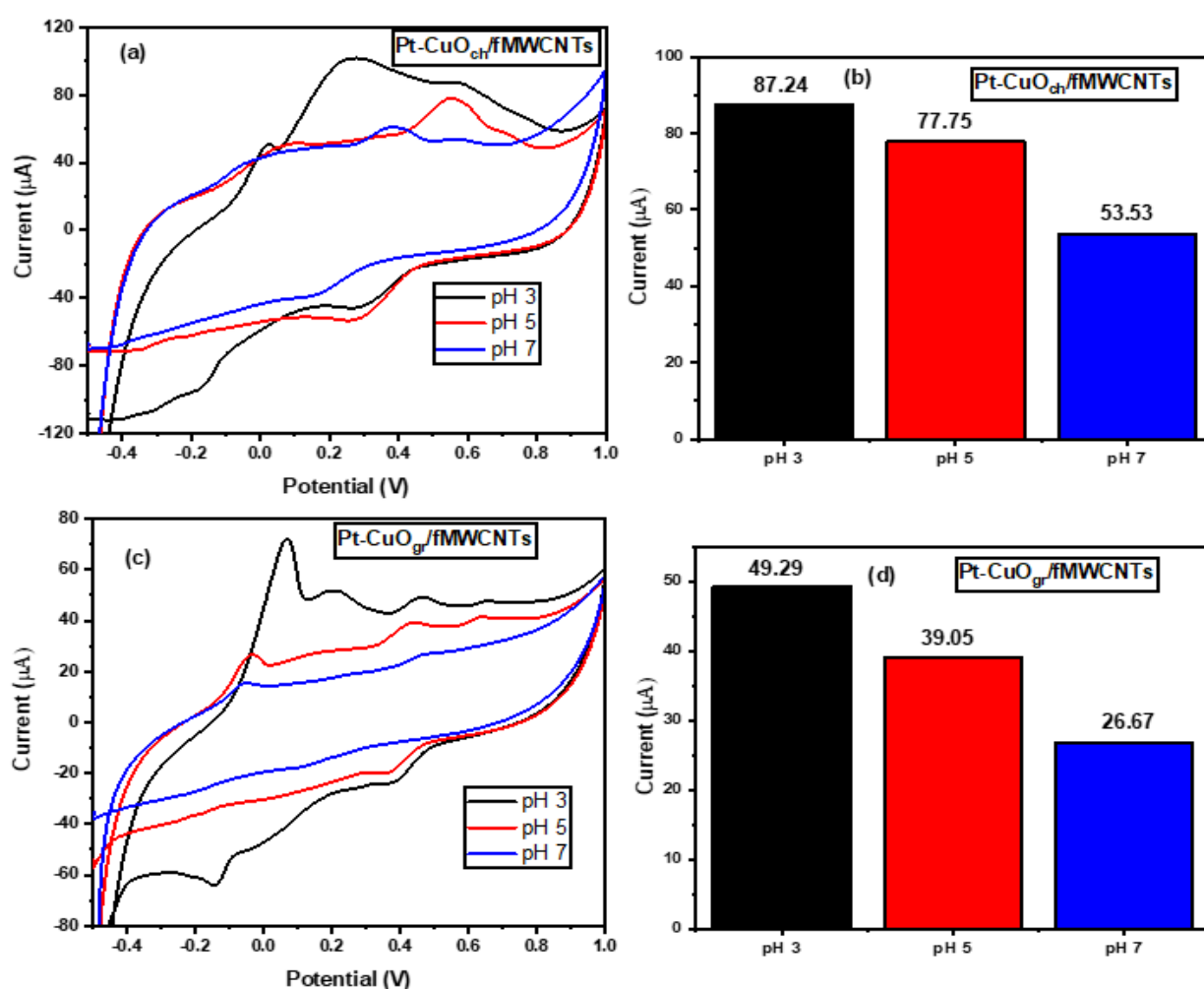


Figure 4.12: CV spectra for the pH studies on the (a) Pt-CuO_{ch}/fMWCNTs and (c) Pt-CuO_{gr}/fMWCNTs electrodes with their current vs pH bar graphs (b) and (d)

4.8.2 Electrochemical comparative studies on the unmodified and modified platinum electrodes utilizing cyclic voltammetry in CAP probe.

Comparative studies were conducted with the utilization of CV at scan rate of 25 mVs^{-1} to understand the electrochemical behaviour of capsaicin at different modified and unmodified electrodes. The studies were conducted in 60:40 PBS: EtOH solution containing 32.7 mM CAP. Figure 4.13 shows the obtained comparative cyclic voltammogram, it was observed that the I_{pa} and E_{pa} increase in the following trend: Pt-CuO_{ch} NPs ($I_{pa} = 5.38 \text{ } \mu\text{A}$; $E_{pa} = 0.41 \text{ V}$), Pt-Bare ($I_{pa} = 5.95 \text{ } \mu\text{A}$; $E_{pa} = 0.42 \text{ V}$), Pt-CuO_{gr} NPs ($I_{pa} = 7.11 \text{ } \mu\text{A}$; $E_{pa} = 0.45 \text{ V}$), Pt-fMWCNTs ($I_{pa} = 58.74 \text{ } \mu\text{A}$; $E_{pa} = 0.47 \text{ V}$), Pt-CuO_{ch}/fMWCNTs ($I_{pa} = 70.31 \text{ } \mu\text{A}$; $E_{pa} = 0.48 \text{ V}$), and Pt-CuO_{gr}/fMWCNTs ($I_{pa} = 104.73 \text{ } \mu\text{A}$; $E_{pa} = 0.50 \text{ V}$). The anodic peak current response regarding the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs electrodes were 12 and 18 times higher than that of the Pt-bare electrode respectively, indicating successful modification and enhanced electrocatalytic processes on both nanocomposite electrodes and this enhancement can be ascribed to the presence of the fMWCNTs. From Table 4.2, the I_{pa}/I_{pc} ratios indicate that there is a reversible reaction in all the electrodes. The ΔE_p value for all the electrodes increased in the following manner $0.09 < 0.1 < 0.11 < 0.15 \text{ V}$ for Pt-Bare, Pt-fMWCNTs, Pt-CuO NPs, and Pt-CuO/fMWCNTs, respectively which were greater than the 0.059 V for a fast one electron transport processes.

