

**FABRICATION OF FUNCTIONALISED-MWCNTS/METAL
CHALCOGENIDES COMPOSITE NON-ENZYMATIC
ELECTROCHEMICAL SENSOR FOR THE DETECTION OF GLUCOSE**

by

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2024

DECLARATION

I hereby declare that this dissertation, which I am submitting to the University of Limpopo for a Master of Science in Chemistry, is entirely original work of mine, that all sources used in its creation have been properly cited, and that it is my original work in design and execution.

A handwritten signature in black ink, appearing to be 'MAB' with a horizontal line through the middle.

SURNAME, INITIAL

11 JULY 2024

DATE

DEDICATIONS

I dedicate my work to my parents, my father July Motshabapula Maphake and my mother Maleke Naida Maphake for the financial and emotional support, and encouragement, inspiration, and support in every decision that I have made in life. A special feeling of gratitude to my siblings Mashego Maphake and Dimpho Maphake, my nephew Kgotlelelo Maphake and my daughter, Oratile Delight Maphake for always believing in me and cheering from the sidelines.

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ABSTRACT

One of the most significant analysis in medical diagnosis is blood glucose measurement. In other scientific and industrial domains, such as environmental monitoring, pharmaceuticals, and the food sector, they are equally indispensable, however commercial glucometers that are sold on the market frequently have a lot of flaws and limits. These can be caused by a variety of challenges, such as poor material design, incorrect storage, coding mistakes, issues with device calibration, manufacturing processes, and the substantial influence of ambient elements like pH and temperature. Some of these issues might be resolved by creating brand-new hybrid materials with enhanced characteristics (such stability and adsorption capabilities).

The metal sulfide-decorated-functionalised-multi-walled carbon nanotube modified non-enzymatic glucose detecting sensor was studied. Many physicochemical and structural investigations, such as Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and Raman spectroscopy, were performed to characterize the synthesized nanocomposites. Electrochemical methods such as square wave voltammetry, cyclic voltammetry, and electrochemical impedance spectroscopy were used to comprehensively test the produced sensors.

The p-MWCNTs were purified and functionalised by acid treatment into COOH-MWCNTs. Amine functionalise MWCNTs (NH₂-MWCNTs) were donated by Mintek. Metal sulfides nanoparticles (FeS₂ and ZnS) were synthesised using hydrothermal method under the same experimental conditions as the metal sulfides/f-MWCNTs nanocomposites for comparison in the electrochemical studies. Fourier transform infrared spectroscopy (FTIR) verified that carboxyl (COOH) and amine (NH₂) functional groups were detected on the surface of the f-MWCNTs, which appeared at lower wavenumbers, i.e., below the fingerprint due to the presence of metal sulfides. Transmission electron microscopy (TEM) was used to confirm that FeS₂ and ZnS nanoparticles successfully decorated MWCNTs in situ. The functionalized MWCNTs surface exhibited a well-dispersed distribution of metal sulfides, as demonstrated by TEM pictures. X-ray diffraction (XRD) was used to confirm the chemical composition of the as-prepared MWCNTs, indicating the existence of MWCNTs and metal sulfides.

The XRD pattern of FeS₂/COOH-MWCNTs nanocomposites represented crystal planes of pyrite FeS₂. The ZnS/COOH-MWCNTs nanocomposites' XRD pattern agrees with published data and confirms that ZnS forms in the cubic (sphalerite) phase with acceptable crystallinity. The Raman spectra of pristine MWCNTs, COOH-MWCNTs, FeS₂/COOH-MWCNTs, FeS₂/NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs all showed three vibration bands namely, D, G, and G', further confirming the presence of the carbonous nature of the MWCNTs.

The electrochemical characteristics of the nanocomposites formed by metal sulfides/functionalized-MWCNTs were investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). These analyses were performed in a solution containing 5 mM K₃Fe(CN)₆ /K₄Fe(CN)₆ as the redox probe and 0.1 M KCl as the supporting electrolyte. The resultant nanomaterials had redox active groups that produced a signal on the electrode surface, and these groups could be used to build electrochemical sensors and biosensors. The cyclic voltammograms illustrate the comparative current in this decreasing order: ZnS/COOH-MWCNTs-GCE > FeS₂-GCE > p-MWCNTs-GCE > BARE-GCE > ZnS/NH₂-MWCNTs-GCE > FeS₂/NH₂-MWCNTs-GCE > GCE-ZnS-GCE > GCE-COOH-MWCNTs-GCE > FeS₂/COOH-MWCNTs-GCE > NH₂-MWCNTs-GCE.

The fitted impedance data reveals that the GCE-ZnS/COOH-MWCNTs electrode has the lowest R_{ct} value of 33 Ω , proposing a swift electron transfer of the GCE-ZnS/COOH-MWCNTs electrode as in comparison to the remaining electrodes, consistent with the findings from cyclic voltammetry. Consequently, the ZnS/COOH-MWCNTs nanocomposite showed better electroactivity and great potential to be employed for glucose detection. Square wave voltammetry (SWV) was used for the electroanalysis of the analyte (glucose) at the GCE-ZnS/COOH-MWCNTs. The results showed that the GCE-ZnS/COOH-MWCNTs had better electrocatalytic behaviour resulting from the higher oxidation peak currents (I_{pa}) and faster electron transport. The results from scan rate studies demonstrate that the scan rate (v) correlates directly with the glucose anodic current responses (I_{pa}) at GCE-ZnS/COOH-MWCNTs. The glucose oxidation mechanism at GCE-ZnS/COOH-MWCNTs was diffusion-controlled, indicated by the linear relationship observed between oxidation peak currents (I_{pa}) and the square root of the scan rate ($v^{1/2}$). Employing square wave voltammetry, a limit of detection (LoD) of 0.83 mM was achieved for GCE-ZnS/COOH-MWCNTs within a

linear dynamic range (LDR) of 1-9 mM. The sensitivity of the sensor was measured at $7.2 \times 10^6 \text{ A.mM}^{-1}$.

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LIST OF ABBREVIATIONS

CCVD - Catalytic Chemical Vapor Deposition

CE - Counter Electrode

CNTs - Carbon Nanotubes

COOH - Carboxylic acid

CV - Cyclic Voltammetry

DI - Distilled/ De-ionized

DMF - N, N-Dimethylformamide

EIS - Electrochemical Impedance Spectroscopy

FeS - Iron sulfide nanoparticles

FTIR - Fourier-Transform Infrared

Fe (Cl)₃ .6H₂O - Iron (III) chloride hexahydrate

GCE - Glassy Carbon Electrode

H₂O - Water

H₂SO₄ - Sulphuric acid

HNO₃ - Nitric acid

IDF - International Diabetes Federation

KCl - Potassium Chloride

MWCNTs - Multi-Walled Carbon Nanotubes

NaBH₄ - Sodium Borohydride

NEG - non-enzymatic glucose

NaCl - Sodium Chloride

OH⁻ - Hydroxyl ion

RE - Reference Electrode

SEM - Scanning Electron Microscopy

SWCNTs - Single-Walled Carbon Nanotubes

TEM - Transmission Electron Microscopy

WE - Working Electrode

XRD - X-Ray Diffraction

$\text{Zn (Cl)}_2 \cdot 6\text{H}_2\text{O}$ - Zinc (II) chloride hexahydrate

ZnS - Zinc sulfide nanoparticles

CHAPTER 1

1. Introduction

1.1. Background and motivation

One of the problems with human health is diabetes, which is a syndrome rather than a single disease. It involves high blood sugar levels and, with time, an elevated likelihood of harm to the kidneys, eyes, nerves, heart, and medium and large blood arteries, to name a few organs. Diabetes comes in four primary forms: type 1, type 2, secondary type 3c, and gestational [1]. Insulin therapy is required for type 1 diabetes, an autoimmune illness in which the immune system mistakenly recognises and kills the pancreatic beta cells that produce insulin [2]. Chronically consuming too much glucose lowers the body's sensitivity to insulin in type 2. The term "gestational diabetes" refers to diabetes discovered during pregnancy stage [3]. Secondary type 3c diabetes has a wide range of specific causes, including infections, rare immune-mediated and other genetic disorders, abnormalities in insulin action or structure, genetic defects in beta cell function, pancreatic diseases such as hemochromatosis and pancreatitis, drug/chemical and surgically induced endocrinopathies, and infections [4].

The prevalence of diabetes worldwide has been rising over time because of significant lifestyle changes in recent decades [5]. A significant increase of diabetic of almost 10% population to individuals whose age ranges between 18 and 99 has been predicted by the International Diabetes Federation (IDF). The increase is expected by 2045 [6]. The societal health burden and family health care costs are undoubtedly exacerbated by the increased incidence of diabetes.

Patients with diabetes require ongoing blood glucose monitoring as well as possible daily medication or insulin administration. Therefore, maintaining the blood glucose level of humans is crucial for clinical purposes, and as a result, the market for glucose biosensors is expanding quickly. Electrochemical enzyme glucose oxidase-based biosensors have been created and made accessible over the counter to solve this problem [7]. These platforms do, however, have limitations, such as difficult immobilisation procedures, ideal operating pH and temperature, and chemical

instability. The usage of enzyme-based electrodes is restricted by the fact that they are typically expensive and difficult to sterilise [8].

Many efforts have been made over the past 30 years to create sensitive and selective glucose biosensors. To encourage and increase glucose oxidation, non-enzymatic glucose (NEG) biosensors have been created [8]. Non-enzymatic glucose sensing is a simple technique that measures blood glucose by oxidizing or reducing glucose without the need of enzymes. This process is quick and cost effective. Typically, non-enzymatic biosensors have been made with noble metals like Pt and Au and their composites [9]. Noble metals are difficult to use in NEG sensors despite their adequate performance because of their expensive cost and limited availability, which prevents widespread use [10]. Noble metals also experience surface poisoning from the solution's intermediates, which can instantly cover the electroactive surface and lower the glucose sensor's selectivity and sensitivity [11].

Efforts have been directed towards discovering novel materials with enhanced electrical and surface properties to improve NEG biosensor performance [12]. Conducting carbon nanomaterials, renowned for their high electrical conductivity, low energy optical transitions, elevated electron affinity, and low ionization potential, stand out as a unique group. Multi-walled carbon nanotubes (MWCNTs) have been favoured for their exceptional electrical conductivity and surface area in the development of optoelectronic devices. Since most solvents cannot dissolve pure multi-walled carbon nanotubes, their use is restricted. Multi-walled carbon nanotubes (MWCNTs) surface modification will subsequently increased their ability to dissolve in both aqueous and non-aqueous solvents for a variety of applications [10].

Metal chalcogenides have been used in the past as electrode choices for the detection of glucose due to their ease of use, high synergistic action, and great comparability [13]. These metal chalcogenides, however, have a propensity to aggregate without support [14]. To create a non-enzymatic electrochemical sensor for the detection of glucose, it is significant to incorporate metal chalcogenides in functionalised-MWCNTs. In this project, MWCNTs were functionalised, characterised and applied as mediating materials to enhance the surface conductivity and sensitivity of the sensing platform. Subsequently, zinc sulfide (ZnS) and iron sulfide (FeS) were used as electro-

catalysts to promote the oxidation of glucose, hence, hybrid nanocomposites will be produced possessing both these properties.

1.2. Problem statement

In the world, diabetes is a common cause of death and disability, and it has been related to several other illnesses, including heart disease, renal failure, and blindness. According to estimates, 100 million people worldwide have diabetes, making the development of glucose detecting devices crucial for the medical and food industries [15]. To solve the problem, electrochemical enzyme glucose oxidase-based biosensors were created, and they are now sold without a prescription as diabetic self-monitoring devices. The focus of this research is currently changing toward the creation of chemical glucose sensors without enzymes that are more stable and sensitive using functional materials [16].

Nano and micro-materials have been used to create enzyme-free biosensors that can improve and facilitate the oxidation of glucose [17]. Metal-carbon nanotube nanocomposites, inert metal catalysts including Pt, Au, and Ni and their alloys, metal oxides, and metal nanofibers have all been used to accomplish this successfully [18,19]. Noble metals are problematic for non-enzymatic glucose sensors because they are expensive to produce and have a limited supply, which limits their application while being effective [20]. Multi walled carbon nanotubes (MWCNTs) have been used in the construction of opto-electronic devices because of their better electrical conductivity. In this project or study, multi-walled carbon nanotubes (MWCNTs), were functionalised (COOH-MWCNTs). Subsequently, metal chalcogenides (ZnS and FeS) decorated functionalised-MWCNTs composites were then produced from the as-synthesized nanomaterials to develop a highly sensitive non-enzymatic sensor for glucose detection.

1.3. Aim and objectives

Aim

The aim of this research project is to fabricate metal chalcogenides decorated functionalised-MWCNTs composite non-enzymatic electrochemical sensor for glucose detection.

To achieve the above aim, the following objectives were outlined:

- i. Characterise multi walled carbon nanotubes.
- ii. Chemically functionalise and characterise MWCNTs (i.e., COOH-MWCNTs)
- iii. Synthesise and characterise metal chalcogenide (ZnS and FeS) decorated functionalised (COOH)-MWCNTs using XRD, UV-Vis, TGA, TEM, SEM, FTIR, CV and EIS.
- iv. Develop a sensor using the functionalised-MWCNTs/metal chalcogenides (ZnS and FeS) nanohybrid composite for detecting glucose via electrochemical methods

Glucose analysis was studied using metal chalcogenides decorated functionalised-MWCNTs-GCE in comparison to functionalised-MWCNTs-GCE and bare SPCEs. Glucose sensing experiments of the metal chalcogenides decorated functionalised-MWCNTs-GCE based sensor were carried out at different concentrations of glucose using cyclic voltammetry and other electrochemical techniques to determine parameters such as sensitivity, durability, detection limit, and the operational concentration range of the sensor. The metal chalcogenides decorated functionalised-MWCNTs-GCE non-enzymatic sensor performance will then be benchmarked against other non-enzymatic sensors for glucose in literature.