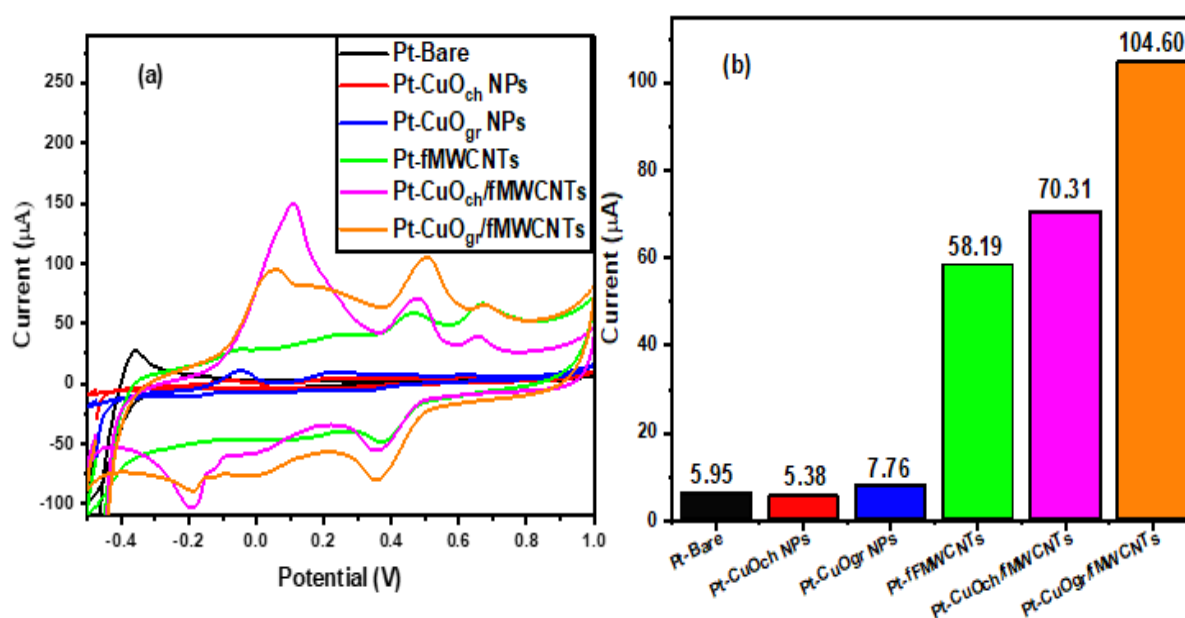


Figure 4.13: CV spectra of the comparative studies of the fabricated platinum electrodes

Table 4.2: Summarized parameters of the comparative studies in 32.7 mM CAP probe. Table 5.2: Capsaicin recovery from the Nando's sauces and oestoeze rub using Pt-CuO_{ch}/fMWCNTs electrode

Electrodes	I _{pa} (μA)	I _{pc} (μA)	I _{pa} /I _{pc}	E _{pa} (V)	E _{pc} (V)	E ^{1/2} (V)	ΔE (V)
Pt-Bare	5.95	-2.19	-2.72	0.42	0.33	0.38	0.09
Pt-CuO_{ch} NPs	5.38	-3.58	-1.50	0.41	0.33	0.37	0.08
Pt-CuO_{gr} NPs	7.76	-5.33	-1.46	0.45	0.34	0.40	0.11
Pt-fMWCNTs	58.19	-49.83	-1.17	0.47	0.37	0.42	0.1
Pt-CuO_{ch}/fMWCNTs	70.31	-57.14	-1.23	0.48	0.36	0.42	0.12
Pt-CuO_{gr}/fMWCNTs	104.60	-80.23	-1.30	0.50	0.35	0.43	0.15

4.8.3 Electrochemical impedance spectroscopy on the unmodified and modified platinum in CAP probe.

To further examine the electron behaviour of the unmodified and modified electrodes, electrochemical impedance spectroscopy was utilized in 60:40 PBS: EtOH solution containing 32.7 mM CAP in pH 3, the parameters were set as follows; potential was set a 0.35 V, the frequency range was 10 and 100 Hz. Figure 4.14 shows the representative of the Nyquist plot of the imaginary part of the impedance (-Z'') against the real part of the impedance (Z') for all of the electrodes. From the Nyquist plot, a decrease in charge transfer resistance reflected by the semi-circle and arc is observed in this order Pt-CuO_{ch} NPs > Pt-Bare > Pt-CuO_{gr} NPs > Pt-CuO_{gr}/fMWCNTs > Pt-CuO_{ch}/fMWCNTs > Pt-fMWCNTs.

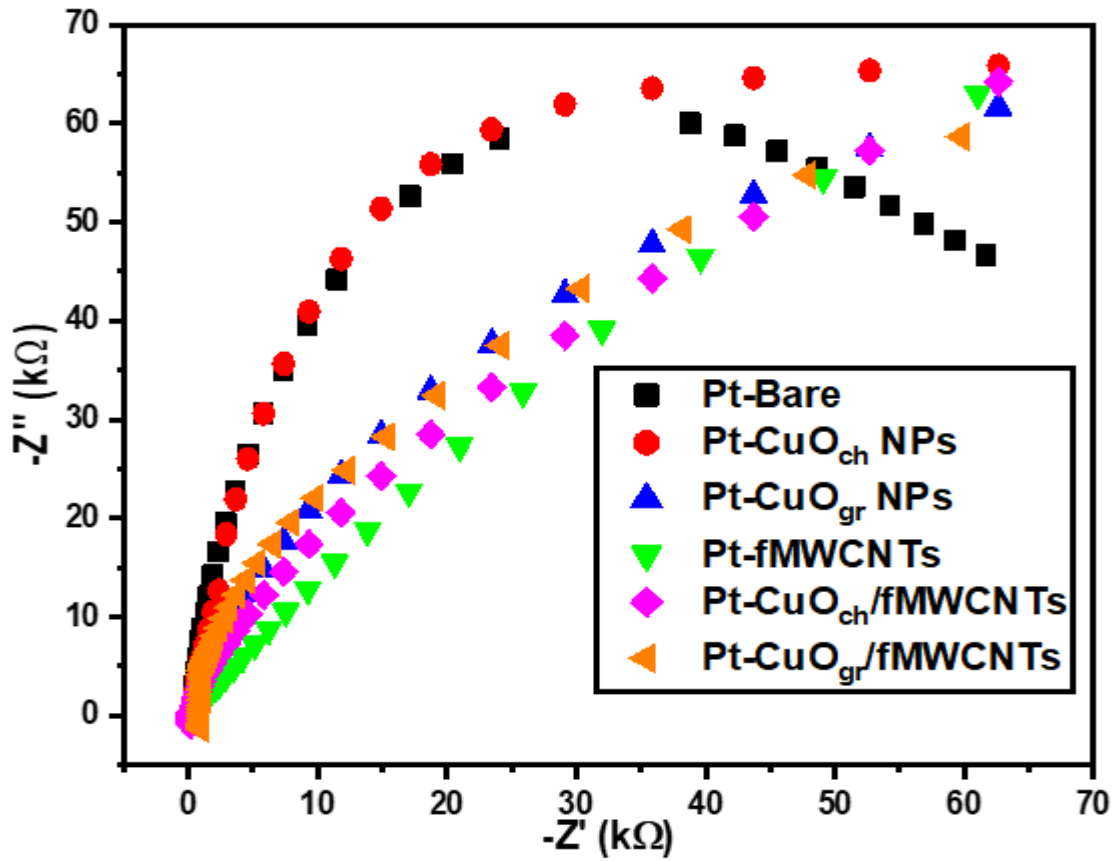


Figure 4.14: Nyquist plot of the fabricated platinum electrodes

4.8.4 The effect of scan rate variations on the nanocomposite modified platinum electrodes using cyclic voltammetry (CV)

4.8.4.1 Scan rate effect at the Pt-CuO_{ch}/fMWCNTs on CAP probe.

The effect of the scan rate ranging from 15 – 100 mVs⁻¹ on the anodic and cathodic peak current response of the Pt-CuO_{ch}/fMWCNTs modified electrode towards 60:40 PBS: EtOH solution containing 32.7 mM CAP at pH 3 were conducted with the utilization of CV. There was an increase in the anodic peak current as the scan rate increases, which indicated that there was adsorption-controlled process on the electrode's surface [121]. From the obtained scan rate voltammograms, the linear plots correlating to the square root of scan rate ($V^{1/2}$) vs anodic (I_{pa}) and cathodic (I_{pc}) current were obtained as depicted in Figure 4.15, with the linear regression equations 9 and 10,