1.4. Dissertation outline

The dissertation is organized into six chapters altogether. The problem statement, research aim, objectives, and basic background and motivation of the study are presented in Chapter 1. A summary of the literature reviewed that is pertinent to finishing this assignment is given in Chapter 2. An overview of the various forms of diabetes and the various sensor techniques for continuous glucose monitoring opens this chapter. Next, different electrochemical principles applied in the field of biosensors, and in depth the evolution of glucose sensor, the distinction between enzyme-based glucose sensors and enzyme-free glucose sensors. Lastly, this chapter is concluded with an overview of conductive carbon nanotubes and electrocatalytic metal chalcogenides as material of interest for the detection of glucose. Chapter 3 provides the different characterisation techniques principles necessary to investigate the properties and microscopic structures of nanoparticles. In chapter 4 the conditions for the chemical oxidation of MWCNTs, synthesis of metal sulfides nanoparticles, in-

situ synthesis of nanocomposites, and modification of the GCE surface for the detection of glucose are explored. The characterisation techniques used to confirm the structural, functional, and optical properties of the nanocomposites are discussed in chapter 5. The electrochemical activity of the nanocomposites is another topic covered in this chapter, along with sensor optimization for ideal pH, stability, concentration, and electrode scan rates. Chapter 6 discusses the study's conclusions based on the data gathered and the suggestions for additional research.

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CHAPTER 2: LITERATURE REVIEW

2.1. Diabetes mellitus

Diabetes mellitus is a metabolic disease with multiple aetiologies that is defined by chronic high blood sugar levels and alterations in protein, lipid, and carbohydrate metabolism resulting from issues with insulin secretion, function, or both [1]. Diabetes mellitus causes long-term harm to the body, malfunction and impairment of multiple organs. Diabetes mellitus is commonly characterised by thirst, polyuria, blurred vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketosis hyperosmolar state may manifest, resulting in stupor, coma, and, in the event that appropriate treatment is not received, death [2].

Long-term consequences of diabetes mellitus include the progressive development of its particular complications: retinopathy, which may result in vision loss, kidney disease leading to renal failure, and nerve damage increasing the chances of foot ulcers, amputation, and charcot joints and autonomic dysfunction symptoms like erection issues. Diabetics are more likely to develop cerebral, peripheral vascular, and cardiovascular disease [3]. Diabetes is caused by pathogenetic pathways that include actions that destroy beta cells in the pancreas, leading to inadequate insulin production, and other actions that make the body resistant to the effects of insulin [4].

Insulin's insufficient impact on target tissues due to insulin sensitivity or lack results in abnormalities in the metabolism of proteins, lipids, and carbohydrates. Type 1, type 2, gestational diabetes, and secondary type 3c are the four primary forms of diabetes mellitus [5].

2.1.1. Type 1 diabetes (insulin–dependent diabetes mellitus”)

Type 1 diabetes affects about 5% of all instances of diabetes. Most type 1 diabetes cases have an autoimmune attack on the pancreatic beta cells (β), which leads to severe insulin insufficiency. Thymus (T) cells are primarily responsible for beta cell damage and death, even though β cell-formed autoantibodies to islet antigens are used as disease markers and may have a pathogenic function [6, 7].

In short, type 1 diabetes is a chronic disease caused by the destruction of insulin-beta cells in the islets of Langerhans in the pancreas. Type 1 describes the beta cell degeneration processes that could eventually result in diabetes mellitus, in which "insulin is essential for survival" to avoid the onset resulting in ketoacidosis, coma, and potentially fatal outcomes. Before the disease becomes clinically evident in a person with a Type 1 process, their metabolism may be normal, but the beta-cell death process can still be seen. This kind can also be divided into immune-mediated and idiopathic subtypes [8].

In addition to frequent ketosis and severe hypoglycaemia, type 1 diabetes can also be accompanied by unpredictable and erratic hyperglycaemia. A compromised counter-regulatory response to hypoglycaemia, infection, gastroparesis, and endocrinopathies (such as Addison's disease) are some additional challenges [9-11]. Numerous genes, including certain human leukocytes antigen (HLA) genotypes known to affect diabetes risk, are partially inherited in type 1 diabetes. When someone is genetically predisposed to diabetes, it might be triggered by one or more environmental factors, such as diet or a viral infection. Unlike type 2 diabetes, type 1 diabetes does not correlate with lifestyle decisions [12,13].

2.1.2. Type 2 diabetes (“non–insulin–dependent diabetes mellitus”)

The second most common kind of diabetes, type 2, accounts for 90 to 95 percent of all occurrences of diabetes globally [14]. In type 2 diabetes mellitus, the amount of insulin generated is insufficient at providing the cells with glucose, depriving them of energy. Lack of insulin leads to accumulation of glucose in the blood, which is why this condition is known as "insulin resistance" [15].

This is also the main disorder that occurs in the early stages of type 2 diabetes, however people can control the amount of glucose they make by exercising more, eating less high-carbohydrate meals, and losing weight [16]. However, insulin resistance might persist, therefore patients should continue to modify appropriately through specialized training, physical activity, food, body weight control, and the addition of medication (antidiabetic tablets) [17].

2.1.3. Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is characterised as a glucose intolerance that manifests as hyperglycaemia of varying severity that is first seen during pregnancy

[18]. The primary reason for negative pregnancy outcomes in gestational diabetes mellitus (GDM) is macrosomia, caused by fetal hyper-insulinism triggered by elevated maternal blood glucose levels due to hyperglycemia [19]. Pre-existing diabetes, occurring before conception, adds the risk of fetal abnormalities due to the teratogenic effects of glucose and/or related metabolic disturbances during crucial stages of organ development in early pregnancy [20].

Changes in maternal body fat mass show an inverse correlation with alterations in insulin sensitivity. An insulin action insufficiency may also influence the liver, as seen by increased hepatic glucose generation during pregnancy. Physiological changes in pregnant women are influenced by placental factors, as indicated by the significant rise in insulin sensitivity shortly after delivery [21, 22]. Placental hormones, including progesterone, oestrogen, and human placental lactogen (hPL), have traditionally been blamed for changes in maternal metabolism [23].

2.1.4. Secondary type 3c diabetes mellitus

While type 1 and type 2 diabetes affect most individuals with the diabetic condition, not all cases of diabetes fit strictly into these categories. A smaller fraction of patients with clinically severe diabetes may develop the disease due to a different pathophysiology [24]. Genetic mutation that increases susceptibility to type 1, type 2, or a hybrid form may cause these forms to develop at any stage of life, and they can also arise from additional metabolic conditions where hyperglycaemia is more probable to occur [25].

Diabetes has long been associated with congenital viral infections, primarily rubella and cytomegalovirus (CMV) [26]. A potential significant cause for rubella continues to exist given the variability in immunization rates. Numerous other viruses have been suggested as possible pathogens in either directly infecting the pancreas or inducing the autoimmune response. About 12% of children, and 33% of adults have been reported to have developed cystic fibrosis-related diabetes (CFRD). Since patients with CFRD have a higher risk of death and therapy with insulin improves clinical outcomes, diagnosing and treating CFRD is crucial [27].

Pancreatitis-associated diabetes may account for approximately 0.5–1% of all new diabetes cases in the general population. Because of their low quality of life and ongoing pancreatitis flare-related discomfort, some of the affected people prefer to

have their pancreas removed. Islet auto transplantation is a treatment that is still being developed to treat these patients' diabetes [28].

2.2. Glucose sensing technology for continuous glucose monitoring

Patients have historically been monitored for glucose using an intrusive finger-prick technique, in which a small lancet is used to puncture the patient's finger. A home glucose monitor or self-monitoring blood glucose meter (SMBG) (Figure 2.1a) is then used to analyse the patient's blood glucose levels [29]. Commercial glucose test kits have completely changed the way people manage their diabetes since home glucose testing meters became widely available in the late 1970s [30]. Since then, the market for glucose sensors has grown significantly, and it now represents over 85% of the global biosensor market [31, 32].

There are currently more than 40 distinct glucose test kits on the market that are sold commercially. Although SMBG has many advantages, it also has several significant disadvantages, including (i) frequent finger prick discomfort for patients and (ii) inability to obtain a thorough picture of the patient's glucose dynamics within the time intervals between measurements. Consequently, there is a chance that the patient will not be aware of their glycaemic swings throughout the non-measured interval, which could have deadly consequences. The continuous glucose monitoring (CGM) device, which is an analytic tool with a continuous in vivo glucose measurement has been a major aid for the past 20 years. This tool is now being considered as a solution to the issues to help with effective diabetic management. This device is attached to the body and delivers glucose readings every 1-2 minutes, along with glucose trends and alerts for hypoglycaemic and hyperglycaemic conditions (Figure 2.1b) [33]. An insulin pump can ideally be connected to one of these CGMs so that the monitor can act as an artificial pancreas by automatically adjusting insulin supply in reaction to abrupt fluctuations in glucose levels.

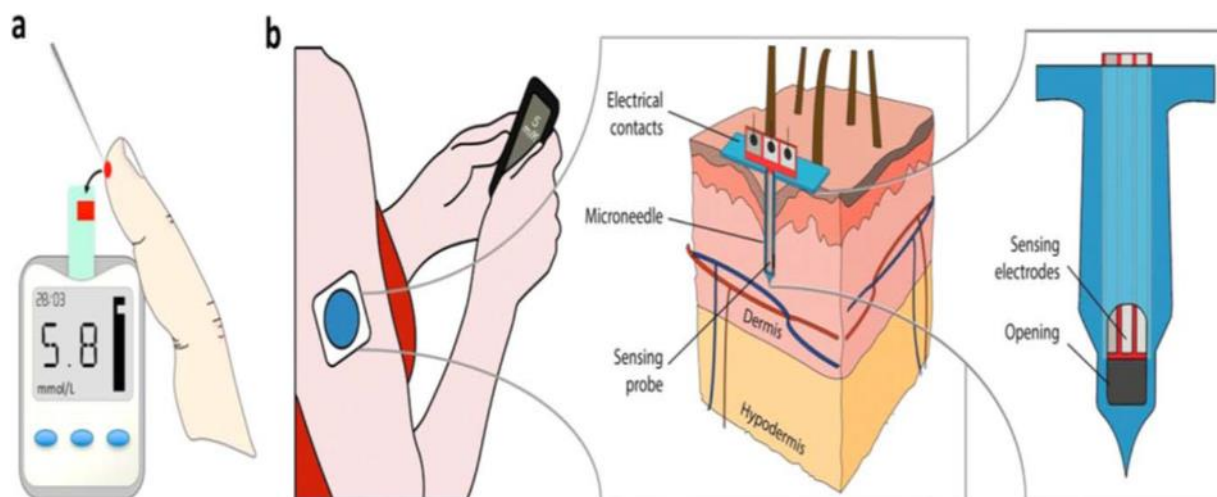


Figure 2.1: The finger-pricking equipment consists of a lancet needle, a glucose meter that shows the glucose concentration in mmol/L, and a blood sample on a test strip [29]. Continuous glucose monitor (b) [33].

Additionally, CGM can be utilised to obtain "time in range" targets by capturing a holistic model of glycaemic management, as advised by numerous international consensus papers [34]. In the end, CGM is a crucial tool for getting patient-specific huge data sets that will support improved adherence to lifestyle behaviours and individualised therapy [35].

2.2.1. Electrochemical glucose sensing technology

The basis of the majority of commercial blood glucose assays is the electrochemical enzymatic reaction of glucose with either glucose dehydrogenase or glucose oxidase (GOx) [36]. Using an enzyme to catalyse the O_2 -oxidation of glucose, the initial generation of electrochemical glucose biosensors is developed. But there are two main challenges facing the first-generation glucose biosensor. The first-generation biosensor faces several challenges, primarily related to its high working potential required for detecting hydrogen peroxide, which also oxidizes biological interferences and produces significant current densities. The remaining barrier is that straight electron transport from GOx's $FADH_2$ centre to the operational electrode's surface is too sluggish to be effective in a timely manner in the absence of a redox mediator (Figure 2.2). The redox mediator, which can lessen reliance on ambient oxygen, was added by the second-generation glucose biosensor [37] to get around the afore mentioned problems. Thus, to mitigate the effects of biological interferences, the measurement might be performed at a lower applied potential [38,39]. The

employment of electrochemical sensing technology for CGM applications has been extensively documented in literature. For instance, because they cause less pain and tissue damage, CGM biosensors based on microneedles are becoming more and more popular [40]. Devices based on electrochemical microneedles can provide simultaneous therapy and glucose monitoring [41]. The nonenzymatic glucose sensors are free from oxygen constraint, simple, reproducible and easy to fabricate, stable in a variety of environmental conditions, and inexpensive [42]. Yoon *et al.*, for instance, reported a wireless CGM module and a nonenzymatic glucose sensor utilizing nanoporous Pt as the catalytic material [43]. Electrochemical biosensors are superior to optical transducers in several aspects of CGM. They are easily made smaller for a wearable continuous monitoring system since they lack optical components.

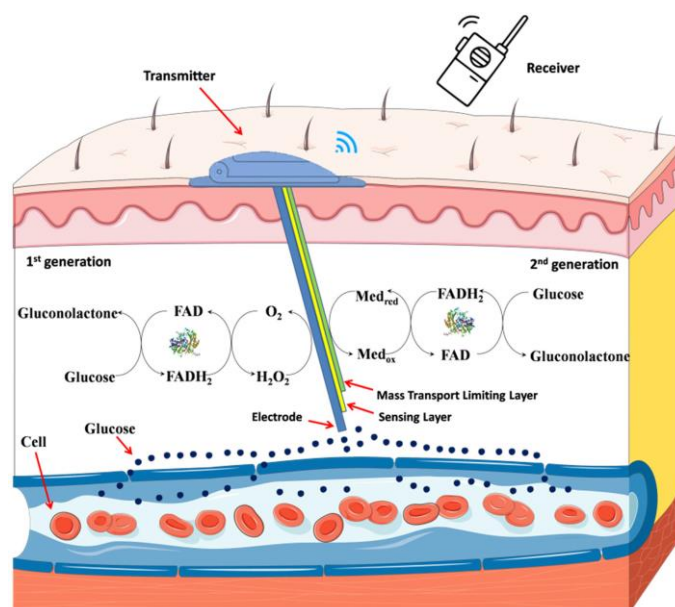


Figure 2.2: The overall design and operation of an electrochemical CGM biosensor. Diagrams demonstrate the development of several generations of enzyme electrochemical sensors from left to right. [37].