$$I_{pa} = 34.097 v^{1/2} - 104.84 (R^2 = 0.997) \quad (9),$$

and,

$$I_{pc} = -25.741 v^{1/2} + 76.01 (R^2 = 0.997) \quad (10).$$

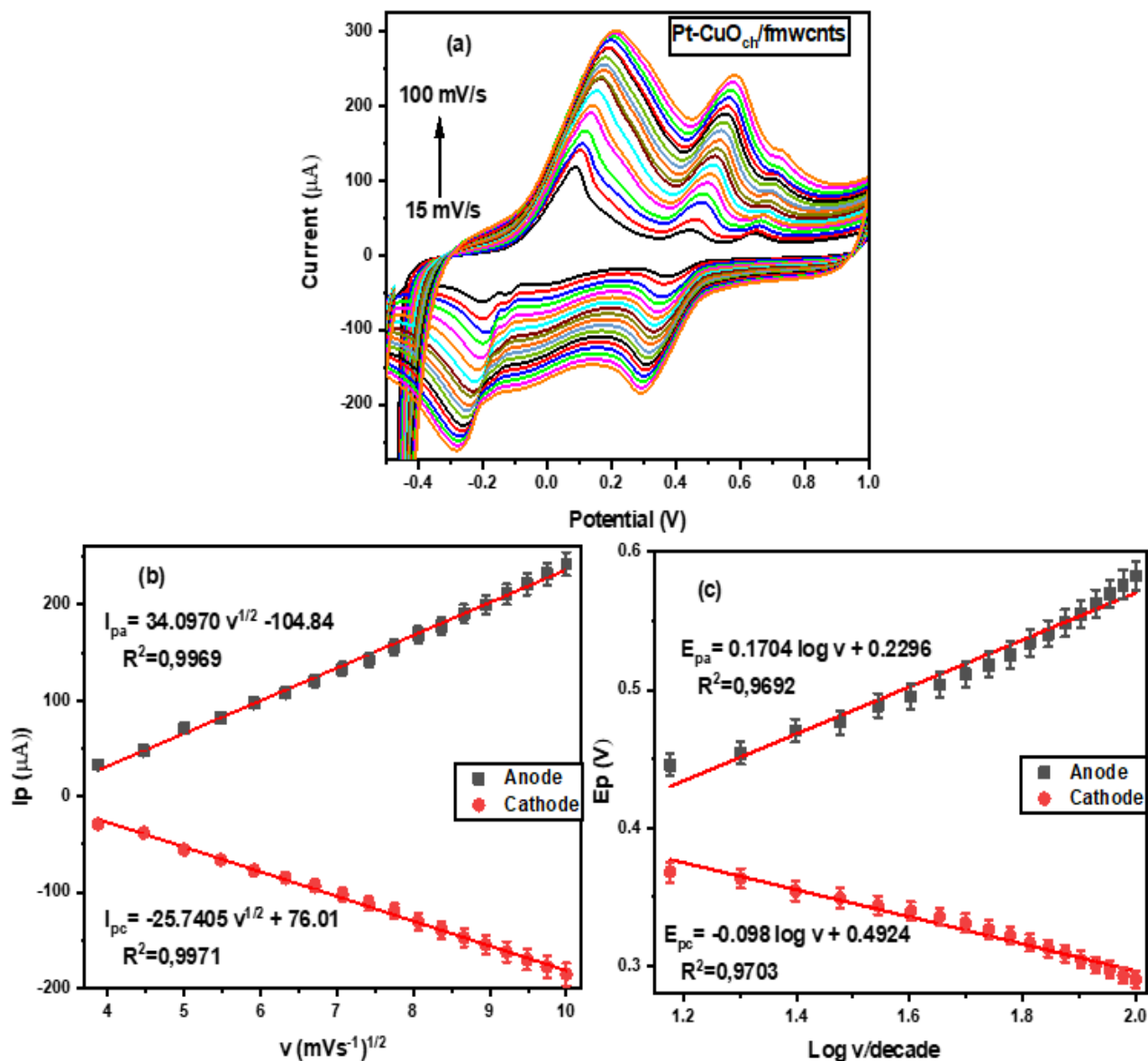


Figure 4.15: CV spectra for the effect of scan rate at Pt-CuO_{ch}/fMWCNTs electrode and its regression plots

4.8.4.2 Scan rate effect at the Pt-CuO_{gr}/fMWCNTs on CAP probe.

The effect of the scan rate ranging from 25 – 3500 mVs⁻¹ on the anodic and cathodic peak current response of the Pt-CuO_{ch}/fMWCNTs modified electrode towards 60:40 PBS: EtOH solution containing 32.7 mM CAP at pH 3 were conducted with the utilization of CV. There was an increase in the anodic peak current as the scan rate increases, which indicated that there was adsorption-controlled process on the electrode's surface [121]. From the obtained scan rate voltammograms,

the linear plots correlating to the square root of scan rate ($V^{1/2}$) vs anodic (I_{pa}) and cathodic (I_{pc}) current were obtained as depicted in Figure 4.16, with the linear regression equations 11 and 12 of