2.2.2. Optical glucose sensing technology

A viable platform for glucose sensing is the optical sensor, particularly for non-invasive measurements. Optically based biosensors, in contrast to electrochemically based biosensors, primarily rely on the photon detection as opposed to electron detection [44]. With benefits in sensitivity and adaptability, it is a good candidate approach for CGM biosensors and enables quicker and continuous monitoring [45].

Optical sensors are given more attention as compared to other technologies for the application of continuous quantification of glucose in biofluids. They offer real time monitoring systems for glucose that are label free, free from electromagnetic interferences and are internally calibrated [46].

According to transducer systems, optical biosensors can be categorized into various types, such as fluorescence, surface plasmon resonance (SPR), Raman spectroscopy, near-infrared spectroscopy, Fourier-transform infrared spectroscopy (FTIR), optical coherence tomography, and surface-enhanced Raman scattering (SERS) biosensors [47]. Fluorescence and SPR have been pivotal in optical glucose sensing technology for continuous monitoring. Fluorescence sensing relies on glucose-induced alterations in the inherent fluorescence of enzymes, as well as fluorescence resonance energy transfer or quenching between a fluorescent donor and acceptor [48].

Schultz *et al.*, has shown in Figure 2.3(a), a fluorescence affinity hollow fibre sensor for transdermal fluorescence-based glucose monitoring that included coloured beads and Concanavalin A. With reaction times ranging from five to seven minutes, the manufactured sensor can measure glucose within the normal physiological range [49]. The Senseonics CGM sensor is a wearable transmitter and fully implanted glucose sensor that uses fluorescence to monitor glucose in the interstitial fluid (ISF) for commercial use [50].

Because SPR is simple and affordable, it has drawn a lot of interest in the field of biosensors. Changes in refractive index resulting from interactions between glucose molecules and a surface plasmon wave form the foundation of surface plasmon resonance (SPR) sensing technology. Yang et al. introduced an optical tapered fiber glucose sensor in 2020, utilising localised surface plasmon resonance (Figure 2.3(b)). The sensor demonstrated a detection limit of 322 μM and a linear measurement range for glucose from 0 to 10 mM [51].

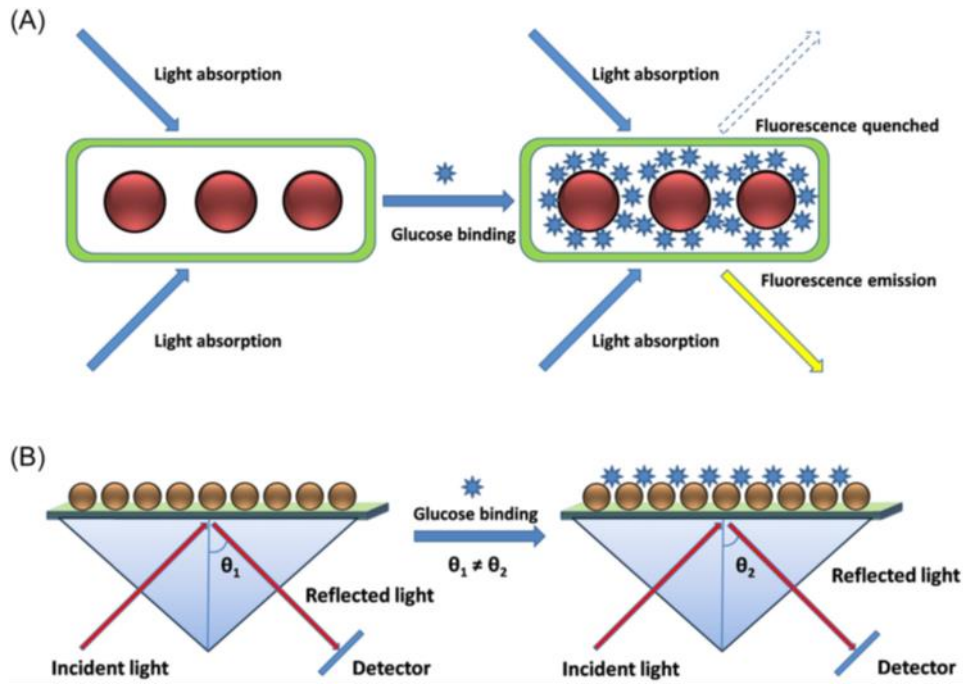


Figure 2.3: A general framework for surface plasmon resonance and fluorescence-based optical glucose monitoring technologies [39].

Despite improvements in optical sensing technology for CGM, several significant obstacles must still be overcome before achieving commercial maturity. Currently, most of the optical equipment are expensive and challenging to miniaturise. Furthermore, optical glucose sensing technology often faces challenges such as a low signal-to-noise ratio and the necessity for invasive sensor calibration, owing to the complexities of physiological fluids. Additionally, non-invasive optical glucose sensing technology lacks a universally applicable algorithm model that accommodates clinical requirements, influenced by individual factors like skin thickness, body fat, and blood volume.

2.3. Wearable technology in CGM

Advances in wearable and digital technology allow people to regularly and non-intrusively monitor their fitness and health status. Technologies classified as minimally invasive (MI) need the removal of bodily fluids as shown in Figure 2.4 [52] such as tears, saliva, sweat, and interstitial fluid (ISF) to detect the glucose levels through the use of enzymes. Biological fluids are acquired *via* minimally invasive or non-invasive techniques, and they contain glucose at concentrations similar to those in blood

quantities. This relationship has made additional bodily fluids viable targets for painless blood glucose monitoring [53].

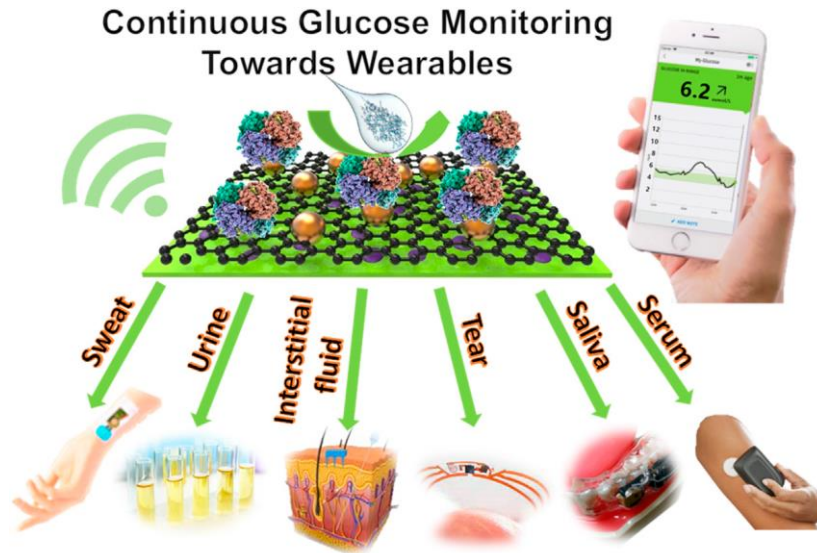


Figure 2.4: the alternative biofluids used as potential analytes for wearable CGM devices [52].

Sweat and interstitial fluid (Figure 2.5) are the most used sources of CGM under wearable technology, are discussed below.

2.3.1. Sweat as an alternative source of CGM

Eccrine and apocrine sweat glands release sweat, a colourless biofluid [54]. Sweat contains glucose in concentrations from 10 to 200 M, which is equivalent to 1 to 2 percent of blood glucose levels. Blood glucose and sweat glucose for people living with diabetes are known to be closely connected. Sweat is highly regarded for applications of continuous, non-invasive monitoring due to its characteristics nature. It is widely accessible from external sample sites, easily obtained, conducive to device placement, and contains physiologically significant electrolytes and metabolites, making it the most readily available source of glucose [55].

Since sweat may easily be collected and continually monitored, sweat biosensing has attracted a lot of attention recently for non-invasive CGM [56]. For instance, Lee *et al.* [57], created a closed-loop system for pH, temperature, and humidity measurements, as well as for microneedle-based point-of-care therapy, non-invasive sweat glucose monitoring, and other measurements. Gao *et al.* [54], developed a completely

integrated wearable flexible sensing array for the simultaneous real-time detection of glucose, lactate, and electrolytes (sodium and potassium ions) in human sweat. To calibrate the glucose sensor's reaction, skin temperature was also measured.

Despite recent improvements in the field of sweat biosensing for continuous glucose monitoring (CGM), there remain several significant challenges. For instance, the diluted glucose levels in sweat that continue to be secreted and the variations in sweat production rates between persons. There are still several obstacles that must be overcome to collect correct sweat glucose data, including temperature variations, skin contamination, infrequent sampling without iontophoretic stimulation, low collection efficiency, and the contamination of older and newer samples [58].

2.3.2. Interstitial fluid as an alternative source of CGM

Glucose is delivered to interstitial fluid (ISF), a type of extracellular fluid, through continuous capillaries from the blood. The three primary artificial intelligence (AI) uses for CGM biosensors are centred around ISF: calibrations, glucose predictions, and closed-loop control algorithms (Figure 2.5). People with diabetes and those without the disease typically have blood glucose levels between 2 and 30 and 4 and 8 mM, respectively. ISF which benefits CGM in two keyways, namely (i) comparatively high glucose concentration and (ii) improved detection accuracy, has almost the same amount of glucose as blood does [59]. The key benefit of ISF over other non-invasive biofluids such as saliva and tears, is that ISF has a comparatively high glucose concentration, improved detection accuracy and dependability. Additionally, CGM technology in ISF is now an established field because it is less invasive to implement than blood [60].

The first wearable ISF enzymatic biosensor uses metallic electrodes and microneedle technology as a detection method for glucose with the goal of being minimally intrusive and causing minimum pain [61]. The microneedles are less invasive because they are in the surface vascular plexus rather than subcutaneous tissue, which may also lessen pain.

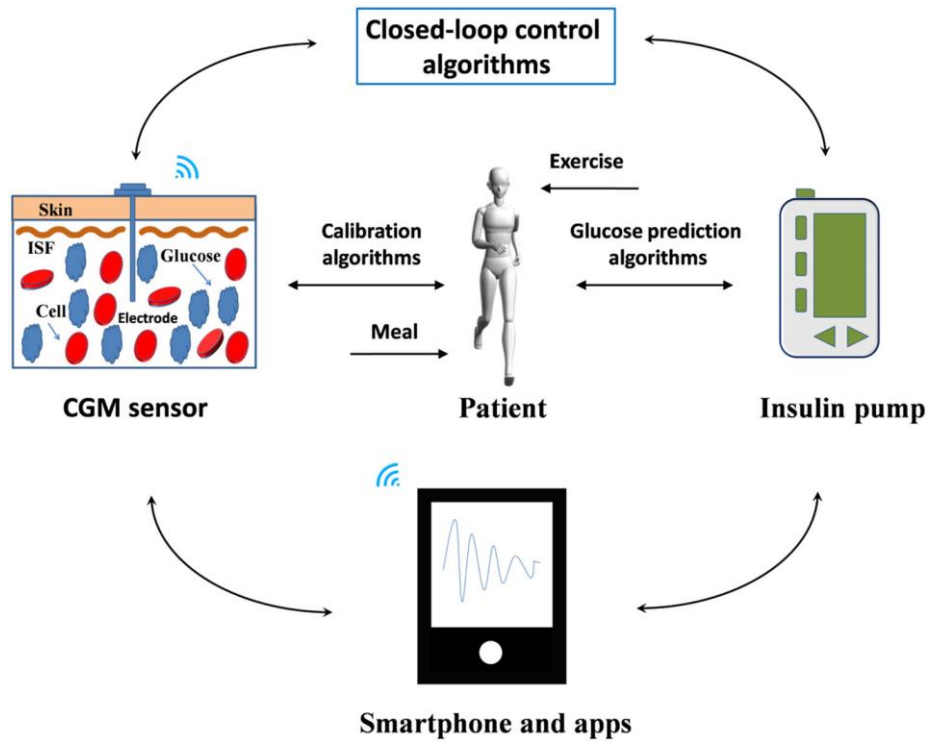


Figure 2.5: Diabetes control through the use of artificial intelligence systems. ISF, or interstitial fluid; CGM, or continuous glucose monitoring [39].

Microwave resonator-based sensors for continuous glucose monitoring from ISF were reported by Baghelani *et al.* [62]. The sensor is made up of a substrate-less split ring resonator tag that is connected to a reader electromagnetically. Implantable glucose biosensors based on microwave resonators have the potential to be integrated into artificial pancreas systems because of the sensing element's low power consumption [63].

Although CGM in ISF is becoming more and more well-known, there are still a number of serious issues with the closed loop. Firstly, there is a delay of five to ten minutes between blood glucose levels and interstitial fluid (ISF) glucose levels due to the time required for glucose to transit from plasma to ISF. Secondly, during the ISF biosensors' development as a subcutaneous glucose sensor, biocompatibility and electrode material loss are major challenges [64].

2.4. Biosensing concept

Target analytes are recognized by receptors in biosensors, and this recognition is converted into a detectable signal by transducers. A typical sensor's operational

profiles are divided into five stages, from analyte (input) to signal (output) [66], as shown in Figure 6:

1. An intriguing biological component that requires to be detected, such as glucose, is called a bio-analyte.
2. A bioreceptor is a molecule, such as an antibody, aptamer, enzyme, nucleic acid, etc., that identifies the analyte precisely.
3. The bio-transducer is a component that transforms one type of energy into another and operates on conversion principles. Another name for this procedure is indication.
4. converter: a component that prepares the transducer's indication for visual presentation.
5. detection system or display: a technique for interpretation that can recognize and locate the converted data and outputs after processing that can be read, including images, tabular data, graphics, or numbers.

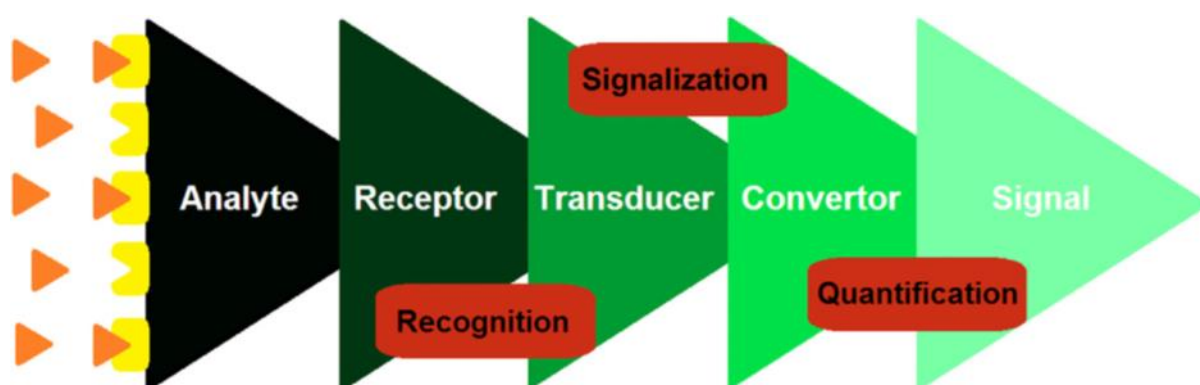


Figure 2.6: Steps in sections of a standard sensor [66].

Regarding sensitivity and selectivity to the intended analyte, a biosensor that has all the afore-mentioned functional sections offers superior advantages. The functionality of the sensors is controlled by the interaction between the bioreceptor and bioanalyte. This interaction keeps signals from unrelated substances from obstructing the target signal in a biosensor.

2.4.1. Electrochemical biosensors

The first multi-analyte biosensors to be scientifically suggested and successfully marketed were electrochemical biosensors [67]. Electrochemical biosensors are constructed using electrochemical transducers and bio-analytes. They harness the

chemical interactions between the immobilized biomolecule and the target analyte, which either generate or consume ions or electrons, thereby influencing the measurable electrical properties of the solution [68]. Faraday's equations for electrolysis and Faradaic current, which are generated at the heterogeneous electrode-solution interface by direct electron transfer during redox reactions, serve as the fundamental foundation for these biosensors [69]. To avoid influencing the working electrodes or being impacted by the solution, reference electrodes, such as silver tetramethylbis (benzimidazolium) diiodide [70], must be kept stationary during such redox processes at a stable potential. Because of the enormous increase in commercial demand for electrochemical biosensing-based technologies, it is imperative to produce reusable and miniaturized reference electrodes [71].

Numerous advantages of electrochemical biosensors have been discovered, such as low power consumption, low noise levels, low production costs, low signal-to-noise ratios, compatibility with downscaling, and the capacity to work with small sample volumes [72]. Nevertheless, the reported limitations include the unstable dynamic range of measurement caused by enzyme saturation kinetics, the potential for other substances to interfere with the measurement, the oxygen requirement and concentration fluctuation in the solution, particularly for glucose measurement, the influence of pH levels or ionic forces on the enzymatic activities, and the biochemical and slow electron transfer processes that limit efficiency and speed [73].

Some of the primary subcategories of electrochemical biosensors include, amperometric, voltametric, potentiometric, conductometric, impedimetric, capacitive biosensors, and are described below.

2.4.1.1. Types of electrochemical biosensors

2.4.1.1.1. Amperometric and voltammetric biosensors

Amperometric biosensors continuously monitor the current (I) or the current density ($J = I/A$) (according to unit area A) which is generated by either oxidation or reduction by a biological process at the outermost portion of a working electrode. Graphite, noble metals, conducting polymers, and modified forms of carbon can all be found in the electrode species. Direct current (DC) measurements are the most basic type of amperometric biosensing, and they are ideally modelled by their ancestor Clark oxygen electrodes.

Amperometric biosensors with linear concentration dependency over a predetermined range have the benefits of being quick, portable, highly sensitive, inexpensive, and with a low limit of detection [75]. However, it is necessary to provide suitable analytes to facilitate an analyte's electrochemical reaction at the working electrode. Some analytes, such as those based on proteins, might not be suitable redox partners in these reactions [76]. It has also been mentioned that due to the uncompensated intrinsic resistance of the DC tests, the accuracy and choice of this method might not be appropriate for purposes requiring high sensitivity [77].

Voltammetry, which measures the current during controlled variations in the potential, is likewise a part of the amperometric biosensing subclass. Through voltammetric biosensors, promising voltammetric properties created considerable prospects in electrochemically transducing biosensing devices. With the help of an electro-analytical technique called voltammetry, information about an analyte may be obtained by varying its potential, and the resulting current can then be measured to determine its value. In addition to polarography [78], cyclic voltammetry (CV), normal pulse, reverse pulse, linear sweep, differential staircase and differential pulse, and square wave [79], other voltammetry techniques are produced from different techniques of potential modulation. Cyclic Voltammetry is one of these techniques that offers information on the Analyte solutions' redox potential and electrochemical action rates.

Voltammetric biosensors work by using the variation in current in relation to changing potential. This concept is necessary to identify solutions containing analytes because peak current density is proportional to analyte concentration and maximum current value serves as identification information. Voltammetric biosensors have the advantages of high measurement sensitivity and simultaneous detection of several analytes [80]. Numerous logistical aspects and elements, including the simplicity of changing the structure and its accessibility, affordability, physical strength, and stability, have been reported. Graphene [81], glassy carbon, polycrystalline boron-doped diamond [82], carbon nanotubes, multi-walled carbon nanotubes, carbon-paste electrodes (CPEs) alongside graphite or carbon microspheres [83] are a few of the materials that can be used to construct these kinds of biosensors.

2.4.1.1.2. Potentiometric and conductometric biosensors

Biosensors that utilize potentiometric transducers evaluate the potential distinction (oxidation or reduction potential) formed between two solutions separated by an ion-selective membrane in order to detect different analyte activities [84]. The operation of potentiometric biosensors depends on the flow of current that results from an electrochemical process in which the electrodes in the solution are subjected to a ramp voltage. A "Boltzmann distribution applied to the redox-active molecular systems," or the Nernst Potential in its simplest form (equation (1) below), is how potentiometric transduction works [85]:

$$V = V^0 + K_B T / e \ln [Ox]/[Red] \dots \dots \dots (1)$$

The ratio $[Ox]/[Red]$, at which V represents the electrode potential, V^0 is the formalized potential of redox instruments, T is the temperature at the absolute level, K_B is the Boltzmann coefficient, and e is the electrical charge of a single electron, represents the amount ratio of oxidized and reduced ions. Potentiometric biosensors primarily work with the potential variation $(V - V^0)$ in equation (1) that arises from a biological activity which alters the redox reaction's equilibria. It can therefore be described by definition as a modification of the Gibbs free energy, as seen in the following equation (2) [86].

$$\Delta G = e (V - V^0) = K_B T \ln [Ox]/[Red] \dots \dots \dots (2)$$

Potentiometric transducers are a subclass that includes conductometric transducers for biosensors. The goal of conductometry is to quantify how the conductivity of ionic species changes in response to a bio-recognition occurrence. Numerous analytes, including glucose, urea, and arginine, have been proved to be detectable using conductometric biosensors [87]. The benefits of conductometric transducing methods include low sensitivity to light, compatibility with low amplitude changing voltages that prevent Faraday interactions on electrodes, low resolution, high cost, and the ability to operate without reference electrodes. However, the lower sensitivity caused by higher signal-to-noise ratios ($>2\%$) has limited the applications of biosensing techniques. Additionally, factors including pH, ionic strength, and buffer capacity play a significant role in how well these biosensors respond [88]. Therefore, it is assumed that the conductometric approach has low selectivity, which presents a technological challenge for its prospective usage in many wearable applications.

2.4.1.1.3. Impedimetric and capacitive biosensors

Because of biological recognition, impedimetric biosensors rely on impedance readings from redox reactions at the analyte/electrode contact. This method utilizes a moderate perturbation bias to identify changes in the current response's fluctuations [89]. The electrochemical impedance spectroscopy (EIS) approach is a popular biosensing technique that measures the resulting impedance changes at the interface between the bio-receptor and analyte by scanning over the perturbation frequency is another byproduct of impedimetric transducing [90].

Capacitive biosensors, also known as transducing, are another intriguing bio-detection method. More studies have been done on how these biosensors might be integrated into wearables to monitor people's health. Analytes attach to an electrode surface in a biosensor that uses capacitive transducing by interacting with immobilized or grafted bio-receptors [91]. By recording the signal changes caused by bio-events altering the thickness or dielectric characteristics of the attached sensor layer, capacitive transducers have been shown to be compatible in identifying analytes, such as hormonal or fragments of DNA [92]. Thus, the first empirical support for the creation of affinity-based capacitive biosensors came from the discovery of an antibody/antigen conjunction on the outermost layer of an electrode in the 1980s, which depended on changes in the dielectric properties, dimension, shape, and charge distribution [93].

Similar to parallel plate capacitors, the active surface (equation (3)) of capacitive biosensors observes variations in capacitance which could be explained as follows. It serves to form one of the sheets of the parallel-plate capacitor:

$$C = \epsilon \cdot A / d \dots \dots \dots (3)$$

where A is the area of the plates, d is the distance between them, and ϵ is the permittivity of the dielectric medium. To adjust the capacitance between the plates, one only needs to modify the values of d, ϵ , or A. Ideally, a bridge circuit can be used to measure the capacitance, where the transducer's output impedance (equation (4)) is given by:

$$X_C = 1 / 2\pi f \cdot C \dots \dots \dots (4)$$

Where f is the excitation frequency and C is the capacitance. Due to changes in the electric field surrounding them, usually, affinity-based capacitive biosensors are able to identify target materials that are conductive or nonconductive [94]. These biosensors can function regardless of the readout distance. In the capacitor analogy, the second plate stands in for the analyte that is in the target samples that conduct. In contrast, a metallic plate is used as the sample of interest while a second plate acts as an insulator across both plates in parallel for measuring nonconductive target samples. The accuracy of a capacitive sensor is dependent on several variables, including the analyte composition, the spacing between the electrode plates, and the amount of charged ions at the electrode-analyte interface. This is because the sensor determines changes in the dielectric characteristics and the width of the dielectric layer.

Since the technique of capacitive transducers relies on detecting alterations that take place following the analyte's binding to a ligand or receptor that is placed on the electrode surface, they offer label-free detection [95]. It is possible to take measurements in real time without the need for pricey labels. Capacitive transducer-based biosensors have several new advantages over labelled biosensors in this regard. However, in addition to the link between the analyte and the receptor at the electrode interface, any binding brought on by inadequate sample separation may also produce a subsequent signal that is challenging to differentiate from the original signal [96].

2.5. Glucose sensor evolution

Enzymatic glucose sensors dominate the biosensing market, particularly in light of the ongoing advancements in self-testing and continuous blood glucose monitoring. The earliest enzymatic glucose sensors were created in 1962 by Clark and Lyons and were based on the catalytic oxidation of glucose in the presence of oxygen [97]. In response to problems with variable background oxygen levels, Updike and Hicks [98] enhanced the oxygen sensor by taking background oxygen levels into consideration. Originally developed in 1973 [99], the first amperometric enzyme glucose sensor measured anodic hydrogen peroxide generation instead of the extremely variable oxygen reduction current. Since this seminal work, the field of glucose sensors has advanced

quickly with new and improved electrochemical technologies that are more inventive, efficient, and superior produced annually.

Still under this evolution, the creation of non-enzymatic glucose sensors has advanced significantly during the past three decades. Numerous selective and highly responsive glucose sensors have been developed thanks to the manufacturing of a wide range of nanomaterials. Due to their outstanding qualities, nanocomposites have received a lot of attention in the application of sensors [100]. The features of both nanomaterials and matrices are utilised in the modification of bare electrode surfaces utilising nanocomposite materials [101]. Numerous biomedical applications, such as biosensors, drug delivery, tissue engineering and actuators, utilise conductive nanocomposites. Below is the discussion of the selected types of glucose sensors, i.e., enzymatic, and non-enzymatic glucose sensors.

2.5.1. Enzymatic glucose sensors

Glucose oxidase (GOx), which catalyses the oxidation of glucose in the presence of O_2 to produce hydrogen peroxide, is used in typical enzymatic glucose sensors. Two protons and two electrons are traded during this reaction. Due to their exceptional selectivity and sensitivity, enzyme-based biosensors are both frequently utilised and intensively researched [102].

Enzymatic glucose biosensors come in three generations [103]. In the first generation, oxygen is converted to hydrogen peroxide via an amperometric method that requires immobilising a catalytic enzyme. The primary drawbacks of this type of biosensor are its oxygen dependence and high operating potential [104]. The second-generation glucose biosensor is built to function under low oxygen pressure and high applied potential while overcoming oxygen deprivation. In these biosensors, GOx is immobilised on redox mediators [105]. Ferrocyanide, conducting organic salts, quinones, and derivatives of ferrocene are examples of common mediators of electron redox. In the third generation of biosensors, electrons are directly transmitted between the electrode surfaces and the immobilised enzyme active sites [106].

Comparing this third type of glucose biosensor to the first and second types, the latter two have better detection properties, especially a larger linear range. The properties of GOx affect how well enzymatic biosensors perform. Studies show that at pH values of more than 8 and less than 2, GOx becomes unstable [107]. In addition, humidity

and temperatures higher than 40°C greatly diminish the enzyme activity. Furthermore, enzyme immobilization techniques are also expensive and complicated [108].

2.5.2. Non- enzymatic glucose sensors

Non-enzymatic glucose sensors are becoming more and more popular due to the intrinsic drawbacks of conventional glucose enzymatic biosensors, which include their expensive manufacturing processes, low enzyme stability, pH level dependence, and other restrictions. Also, glucose needs to be directly oxidized on an electrode with electrocatalytic activity and a transition metal centre for non-enzymatic biosensors to work [109].