$$I_{pa} = 45.095 v^{1/2} - 134.88 \quad (R^2 = 0.9986) \quad (11).$$

and,

$$I_{pc} = -31.656 v^{1/2} + 85.11 \quad (R^2 = 0.9996) \quad (12).$$

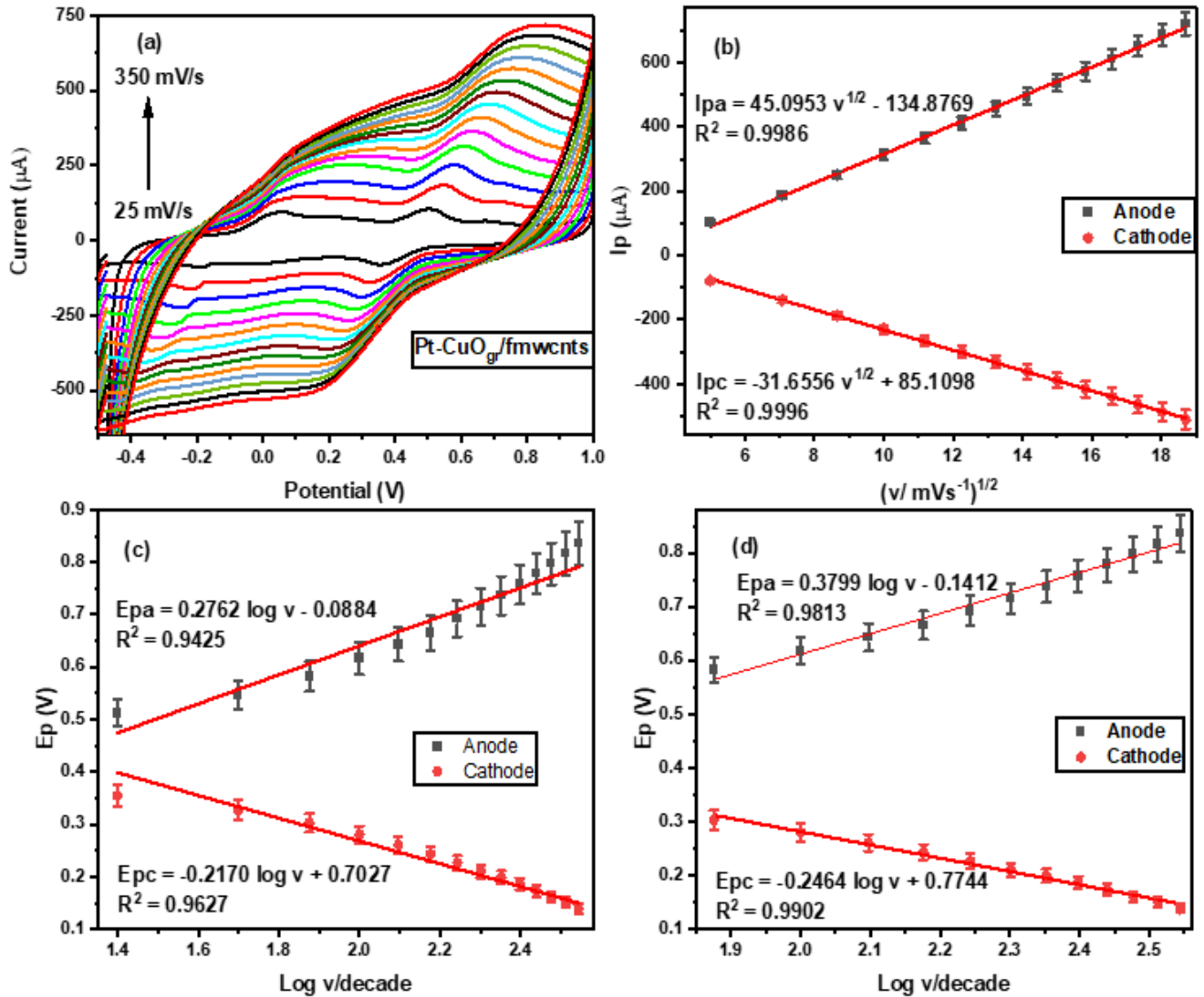


Figure 4.16: CV spectra for the effect of scan rate at Pt-CuO_{gr}/fmWCNTs electrode and its regression plots

4.9 Concentration studies of CAP.

4.9.1 Concentration studies of CAP using the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{ch}/fMWCNTs electrodes

Square wave voltammetry was utilized for the CAP concentration range of 0.357 to 2.73 μM at the (a) Pt-CuO_{ch}/fMWCNTs and (c) Pt-CuO_{ch}/fMWCNTs, (b) and (d) represent the peak currents vs concentration of CAP prepared in pH 3 60:40 EtOH buffer as shown on Figure 4.17. The SWV technique was set at potential window of 0.2 to 0.8 V, 10 Hz for frequency, 0.01 for potential amplitude, and Estep for the potential (0.01). The objective was to study the current response of CAP at different concentrations at the proposed electrodes. For the (a) Pt-CuO_{ch}/fMWCNTs, the results indicated a linear relationship of the current and the concentration, with a linear equation of $I = 1.3109 \text{ CAP} + 27.0962$ and $R^2 = 0.9968$. The detection limit (LOD) of this electrode was found to be 0.0765 μM and the limit of quantification (LOQ) to be 0.232 μM using the equations 13 and 14.

$$LOD = \frac{3.3 \times \text{standard deviation}}{\text{Slope}} \quad (13),$$

$$LOQ = \frac{10 \times \text{standard deviation}}{\text{Slope}} \quad (14).$$

From Figures 4.17 c and d, the results indicated a linear relationship of the currents and the concentrations, with a linear equation and good regression values of $R^2 = 0.99$. The limit of detection LOD for the electrode Pt-CuO_{gr}/fMWCNTs was found to be 0.0881 and LOQ to be 0.294 μM using equations 13 and 14. The results showed that Pt-CuO_{gr}/fMWCNTs had higher sensitivity and detection limits than the Pt-CuO_{ch}/fMWCNTs electrode, thus indicating that the green synthesis route of the CuO NPs played a vital role in their electroactive properties. The comparative values for this study and the reported work on CAP is represented in Table 4.3.

Table 4.6: Comparison of CAP sensors from literature with the current study

Modified electrodes	Method	Linearity (μM)	LOD (μM)	R^2	Ref
MWCNT-BPPGE	AdSV	0.5 – 60	0.31	0.99	[6]
PPS-Grp/SPE	DPSV	0.3 – 70	0.10	0.99	[35]
GCE	CCSWV	0.81 – 80	0.81	0.99	[122]
Pt-CuO _{ch} /fMWCNTs	SWV	0.357 – 2.73	0.0765	0.99	This work
Pt-CuO _{gr} /fMWCNTs	SWV	0.357 – 2.73	0.0881	0.99	This work