Non-enzymatic glucose biosensors are not affected by enzyme immobilization, in contrast to enzymatic sensors. Non-enzymatic biosensors have been impacted by the lack of enzymes to increase their level of stability. It is possible to raise the detection's responsiveness and applicability by functionalised nanoparticles operating as immobilization platforms or catalysts. Using materials devoid of enzymes and a basic method of glucose electro-oxidation, the fourth-generation glucose biosensor was developed [110]. When compared to their enzyme counterparts, these biosensors show noticeably better electrocatalytic performance for glucose detection because of the inclusion of nanomaterials on the electrode surfaces [111]. Non-enzymatic electrocatalysts include, but are not limited to, metallic nanoparticles, metal oxide nanoparticles, bimetallic/alloy nanostructures, carbon nanomaterials, and metal/carbon nanomaterial-based nanocomposites [112]. Selecting the most effective catalyst for glucose detection is the most crucial step in the development of non-enzymatic biosensors. Numerous studies have demonstrated the effectiveness, selectivity, and durability of specific non-enzymatic electrochemical glucose biosensors [113].

2.6. Carbon nanotubes as material of interest for detection of glucose

Graphite sheets rolled into cylindrical shapes are known as carbon nanotubes (CNTs), and they have a diameter of about 100 nm and a length of micrometres [114]. They are thought to be a derivation of both fullerene and carbon fibers, since their molecules are made up of sixty carbon atoms structured closed tubes [115]. Carbon nanotubes are classified into two groups based on the number of carbon layers they contain.

Single-walled carbon nanotubes (SWCNTs) are tubes with a diameter of 0.4 to 2 nm and contain single graphene layer bundles arranged hexagonally. Multi-walled carbon nanotubes (MWCNTs) are tubes with two or more walls made of individual graphene sheets and have a diameter of 1 to 3 nm [116].

Strong mechanical qualities, a large surface area, and outstanding electrical and thermal conductivities characterize CNTs [117]. These characteristics have made them very popular lately, and many researchers have begun utilizing them in different kinds of electrical biosensors. Their single-dimensional empty cylindrical nano-chemistry, which controls the successful capture and enhancement of electrons transmitted from the analyte to the electrode surface, is thought to be responsible for the improved performance [118]. Most CNT-based NEG sensors are made functional by coupling them with metal or metal oxide nanostructured materials. A great deal of research has been done on the various deposition methods that could be used to bond CNTs to the electrode materials [119]. In a nutshell, the creation of diverse non-enzymatic glucose sensors based on carbon nanomaterials is a result of the increased interest in cutting-edge nanotechnologies.

2.6.1. Functionalisation of multiwalled carbon nanotubes

In aqueous medium applications, multiwalled carbon nanotubes (MWCNTs) have proven to be a major negative for both natural and therapeutic purposes. Because functionalization can be achieved through a variety of chemical processes such as absorption, electrostatic interaction, or covalent bonding, it is consequently used as a cure for this problem [120]. These alterations drastically change their biocompatibility profile and make them water soluble. By functionalizing the surface, individual tubes are shortened in length in accordance with the Vander Waals force [121].

2.6.1.1. Covalent functionalisation of MWCNTs

Covalent reactions and the oxidation process can be used to create the function of carbon nanotubes. Oxidizing chemicals such nitric acid, oxygen air, concentrated sulfuric acid, and aqueous hydrogen peroxide are used in the oxidation process [122]. Near the tube's end, carboxyl groups are created as a result of the covalent process that damages the sidewalls. The oxidation processes and the oxidizing agent are impacted by the role of carboxyl groups. Surface functional groups can alter the usefulness, stability, and dispensability of carbon nanotubes (CNTs), in addition to their

surface charge and reactivity. Functionalized MWCNTs are less dangerous than unfunctionalized or pure MWCNTs. Functional MWCNTs with -COOH influence the properties and bioactivity of CNTs [123].

2.6.1.2. Noncovalent functionalisation of MWCNTs

Surfactant molecules or polymers in carbon nanotubes (CNTs) are coated by non-covalent functionalization, as opposed to covalent functionalization. The physical characteristics of CNTs are successfully preserved by the non-covalent technique, and the length restriction brought on by sonication during the functionalization process has no effect on the network's chemical structure [124]. The majority of this functionalisation techniques' components are polymer wrapping, biomacromolecules, or surfactants. Hydrophobic micelle and polymer components should be contained when supramolecular complexes form on the CNTs [125]. Soluble CNT maintains its electrical characteristics and aromatic structure due to non-covalent dispersion. Because they have distinct oxidation structures, polyaniline, polypyrrole, polythiophene, and its derivatives are excellent materials for altering the surfaces.

2.7. Metal chalcogenides as material of interest for the detection of glucose.

In recent years, significant progress has been achieved in the creation of non-enzymatic glucose sensors using transition metal hydroxides, phosphides, nitrides, oxides, and chalcogenides [127]. Among the metal chalcogenides that have garnered the greatest attention in recent decades are metal sulfide, metal selenide, and metal telluride. Metal chalcogenides combine optical, thermomechanical, electrical, and chemical characteristics. Mixed-metal chalcogenides are also well-known for their remarkable capacity for conversion, physicochemical stability, practically tiny band gap, and variety of structural morphologies [128].

2.7.1. Recent developments: metal chalcogenides-based glucose sensors

In electrochemical devices including fuel cells, supercapacitors, and glucose sensors, transition metal chalcogenides (TMCs) have attracted a lot of interest lately due to their inexpensive, chemical and physical characteristics that vary on the layer, and unprecedented high electrocatalytic activity [129]. The primary causes of this improvement in TMC's electrochemical activity are the lattice's improved covalency and decreased anion electronegativity, which cause the materials' bandgap to

decrease and their electrochemical adaptability to increase. A lowered bandgap improves the transfer of charges within the catalyst composite and across the catalyst-electrolyte interface, while electrochemical adaptability makes it easier for chemically reactive intermediates to adsorb on the outer layer of the catalyst through localized oxidation or reduction of the transition metal active site [130].

Kannan and Rout [131] investigated an outstanding performance non-enzymatic glucose sensor constructed from one-step electrodeposited nickel sulfide. A nanostructured NiS thin coating was created using a single-step electrodeposition technique. Using amperometry and cyclic voltammetry (CV), a non-enzymatic glucose sensor was developed to investigate the electrocatalytic activity of NiS thin layer toward glucose oxidation. With an 8-second reaction time, the built-in sensor displayed great sensitivity and a low detection limit of $7.43 \text{ A M}^{-1} \text{ cm}^{-2}$ and 0.32 M , respectively.

Conversely, a simple hydrothermal approach has been used to synthesize the nanostructured compound of NiSe₂ nanosheets (NiSe₂-NS) for electrocatalytic purposes [128]. To advance the development of electrochemical monitoring applications, the generated NiSe₂-NS was used as a customized electrode substrate for the electrochemical oxidation of glucose. Thus, the amperometric technique and cyclic voltammetry were used to ascertain the electrochemical properties of NiSe₂-NS regarding glucose oxidation. Because of its increased electro-active surface area, electrical conductivity, and stability, the recently discovered NiSe₂-NS showed remarkable electrocatalytic performance for the detection of glucose. The obtained electrochemical parameter readings were compared to previously published modified electrodes, and it was discovered that the linear range ($0.099 - 1252 \text{ } \mu\text{M}$), sensitivity ($5.6 \text{ } \mu\text{A Mm}^{-1} \text{ cm}^{-2}$), and limit of detection (23 nM) had all been significantly greater. This is beneficial since the fabricated glucose sensor has a lower detection limit than current Ni-based modified electrodes. The produced NiSe₂-NS/glassy carbon electrode showed precise recovery figures, suggesting that actual time evaluation of samples applications could employ it for glucose detection.

After synthesizing Cu₂Se via one-pot hydrothermal synthesis, Singh et al. [127] found that it was a very excellent bifunctional sensor that could detect glucose and dopamine together with good selectivity. At different applied potentials, this method's copper selenide may electro-oxidize dopamine and glucose. Dopamine oxidized at 0.2 V ,

while glucose oxidized at 0.35 V. This nonenzymatic copper selenide sensor showed outstanding sensitivity for both glucose ($15.341 \text{ mA mM}^{-1} \text{ cm}^{-2}$) and dopamine ($12.43 \text{ } \mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$) with a relatively small limit of detection (0.26 mM and 84 nM), respectively. This sensor offers an intriguing alternative for determining the presence of glucose and dopamine in human bodily fluids with low quantities of these biomolecules due to its high sensitivity and low detection limit. It is also a great option for inclusion into reduced-power ongoing monitoring systems due to its extremely low practical potential for detection. Along with great selectivity for glucose and dopamine detection, this sensor also showed excellent repeatability, reuse ability, and ongoing functional stability within the presence of other interferents.

2.8. Techniques for synthesis of nanomaterials

Two main approaches i.e., top-down, and bottom-up, shown in Figure 2.7 are normally employed for the synthesis of nanomaterials.

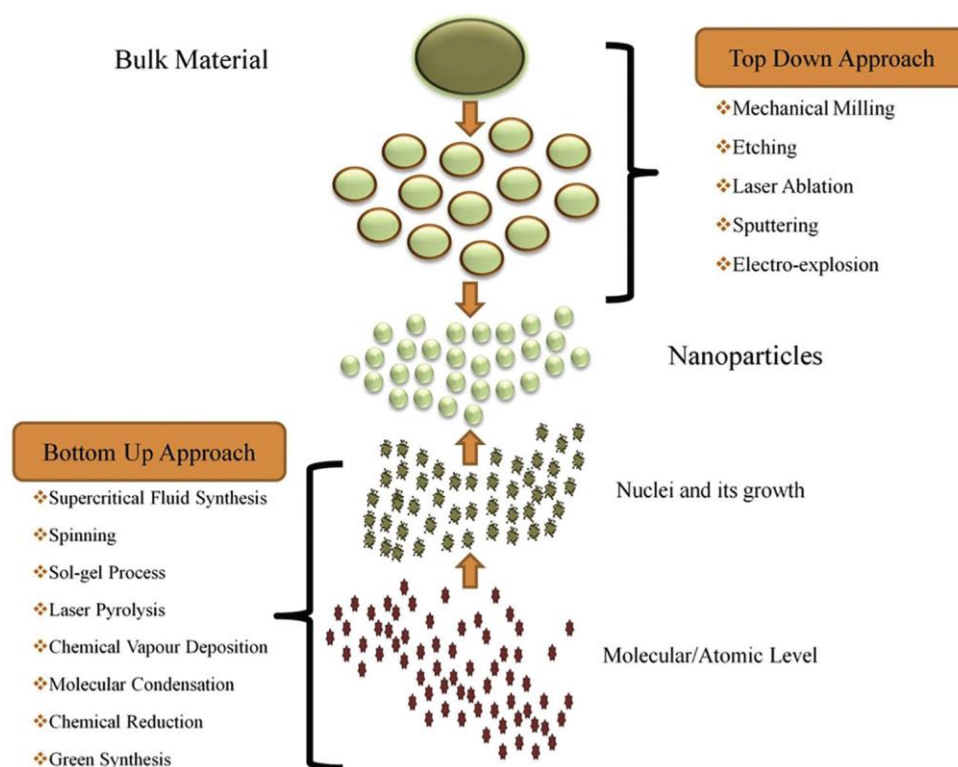


Figure 2.7: The synthesis of nanomaterials via top-down and bottom-up approaches [132].

2.8.1. Top-down approaches

To create nanostructured materials, bulk materials are separated in top-down methods. Researchers have used a variety of techniques in recent decades, including

mechanical milling, laser ablation, sputtering, and electrospinning. The aforementioned techniques are explained in the subsequent sections

2.8.1.1. Mechanical milling

Mechanical milling is a useful technique for converting bulk materials into nanoscale materials. Mechanical milling is a useful method for producing blends of various phases and nanocomposites. Copper, nickel, magnesium, zinc, oxide and carbide-reinforced aluminium alloys, as well as a range of distinct nanocomposite compounds, are among the materials that can be produced mechanically in addition to durable spray coatings [133]. A new class of nanoparticles called ball-milled carbon nanomaterials may have applications in energy storage, environmental remediation, and energy conversion [134].

2.8.1.2. Electrospinning

Electrospinning is one of the simplest top-down methods for producing nanostructured compounds. It is widely used to turn a variety of materials, mostly polymers into nanofibers [135]. An important development in the realm of electrospinning was coaxial electrospinning. In coaxial electrospinning, the spinneret is made up of two coaxial capillaries. In these capillaries, core-shell nanoarchitectures can be created by using two viscous liquids, or a viscous liquid acting as its outer shell and a non-viscous liquid as the core. Coaxial electrospinning is a simple, effective top-down process that can be used to create core-shell ultrathin fibres on a large scale. It is possible to extend these extraordinarily thin nanoparticles by many centimetres. Core-shell and empty polymer, inorganic, organic, and hybrid materials have all been developed using this technique [136].

2.8.1.3. Sputtering

Through the process of sputtering, particles with high energy such as gas or plasma are sprayed onto solid surfaces to create nanomaterials. One practical method for producing thin films of nanomaterials is sputtering [137]. Intense gaseous ions strike the desired layer throughout the sputtering deposition manipulate, and based on the incoming gaseous-ion energy, they may physically expel small atom clusters [138,139]. Different techniques for sputtering can be used, including magnetron, radio-frequency diode, and DC diode sputtering [140].

A chamber which was previously evacuated and then refilled with the sputtering gas is often the site of sputtering. Gas ions are created when electrons that are unbound hit with the gas due to a high voltage delivered to the cathode objective. As the positively charged ions approach the cathode objective in the electric field, they speed rapidly and eject atoms from its surface [141]. WSe₂-layered nanofilms on SiO₂ and carbon paper substrates were created via magnetron sputtering [142]. Sputtering is an interesting process because, compared to other techniques, it is less expensive and produces nanomaterials with a composition similar to the target material but with less impurities.

2.8.1.4. Laser ablation

By striking the intended substance with a powerful laser beam, laser ablation synthesis produces nanoparticles. Throughout the laser ablation process, the source substance or precursor dissipates due to the powerful laser irradiation, generating nanoparticles. Laser ablation is an environmentally conscious way of producing noble metal nanoparticles because it does not require extra chemicals or stabilizing substances [143]. Numerous nanomaterials, such as oxide composites, metal nanoparticles, nanomaterials composed of carbon, and ceramics, can be produced using this technique [144]. Rapid laser ablation in liquids is an interesting technique for generating monodisperse colloidal nanoparticle suspensions without ligands or surfactants. The average size and distribution of nanoparticles may be precisely controlled by altering the fluence, wavelength, and incorporation of laser salt [145].

2.8.2. Bottom-up approach

By building upon individual atoms or molecules, nanostructures are created via the bottom-up method. This approach involves the deliberate separation of atoms or molecules during their assembly into targeted nanostructures. In recent decades, researchers have used a variety of techniques, including chemical vapor deposition (CVD), solvothermal and hydrothermal, sol-gel, and reverse micelle approaches. Below is a discussion of some of the previously described methods.

2.8.2.1. Chemical vapor deposition (CVD)

The process of creating nanomaterials composed of carbon involves the use of chemical vapor deposition techniques. During CVD, vapor-phase intermediates are

used in a chemical reaction to create a tiny layer on the outermost layer of the substrate [146]. If precursors have a long shelf life, good chemical purity, strong evaporation stability, low cost, and no harmful properties, they are considered suitable for CVD. Furthermore, no pollutants should be left behind after the precursor disintegrates [147].

For instance, to create carbon nanotubes using the CVD process, a substrate is heated to extremely high temperatures in an oven. Then, as a precursor, a carbon-containing vapor (such as hydrocarbons) is gradually added to the entire system. Carbon atoms are released during the breakdown of the gas at high temperatures, and these atoms reassemble to produce carbon nanotubes on the supporting material [148]. On the other hand, the kind and shape of the nanomaterial which is produced greatly depend on the catalyst chosen. Ni and Co catalysts produce multilayer graphene during the CVD-based synthesis of graphene, whereas a Cu catalyst only produces monolayer graphene [149]. All things considered, CVD is an excellent technique for creating exceptional nanomaterials [150], and it is widely recognized that it can create two-dimensional nanomaterials [151].

2.8.2.2. Solvothermal and Hydrothermal methods

One of the most popular and widely applied processes for creating nanostructured compounds is the hydrothermal approach [152]. By means of a heterogeneous process carried out in an aqueous solution at elevated temperatures and pressures in the vicinity of the critical point in a confined vessel, the hydrothermal process produces nanostructured materials [153]. The hydrothermal process and the solvothermal technique are comparable. The use of a non-aqueous solution is the only distinction. Generally, closed systems are used for hydrothermal and solvothermal processes [154]. To engineer nanomaterials, the advantages of the hydrothermal and microwave approaches such as rapid volumetric heating, high reaction rates, size and shape control by tuning reaction parameters, and energy efficiency have recently drawn a lot of attention to the microwave-assisted hydrothermal technique [155]. Hydrothermal and solvothermal processes are fascinating and useful methods for generating various nano-geometries of materials, such as nanowires, nanorods, nanosheets, and nanospheres [156].

2.8.2.3. The sol-gel method

The sol-gel procedure is one of the wet chemical techniques commonly used to make novel nanomaterials. This method is applied to produce a range of high-quality metal-oxide-based nanomaterials. The reason this method is called a sol-gel approach is that, while the metal-oxide nanoparticles are being produced, the liquid precursor transforms into a sol, and the sol subsequently transforms into a network structure called a gel [157].

The conventional starting point for the sol-gel method of creating nanomaterials is metal alkoxides. There are multiple techniques by which nanoparticles can be created using the sol-gel methodology. To create a sol, the metal oxide is first broken down in water or achieved with the help of alcohol. In the next phase, the solvent becomes more viscous during condensation, forming structures with pores that allow oxidation to occur. Metal-hydroxo- or metal-oxo-polymers are created in the solution because of the formation of hydroxo- (M-OH-M) or oxo- (M-O-M) bridges during the condensation processes [158]. The structure, characteristics, and permeability of the substance are altered by continuous polycondensation. As one ages, permeability diminishes and the spacing amongst the colloidal particles widens. After the aging process, the gel is dried to remove organic solvents and water. Calcination, the last stage, yields nanoparticles [159].

2.8.2.4. Reverse micelle methods

One helpful way for creating nanomaterials with the appropriate sizes and shapes is the reverse micelle strategy. Typical micelles are produced in a water with oil dispersion; these micelles possess tails with hydrophobic properties pointing toward the centre that contains captured oil droplets. On the contrary, reverse micelles are produced in a water-in-oil solution when the hydrophilic heads are oriented toward a water-containing core [160]. For the creation of nanoparticles, the reverse micelles core functions as nano reactants. It serves as a water reserve to produce nanoparticles. By altering the water-to-surfactant ratio, the size of these nanoreactors may be regulated, which in turn affects the size of the nanoparticles produced using this technique.

When the water concentration is lowered, tiny water droplets develop, which results in the formation of smaller nanoparticles [161]. Consequently, the reverse micelle

technique provides an easy way to make uniform nanoparticles with precisely defined sizes. The remarkably small, monodispersed nanoparticles are created by the reverse micelle process.

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Chapter 3: CHARACTERISATION TECHNIQUES

Characterization is essential to ensure that the produced particles are nanoscale. In material science, "characterisation" refers to the extensive, all-encompassing techniques utilized to look into the composition and characteristics of the material. This fundamental process is necessary in order to comprehend the topic from a scientific standpoint. The term "characterisation" refers to any process used in material analysis, such as density calculation, heat analysis, and mechanical testing, that is required to look into the microscopic structures and characteristics of materials. Newer, more advanced methods of characterization are constantly being developed to complement those that have been utilized for decades. Characterisation lets us determine the content and structure of materials and allows us to assess how well the process worked. Some approaches are qualitative, while others are quantitative. The methods for characterizing nanoparticles that are commonly used and efficient will be the main emphasis of this chapter (Figure 3.1). This chapter's main goal is to give a theoretical and practical explanation of the techniques used to characterize a wide variety of materials.

Scanning electron microscope (SEM) analysis, energy dispersive X-ray analysis (EDX), transmission electron microscope (TEM) analysis, Raman spectroscopy, ultraviolet-visible spectrometry (UV-vis), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), X-ray diffraction (XRD), and zeta potential analysis offer more advantages to observe submicron-sized materials. The basic principles and mechanisms of the techniques are discussed here in detail.

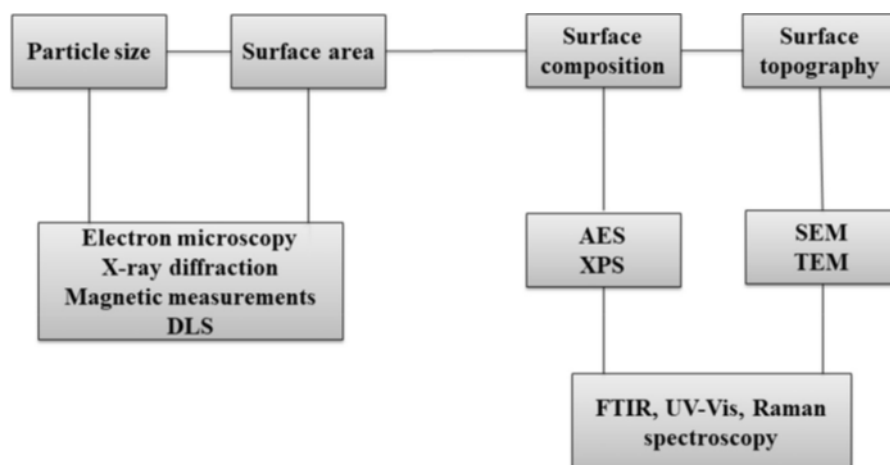


Figure 3.1: Common characterisation techniques for nanoparticles [1].

3.1. Scanning Electron Microscopy (SEM)

Unlike an optical microscope, which uses a light beam to see the object, scanning electron microscopy (SEM) uses an electron beam [1]. At the top of the apparatus is an electron cannon that shoots a highly concentrated electron stream. The two primary forms of electron guns are thermionic guns, which heat the filament until the electrons stream out, and field emission guns, which produce a powerful electric field that tears electrons from the atom.

The SEM scans the surface of the sample using high-energy electron beams [2]. SEMs differ from conventional light microscopes in that they employ light waves to magnify a picture. In SEM, the electron beam interacts with the specimen surface as it passes through it. When the object is impacted by the electron incident beam, it releases X-rays as well as primary, secondary, and Auger electrons. The primary, or backscattered, and secondary electrons are used in SEM. The primary electrons provide valuable information on crystallography, topography and the magnetic field of the sample. Secondary electrons are used in SEM to provide high-resolution images that highlight details at a size of 1-4 nm. Distinctive X-rays are used in an approach known as EDX to identify elemental compositions [3]. The backscattered electrons are also used in this technique to form the picture.

Figure 3.2 displays a schematic diagram illustrating how a SEM operates. Before arriving at the last lens, the electron beam travels via the scanning coils in the electron column. As a result, the beam is deflected in both the vertical and horizontal directions, allowing for raster pattern scanning across the rectangular surface. Signals are

detected and amplified by electronic devices, after which they are shown as pictures on a cathode ray tube. The raster scanning and the microscope are in sync. The image that is shown is a distribution map of the signal strength that is released from the specimen's scanned area [4].

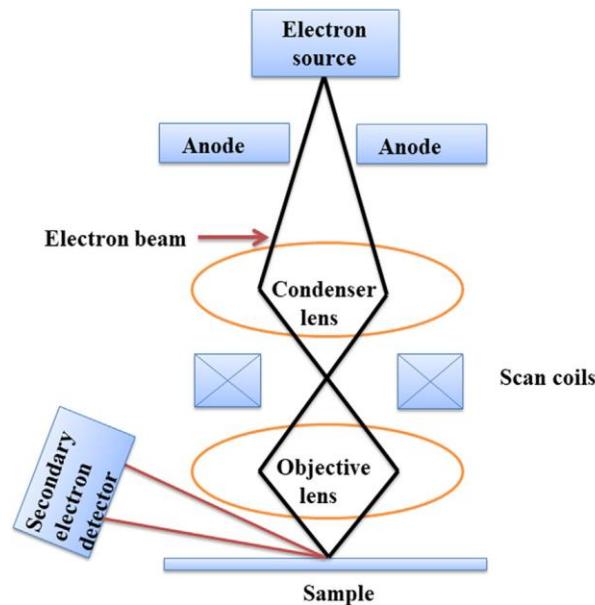


Figure 3.2: A schematic diagram on the working of SEM [4].

3.2. Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is an analytical technique used to visualise the smallest structure in matter. Unlike optical microscopy, which rely on light in the visible spectrum, TEM can reveal the stunning detail at the atomic scale by magnifying nanometre structures up to 50 million times. An objective lens is used to concentrate and magnify the transmitted electrons, which are then utilized to create the image. An electron beam interacts with the sample during transmission. Figure 3.3 displays the fundamental elements of TEM.

Images obtained via TEM and light microscopic imaging have different contrasts. Unlike absorption, diffraction happens when the electron beam interacts with the substance. The plane's orientation with respect to the electron beam affects the diffraction strength [5]. The electron beam is transmitted at certain angles and significantly diffracted from the axis at others. The specimen has holders installed so that it can be tilted to meet specific diffraction requirements. The technique of generating a contrast image by obstructing the electrons that are deflected by the

aperture and permitting the unscathed electrons to pass through is referred to as "light field". The electrons that are deflected can also be used to produce a dark field image [6].

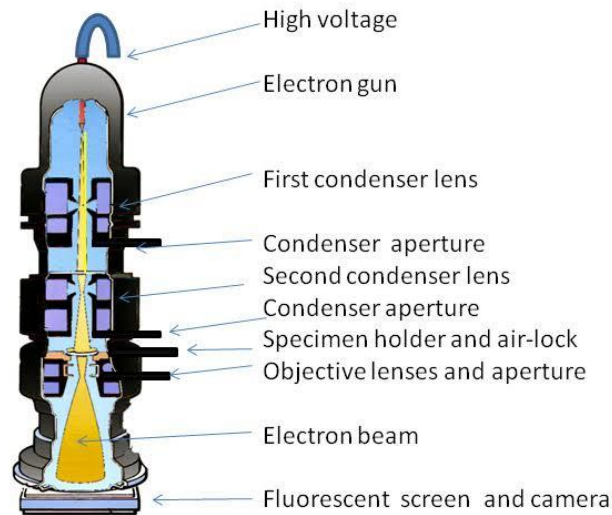


Figure 3.3: Basic components of TEM [6].

3.3. Energy Dispersive X-ray analysis (EDX)

By analysing near-surface elements, this approach provides an overall mapping of the sample and estimates the elemental fraction at various points.

Basic principle

EDX and SEM/TEM are used together. An electron beam with an energy of 10–20 keV strikes the conducting sample, causing the material to produce X-rays. The energy of the X-rays produced is determined by the material under study. EDX is not a surface science technique because the X-rays are generated in a zone that is only a few microns deep. By moving the electron beam over the sample, it is possible to get an image of each element. The chemical analysis performed by EDS is quick. After the areas of the image are selected, a qualitative analysis can be completed in approximately 30 seconds [7]. If there are any heavy metal ions present in the nanoparticles, the EDX can be used to determine their composition or quantity near and at the surface. For example, EDX makes it simple to identify surface nanoparticles such as palladium, gold, and silver. Low atomic number elements are challenging for EDX to identify.

3.4. Ultraviolet-visible spectroscopy

The components of this method include a detector, a monochromator, sample and reference beams, a tungsten lamp for the visible area and a deuterium lamp for the UV (Figure 3.4). The UV spectrum is obtained by subjecting the material to UV radiation. Cuvettes are held inside the device and are used to hold samples that are to be introduced into the light path. Cuvettes can be made of quartz, silica, glass, or plastic. It is not possible to utilise glass and plastic cuvettes for absorbance measurements below 310 nm due to their absorption of wavelengths below that range. Since quartz cuvettes are transparent to wavelengths longer than 180 nm, they are utilized for ultraviolet absorption measurements [8].