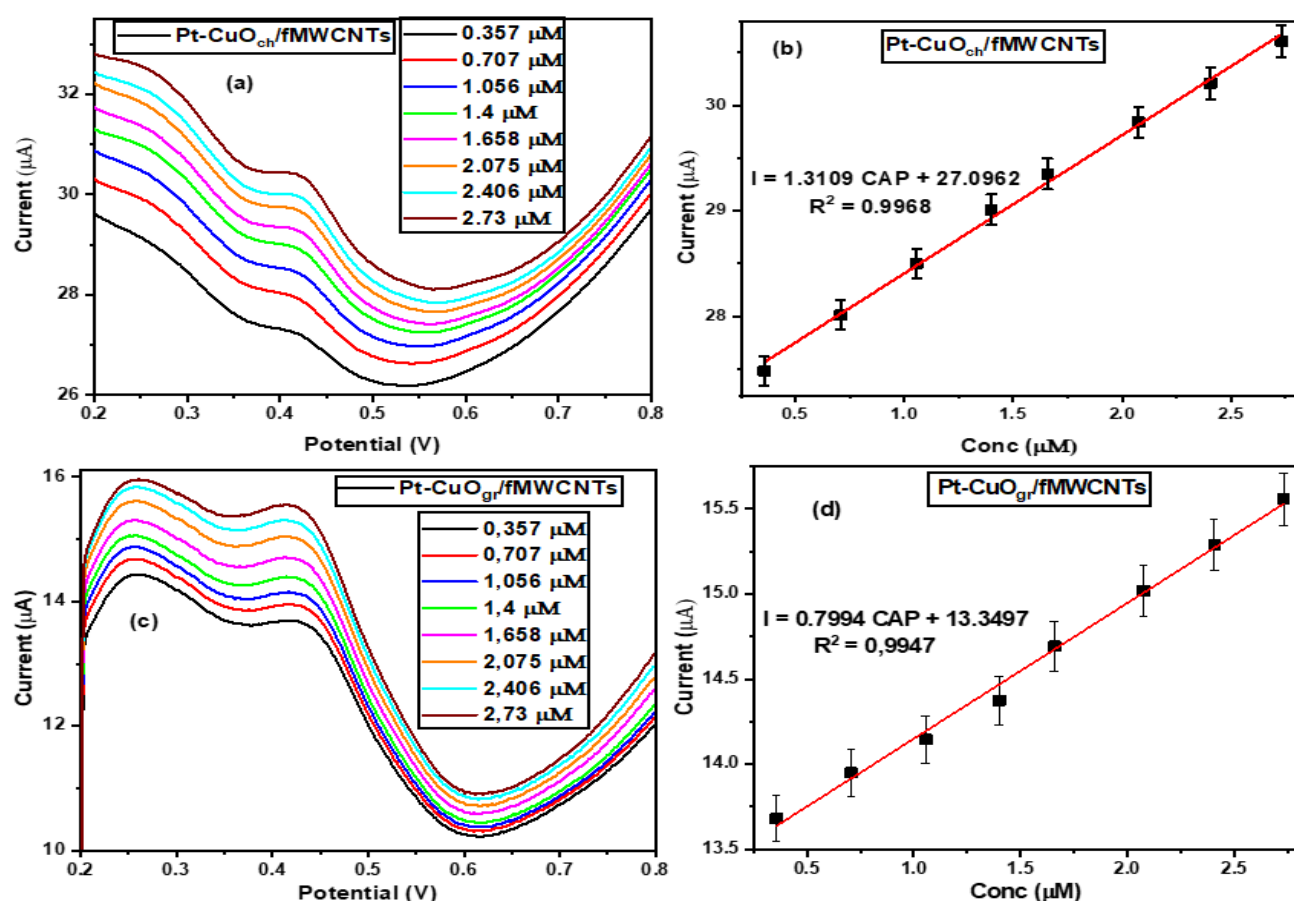


Figure 4.17: SWV spectra for concentration studies of CAP at (a) Pt-CuO_{ch}/fMWCNTs, (c) CuO_{gr}/fMWCNTs electrodes and their corresponding linear plots (b) and (d)

4.10 Interference, reproducibility and stability studies.

4.10.1 Interference studies of CAP using the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs electrodes.

To determine the selectivity of the proposed sensors towards the detection of CAP, interference studies were carried out using SWV. Possible interfering species were selected for this study based on the fact that these species may be present in the pharmaceutical and food capsaicin products. Selected interferants are calcium (Ca⁺), fructose, potassium (K⁺) and sodium (Na⁺). In the SWV analysis, 54.7 μ M capsaicin was spiked with 1 mM of each of the interferants. The current response of the capsaicin was taken to be 100 % and was compared with the current response of the capsaicin spiked with the interferants as shown in Figure 4.18. Figure 4.18a, shows the results for the Pt-CuO_{ch}/fMWCNTs sensor while the results for the Pt-CuO_{gr}/fMWCNTs sensor are shown on Figure 4.18b. The obtained results deduced that the proposed sensors are highly selective as there was no significant interfering behaviour from the selected results.

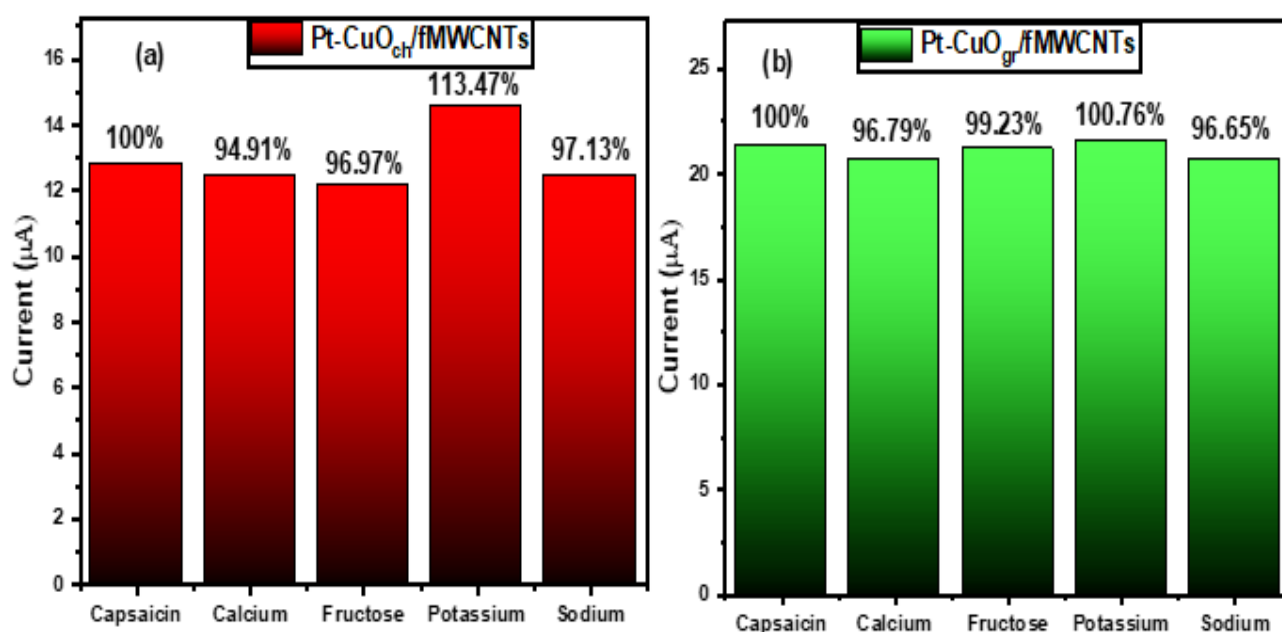


Figure 4.18: Bar graphs for the interferants on CAP at (a) Pt-CuO_{ch}/fMWCNTs and (b) CuO_{gr}/fMWCNTs electrodes