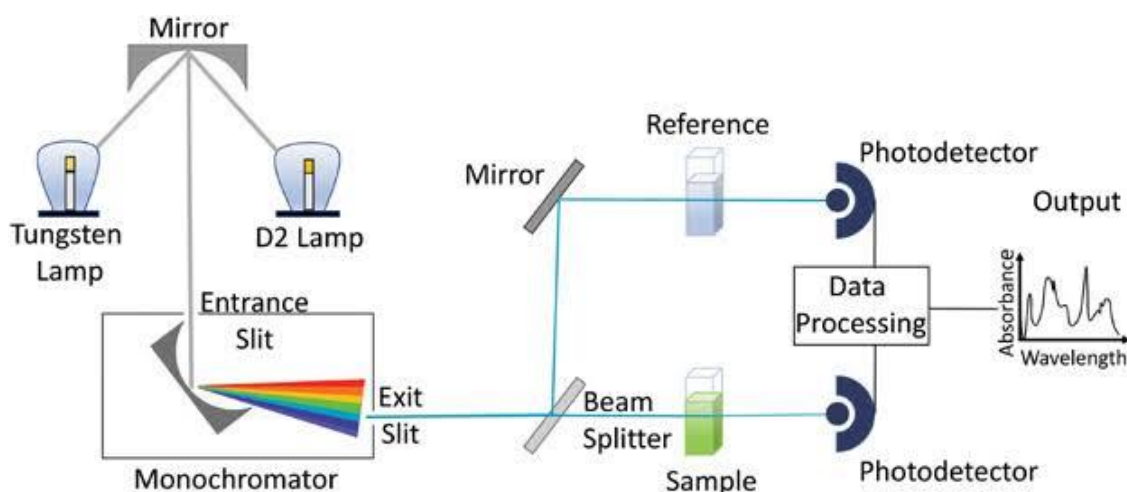


Figure 3.4.: Basic components of UV-Visible spectroscopy [8].

Working principle

A beam that does not come into contact with the sample as it moves from the light source to the detector is known as the reference beam. The sample is exposed to UV light with a continually varying wavelength through interaction between the sample and the sample beam. When the wavelength of light released matches the energy level that elevates an electron to a higher molecular orbital, energy is absorbed. The detector records the ratio of the sample to reference beam strengths. The maximum absorption intensity of the sample determines the wavelength [9]. When there is a discrepancy in the intensities, the wavelength is shown as having the highest UV absorption since the reference beam's intensity is higher than the sample's. UV-vis spectrum refers to the reflectance or absorbance spectra in the UV-visible region.

A portion of the light may be absorbed when it passes through the solution, with the remaining portion being transmitted through it. Transmittance is defined as the ratio of light entering the sample to light leaving the sample at a specific wavelength [10]. Absorbance is the negative logarithm of transmittance. Beer-Lambert Law states that a sample's absorbance at a given wavelength is directly related to the path length and the concentration of the absorbing material, or $A = \epsilon cl$, where c is the concentration, l is the path length, ϵ is the absorptivity, and A is the absorbance.

It is necessary for the incident radiation to be monochromatic and for the absorbing media to be homogenous in order to prevent radiation scattering. Metal nanoparticles having a diameter of less than 100 nm efficiently scatter optical light because of their surface plasmon resonance. This is the collective resonance of the conduction electrons in the metal. The material's composition, surroundings, form, and size all affect the bandwidth of the spectrum, magnitude, and wavelength peak of the plasmon resonance associated with the nanoparticle [11,12]

3.5. Raman spectroscopy

Raman spectroscopy is a type of spectroscopy used to investigate a system's vibrational, rotational, and other modes. It uses the Raman scattering or inelastic scattering of monochromatic laser light. An energy shift is produced by the interaction of the photons with the laser light, and this energy shift tells us something about the phonon modes in the system [13].

The sample is usually illuminated using a laser beam. A lens is used to gather the electromagnetic radiation from the laser hit location, which is then directed through a collimator. The molecule is relaxed into a vibrationally excited state after being excited from a ground state [14]. The Stokes Raman scattering is produced by this. Anti-stokes Raman scattering is what happens if the molecule was already in a vibrational state. The molecule must undergo a shift in polarizability to display Raman scattering. While the Raman shift is determined by the vibrational level involved, the strength of the Raman scattering is dependent on the change in polarizability. Surface enhanced Raman spectroscopy, resonance Raman spectroscopy, and stimulated Raman spectroscopy are examples of advanced versions of the technique [15].

3.6. Fourier transform infrared spectroscopy (FT-IR)

FTIR analysis uses infrared light to scan the samples in order to identify organic, inorganic, and polymeric components. Changes in the distinctive absorption band pattern unmistakably point to a modification in the composition of the substance. Finding additives, recognizing decomposition and oxidation, describing unknown materials, and detecting impurities in a material are all made easier with the help of FTIR [16].

Figure 3.5 illustrates an FTIR schematic diagram. A source, sample cell, detector, amplifier, A/D convertor, and computer are the common components of an FTIR spectrometer. After traveling through the interferometer, radiation from the sources reaches the detector. The A/D convertor and amplifier amplify the signal and convert it to a digital signal. The signal is then sent to the computer to perform the Fourier transform [17].

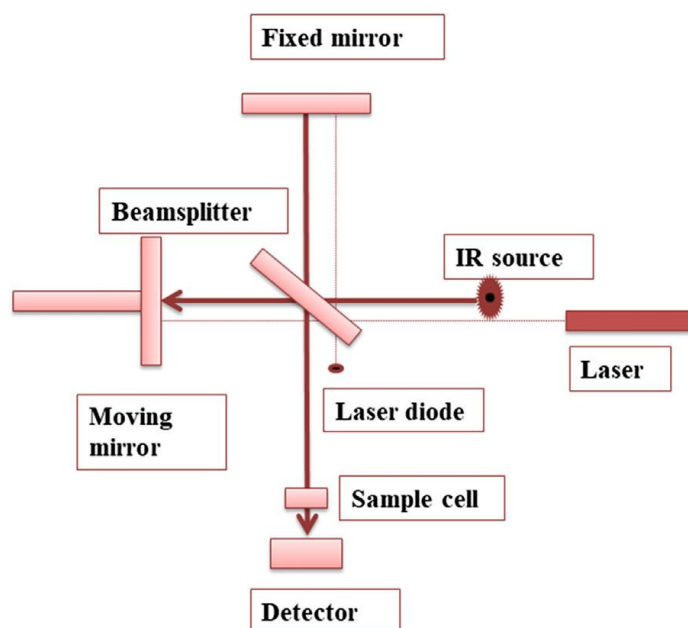


Figure 3.5: A schematic diagram of FT-IR [18].

A portion of the $10,000\text{--}100\text{ cm}^{-1}$ infrared radiation is absorbed by the sample, while the remaining portion passes through. The sample transforms the absorbed radiation into rotational or vibrational energy. The resulting signal, which is the molecular fingerprint of the sample, is typically a spectrum ranging from 4000 to 400 cm^{-1} at the

detector. Since each molecule has a distinct fingerprint, FT-IR is a vital instrument in the chemical identification process [18].

3.7. X-ray diffraction

XRD analysis is used to determine the sample's crystallinity. It can be used to provide additional information in addition to identifying purposes. If the material is in mixture form, the element proportions can be ascertained using XRD patterns. The data analysis can also be used to determine the degree of crystallinity, the departure of a certain element from its ideal composition, and its structural state. The X-ray beam is partially transmitted because of the interaction between the atomic planes and the sample; the remaining portion is absorbed, refracted, scattered, and diffracted. X-rays are diffracted by each element in a different way, depending on the atomic arrangement and the type of atoms [19].

Shorter wavelength electromagnetic radiation is what X-rays are. X-rays are created when electrically charged particles with enough energy slow down. Electrons are drawn to the metal target by the high voltage that is kept between the electrodes. X-rays are produced at the impact spot and radiate outward in all directions. The resulting X-rays are collimated and focused on a finely ground powder sample. The detector picks up X-rays, and either an electronic or computer processes the signals. By changing the angle of the source, sample, and detector, an X-ray or spectrograph scan can be obtained. When the X-ray strikes a crystal lattice, scattering happens. Destructive interference occurs when maximum scattering is removed due to self-interference. Diffraction happens when the scattering is in phase with the scatterings from another plane. Here, constructive interference takes place as new wave fronts are created by combining the reflections. Because every crystalline substance has a different atomic structure, each one diffracts X-rays in a different way. Bragg's equation (5) [20] is used for measuring the angle of diffraction which is given by

$$2d \sin\theta = n\lambda \dots\dots\dots (5)$$

where d denotes the spacing between the planes, θ denotes the angle of incidence, n denotes an integer, and λ denotes the beam wavelength (Figure 3.6).

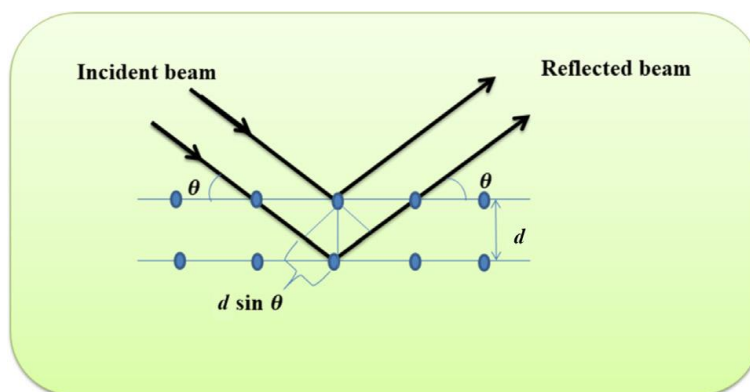


Figure 3.6: Schematic representation of Bragg's equation for XRD [20].

The fundamental geometry of this method consists of an X-ray detector and a radiation source that are positioned around the perimeter of the graded circle that is centred on the powder specimen (Figure 3.7). There are divergent slits between the detector and the specimen as well as between the specimen and the X-ray source. This helps to collimate the radiation, lower background noise, and restrict dispersed radiation. A goniometer is connected to the sample holder and detector so that the rotation of the specimen and the detector rotate in tandem at a 2:1 ratio [21].

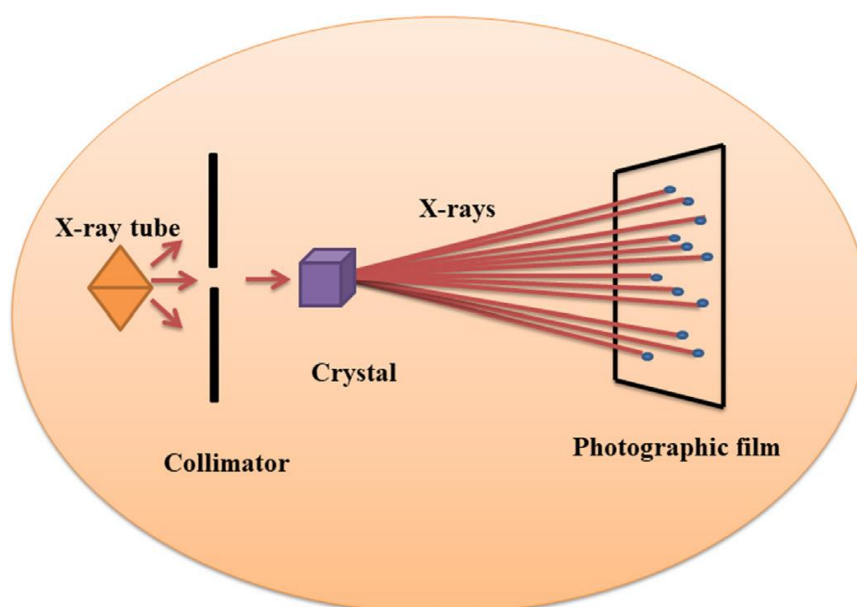


Figure 3.7: X-ray diffraction set-up [20].

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CHAPTER 4: RESEARCH METHODOLOGY

This chapter provides the list of chemicals, and reagents used in the study, followed by the methodological procedures undertaken to prepare chemically oxidised MWCNTs, metal chalcogenides nanoparticles and COOH-MWCNTs incorporated with metal chalcogenides nanocomposites. Lastly, this chapter describes in full details the procedure for the fabrication of non-enzymatic biosensor for the detection of glucose.

4.1. Chemicals and reagents

All chemicals were of analytic grade and were utilised as received without further purification. Distilled water was used as a solvent for the preparation of all the material used in this study. Multi walled carbon nanotubes (MWCNTs, > 98% purity) were purchased from Sabinano (Pty) Ltd (South Africa). Thiourea (NH_2CSNH_2), Sulfuric acid (H_2SO_4 , 98%), Nitric acid (HNO_3 , 55%), sodium borohydride (NaBH_4), sodium hydroxide (NaOH), Nafion, Polyvinylpyrrolidone (PVP), Iron chloride hexahydrate ($\text{FeCl}_3 \cdot \text{H}_2\text{O}$), Zinc chloride hexahydrate ($\text{ZnCl}_2 \cdot 6\text{H}_2\text{O}$), sodium phosphate monobasic, sodium phosphate dibasic, phosphate buffer solution (PBS), Dimethylformamide (DMF), ethanol (EtOH) and glucose were purchased from Merck.

4.2. Functionalisation of the pristine-MWCNTs using chemical oxidation

The chemical oxidation of p-MWCNTs was achieved using the modified method by Chiang, *et al* [1]. An amount of 2.00 g of pristine MWCNTs (P-MWCNTs) was added in 200 mL acidic mixture of H_2SO_4 : HNO_3 with a ratio of 3:1 (v/v). The solution was ultrasonicated overnight at room temperature to open the endcaps of the nanotubes. The solution was further refluxed at 70°C for 24 hours to allow for complete attachment of the -COOH functional group onto the surface of the nanotubes. The oxidised MWCNTs were washed and filtered several times with distilled water until the pH of the filtrate was at pH 7. Followed by drying in an oven at 80°C for 24 hours. The solid MWCNTs were then denoted as COOH-MWCNTs. The NH_2 -MWCNTs were donated by Mintek.