4.10.2 Reproducibility of the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs electrodes.

The reproducibility studies were conducted to evaluate the efficiency and reliability of the proposed sensors by using CV technique. The electrodes were scanned 25 times with the scan rate set at 25 mVs⁻¹ in 5 mM K [Fe (CN)₆]^{3-/4-} in 0.1 M PBS buffer, pH 7.4 as depicted in Figure 4.19. Figure 4.19a shows the cyclic voltammogram of Pt-CuO_{ch}/fMWCNTs which has a current drop of 17 % and Figure 4.19b shows that of the Pt-CuO_{gr}/fMWCNTs which has a current increase of 9.47 %.

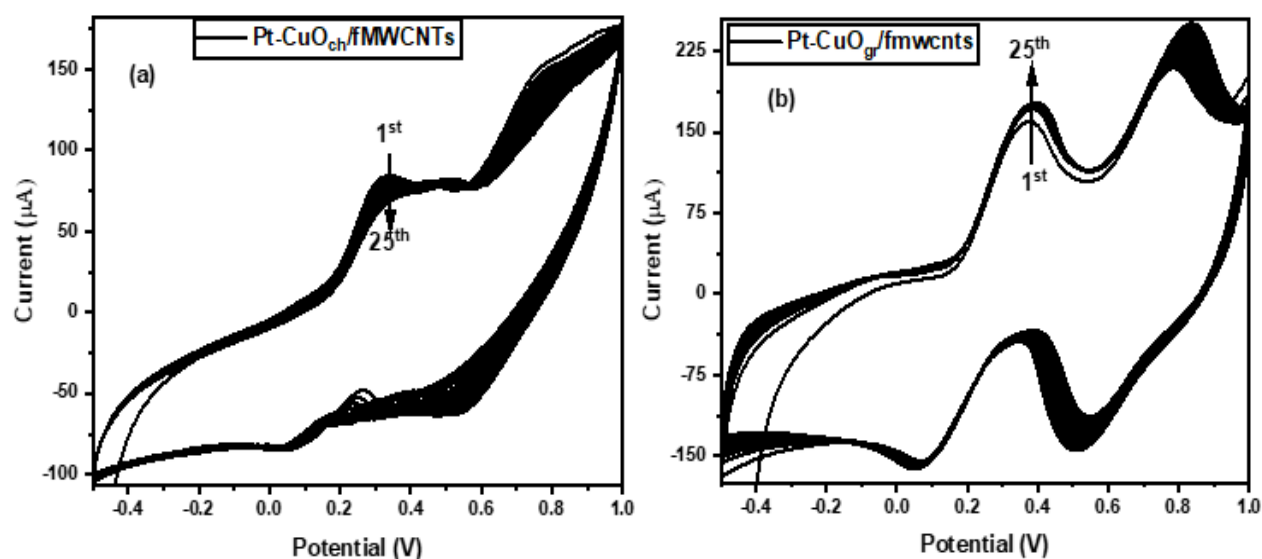


Figure 4.19: CV spectra on reproducibility studies at (a) Pt-CuO_{ch}/fMWCNTs and (b) CuO_{gr}/fMWCNTs electrodes

4.10.3 Stability of the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs electrodes.

With the use of the CV technique, the stability studies of the proposed sensors were carried out. The electrodes were scanned 25 times with the scan rate set at 25 mVs⁻¹ in 32.7 mM capsaicin in 60:40 PBS: EtOH buffer at the pH 3. Figure 4.20 a and b showed that there was a 49 % increase in the anodic peak current of Pt-CuO_{ch}/fMWCNTs while that of the Pt-CuO_{gr}/fMWCNTs increased by 39 %. The results show that neither electrode has a good stability and that they both underwent biofouling during the voltametric experiments.

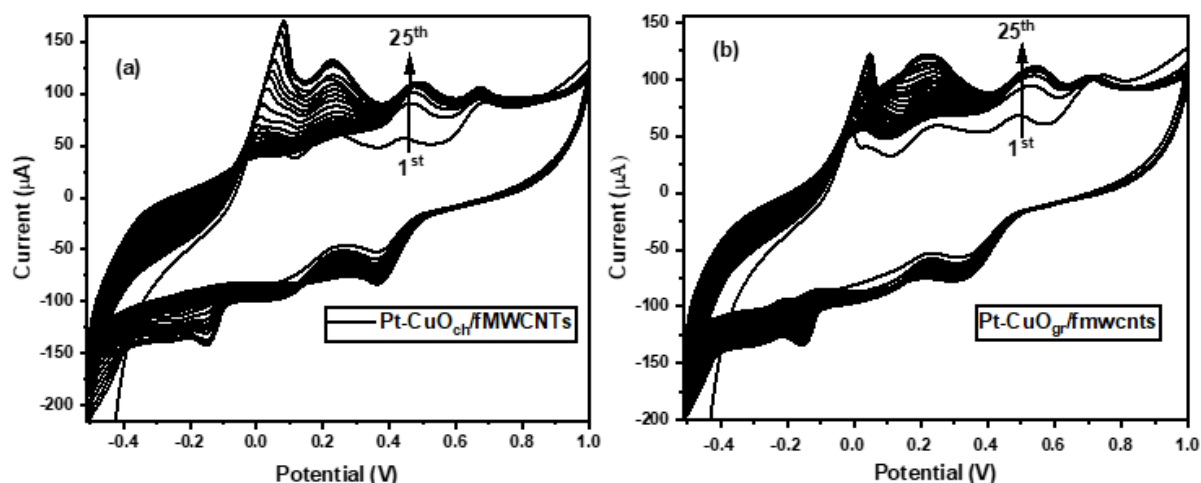


Figure 4.20: CV spectra on stability studies at (a) Pt-CuO_{ch}/fMWCNTs and (b) CuO_{gr}/fMWCNTs electrodes

4. 11 Real sample analyses

The application of the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs sensors were carried out using the SWV towards the detection of CAP in Nando's sauce and Oestoeze rub. Table 4.4 shows the summarized data obtained using the Pt-CuO_{ch}/fMWCNTs, while table 4.5 shows the summarized data obtained using the Pt-CuO_{gr}/fMWCNTs. Pt-CuO_{ch}/fMWCNTs gave a percentage recovery of 98 -110 % with the RSD value of 5.77 (n=3) in the Nando's sauce and percentage recovery of 84 -106 % with RSD value of 12.18 (n=3) in the Oestoeze rub. On the other hand, Pt-CuO_{gr}/fMWCNTs resulted in the percentage recovery of 100 - 116 % with the RSD value of 8.24 (n=3) in the Nando's sauce and percentage recovery of 98-105 % with RSD value of 3.36 (n=3) in the Oestoeze rub. Both sensors gave excellent percentage recoveries, thus confirming that both of the sensors are reliable towards the detection of CAP.

Table 7.4: Capsaicin recovery from the Nando's sauces and oestoeze rub using Pt-CuO_{ch}/fMWCNTs electrode

Sample	Added (μM)	Detected (μM)	Recovery (%)	RSD (%)
Nando's peri-peri sauce (XX hot)	2.97	3.28	110	5.77
		3.11	104	
		2.95	98	
Ostoeze (Rub Capsicum cream)	2.97	2.68	90	12.18
		3.16	106	
		2.51	84	

Table 8.5: Capsaicin recovery from the Nando's sauces and oestoeze rub using Pt-CuO_{gr}/fMWCNTs electrode

Sample	Added (μM)	Detected (μM)	Recovery (%)	RSD (%)
Nando's peri-peri sauce (XX hot)	5.34	5.34	100	8.24
		5.47	102	
		6.19	116	
Ostoeze (Rub Capsicum cream)	5.34	5.24	98	3.36
		5.51	103	
		5.57	105	

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

6. Conclusion and recommendations

In this final chapter, a conclusion is drawn from the obtained results and there is a provision of implications for future studies.

5.1 Conclusion.

The detection of CAP at the CuO_{ch}/fMWCNTs and CuO_{gr}/fMWCNTs nanocomposite fabricated on the platinum electrode was successfully achieved. The morphological and spectroscopic techniques (UV-vis, FTIR, XRD, SEM, EDX and TEM) that were used positively confirmed the synthesized nanomaterials. Electrochemical techniques were utilized to investigate the selectivity, sensitivity and the electron transfer properties of the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs nanocomposite electrodes (CV, EIS and SWV) for the CAP detection. The Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs nanocomposite electrodes, showed remarkable limit of detection of 0.0765 μ M with linear equation of $I = 1.3109 \text{ CAP} + 27.0962$ and LOD = 0.0881 μ M with linear equation of $I = 0.7994 \text{ CAP} + 13.3497$, respectively. The fabricated nanocomposites electrodes displayed remarkable sensitivity and selectivity and these findings motivated that the fabricated electrodes have the capabilities to be applied in sensors. The sensors were used in real sample analysis in the detection of Cap in both Nando's sauce and oestoeze rub. On the Nando's sauce the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs nanocomposite electrodes had an average recovery percentage of 104% with an RSD of 5.77 % and 106 % with the RSD of 8.24 %, respectively. On the oestoeze rub the Pt-CuO_{ch}/fMWCNTs and Pt-CuO_{gr}/fMWCNTs nanocomposite electrodes had an average recovery percentage of 93% with an RSD of 12.18 % and 102 % with the RSD of 3.36 %, respectively.

5.2 Recommendations.

- I. To improve the LOD of the electrode, it is recommended to utilize a three-catalyst system nanocomposite. These nanocomposites could make use of the addition of a conducting polymer such as polypyrrole.
- II. To improve the electrocatalytic activity of the metal oxide nanoparticles, a careful control of the morphology and size is recommended as it has been found that these affect the electrocatalytic properties of the MO NPs.

APPENDIX:

Formula(s) used:

$$D = \frac{0.94\lambda}{\beta \cos \theta} \quad (1)$$

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

$$E_{pa} = \frac{E^0 + 2.303RT}{(1-a)nF \log v} \quad (5a)$$

$$E_{pc} = \frac{E^0 - 2.303RT}{anF \log v} \quad (5b)$$

$$E_p = \left(\frac{b}{2}\right) \log v + \text{constant} \quad (6)$$

$$LOD = \frac{3.3 \times \text{standard deviation}}{\text{slope}} \quad (13),$$

$$LOQ = \frac{10 \times \text{standard deviation}}{\text{slope}} \quad (14).$$

Outputs

1. Presented a poster at the 6th international symposium on electrochemistry, held at University of Johannesburg
2. “Green synthesized copper oxide nanoparticles incorporated with functionalized multi-walled carbon nanotubes based electrochemical sensor for capsaicin”. To be submitted for publication

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