4.3. Synthesis of metal chalcogenides (ZnS and FeS_2) nanoparticles

The synthesis of ZnS and FeS_2 nanoparticles was achieved using the hydrothermal method previously employed by Yan, *et al* [2]. An amount of the selected metal source,

iron chloride hexahydrate or zinc chloride hexahydrate were dissolved in 60 mL of distilled water to make 0.01 M solution. Then, 0.3 g of PVP, and 0.01 M thiourea in 60 mL of distilled water were added to each of the above solutions. After stirring at room temperature for 30 min, 60 mL of 0.25 M NaBH₄ solution was added which resulted in the solution turning black for FeS and milky white for ZnS, followed by further stirring at room temperature for a period of 2 hours. The mixtures were transferred into Teflon-lined stainless-steel autoclave and treated at a temperature of 130°C for 6 h. The solution was then left in suspension overnight. Finally, the precipitates were separated from solutions by centrifugation and washed repeatedly with water and ethanol to remove all soluble salts. The products were then dried at 80°C in an oven for 12 hours.

4.4. In-situ hydrothermal incorporation of functionalised-MWCNTs with metal chalcogenides

The synthesis of FeS₂/COOH-MWCNTs, FeS₂/NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs was achieved using the modified method by Alshrefi, *et al* [3]. An amount of the selected metal source (Zinc chloride hexahydrate or Iron chloride hexahydrate) was dissolved in 60 mL of distilled water to make 0.01 M solution. Followed by the addition of the freshly made stoichiometric thiourea distilled water solution (30 mL, 0.01 M) as the sulphur source. The reaction mixture was then vigorously stirred at room temperature for a period of 30 minutes. Then 0.3 g of PVP was added and stirred for another 30 minutes, followed by the addition of 60 mL of 0.25 M of NaBH₄. Stirring was further continued for a period of 2 hours at room temperature. After 2 hours, a 100 mg of COOH-MWCNTs or NH₂-MWCNTs was added to the mixture and sonicated for an hour to allow for complete attachment of the nanoparticles onto the surface of the f-MWCNTs. The reaction mixture was then transferred into a 100 mL stainless steel autoclave and placed in a preheated oven at 130°C for a period of 6 hours. The solution was then left in suspension overnight. The reaction product was then washed and filtered several times with a mixture of ethanol and distilled water. The product was finally left to dry overnight at 80°C.

4.5. Fabrication of functionalised-MWCNTs incorporated with metal chalcogenides sensor

The glassy carbon electrodes (GCEs) were cleaned with 0.3 mg of alumina powder and then thoroughly rinsed with deionized water prior to modification of the electrode.

The cleaned GCE were then allowed to dry in air. Then, 5 mg of functionalised MWCNTs incorporated with metal chalcogenides was dissolved in a 5 mL DMF solution. The mixture was sonicated at room temperature for 30 minutes to allow complete dispersion. Then, using the drop casting method, 3.0 μ L of the dispersed mixture of functionalized metal chalcogenides was added to the working electrode on the surface of screen-printed carbon electrodes. This combination was then allowed to dry for a few hours. Lastly, about 5% of nafion was added to ensure complete binding of the nanocomposites onto the surface of the electrodes [4].

4.6. Preparation of buffer solution

Sodium phosphate dibasic and sodium phosphate monobasic were used to make the 0.1 M phosphate buffer solution (PBS), which was the buffer employed in this investigation. To make 0.1 M of the solutions, 7.1 g of sodium phosphate dibasic was dissolved in 500 mL of distilled water. After that, 500 mL of distilled water was used to dissolve 6.0 g of sodium phosphate monobasic to create a 0.01 M solution. The solution of 0.01 M sodium phosphate monobasic was gradually added to the 0.1 M sodium phosphate dibasic solution until the required pH was obtained in order to obtain 0.1 M PBS [5].

4.7. References

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CHAPTER 5: RESULTS AND DISCUSSION

In this chapter, synthesis, and characterisation of FeS₂ and ZnS nanoparticles, functionalised-MWCNTs (COOH-MWCNTs and NH₂-MWCNTs) decorated with ZnS

and FeS₂ nanoparticles are named as ZnS/COOH-MWCNT, ZnS/NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs, are characterised. These synthesised materials were employed for the construction of the modified fabricated electrode for enzyme-free glucose sensing application. The sensor performance factors such as sensitivity, stability and dynamic linear range and limit of detection (LOD) of the sensor were systematically investigated and compared with other non-enzymatic glucose sensors reported in literature.

5.1. Characterisation of FeS₂ and ZnS nanoparticles

5.1.1. Morphological Characterisation of FeS₂ and ZnS nanoparticles

The synthesized FeS₂ and ZnS nanoparticles were characterised for their morphology, crystallinity, and purity by using TEM and Raman studies.

5.1.1.1. TEM characterisation of FeS₂ and ZnS nanoparticles

Figure 5.1 (a) shows the TEM image of FeS₂ nanoparticles. Different sizes, as seen in the particle size histogram (Figure 5.2 (a)), were measured using ImageJ software. The particles observed were near-spherical in shape, with an average diameter of 29.15 nm. Figure 5.1 (b) shows the TEM image of ZnS nanoparticles. The spherical-like ZnS nanoparticles with an average diameter of 5.56 nm (Figure 5.2 (b)) were observed. As will be shown, the ZnS and FeS₂ nanoparticles were produced independently, that is, without MWCNTs, in order to serve as reference samples during the electrochemistry stage. These particles were not the ones used to decorate the functionalised MWCNTs.

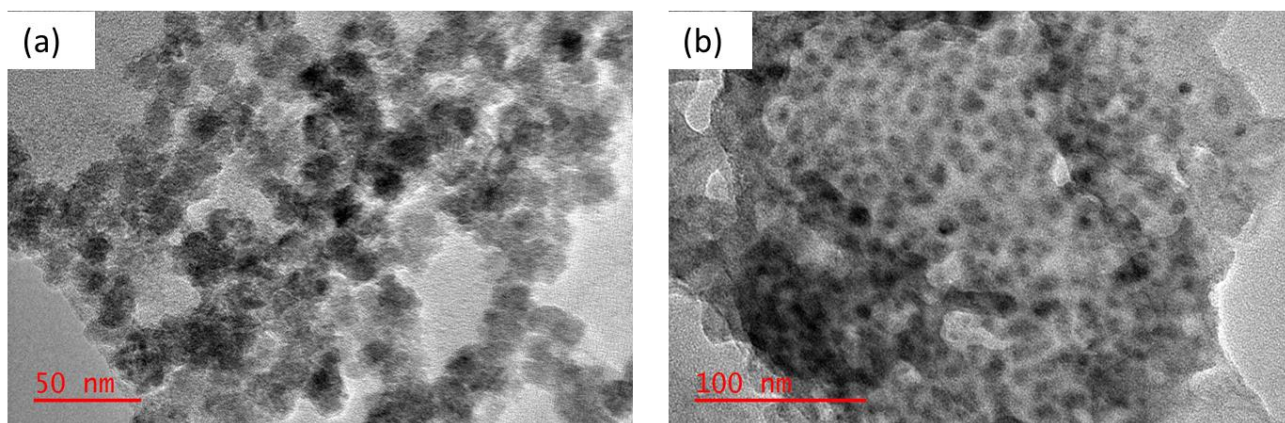


Figure 5.1: TEM images of FeS₂ and ZnS NPs.

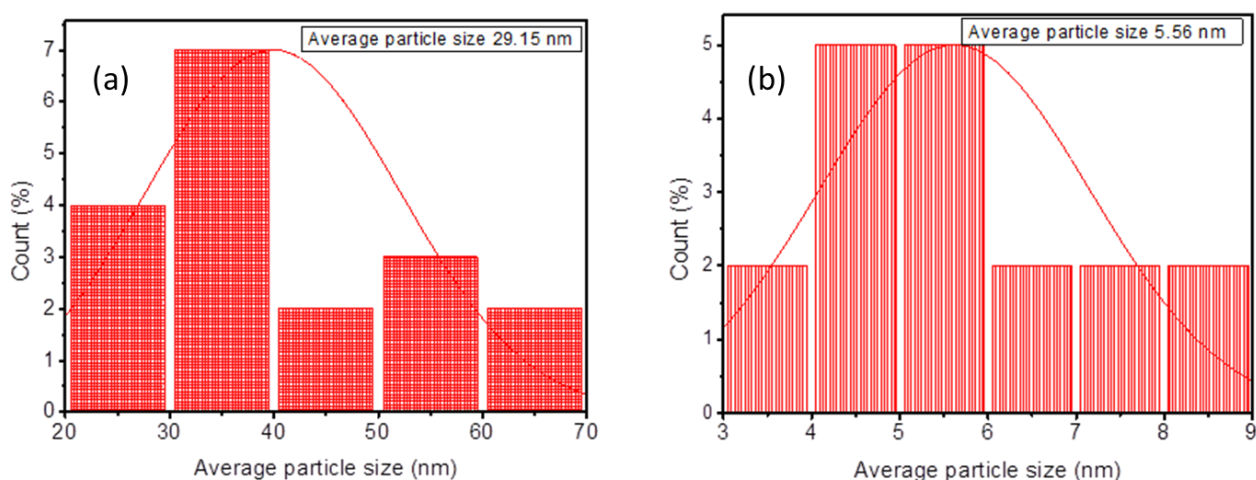


Figure 5.2: Particle size histogram for FeS₂ and ZnS NPs.

5.1.1.2. Raman characterisation of FeS₂ nanoparticles

The Raman spectrum of Figure 5.3 presents the spectral components of FeS₂ nanoparticles. According to Raman peaks at 110 cm⁻¹, 188 cm⁻¹, and 331 cm⁻¹, the substance may be iron sulfide (FeS₂), which has a chemical formula like that of pyrite and marcasite [1]. A prominent peak for FeS NPs can be found at 331 cm⁻¹ (Eg), which corresponds to pyrite FeS₂. The five strong Raman signals of 155, 222, 249, 441 and 472 cm⁻¹ were attributed to the presence of FeS₂ due to sulfur sufficiency and sulfur vacancies [2].

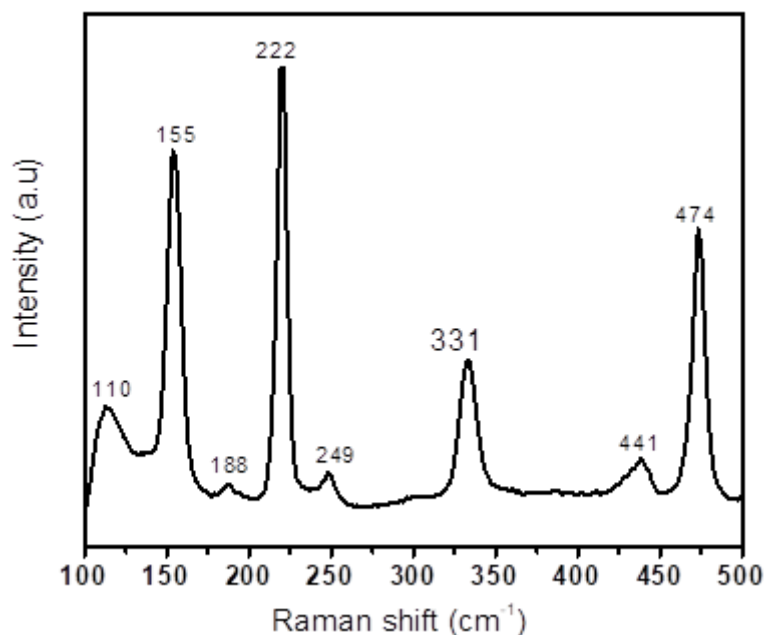


Figure 5.3: Raman spectrum of FeS₂ NPs.

5.1.2 FT-IR characterisation of FeS₂ and ZnS nanoparticles

Figure 5.4 displays the results of an FT-IR analysis of FeS₂ and ZnS NPs in the 4000–400 cm⁻¹ range. The characteristic IR spectrum for FeS₂ shows that there is a peak at 560 cm⁻¹ corresponding to FeS₂ bonds. The FTIR spectra of ZnS samples shows that their transmission bands are nearly the same. The prominent bands are located at 556 and 428 cm⁻¹, respectively, and are caused by ZnS stretching and vibration [3].

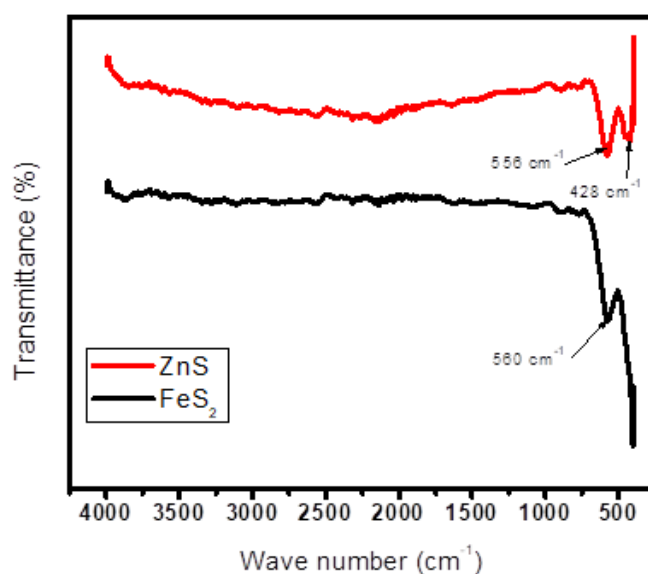


Figure 5.4: FT-IR spectra of FeS₂ and ZnS NPs.

5.1.3 UV-VIS characterisation of FeS₂ and ZnS nanoparticles

Measurements of absorbance in the 200–800 nm region are used to characterise the optical characteristics of FeS₂ and ZnS nanoparticles. The UV-visible absorption spectrum of ZnS NPs is displayed in Figure 5.5, the spectra reveal an excitonic peak for UV absorption at 260 nm. Since this wavelength is significantly blue-shifted from the bulk ZnS band-edge (330 nm), it is suggestive of populations of small monodisperse nanoparticles that are displaying quantum-confinement effects [4]. The linear portion demonstrates that these nanoparticles' method of transformation is direct in nature [5]. FeS₂ NPs were seen in Figure 5.5 to have absorption in a wide range from Vis to NIR with absorption peak at 330 nm, which are assigned to the band-to-band transition of FeS₂ [6].

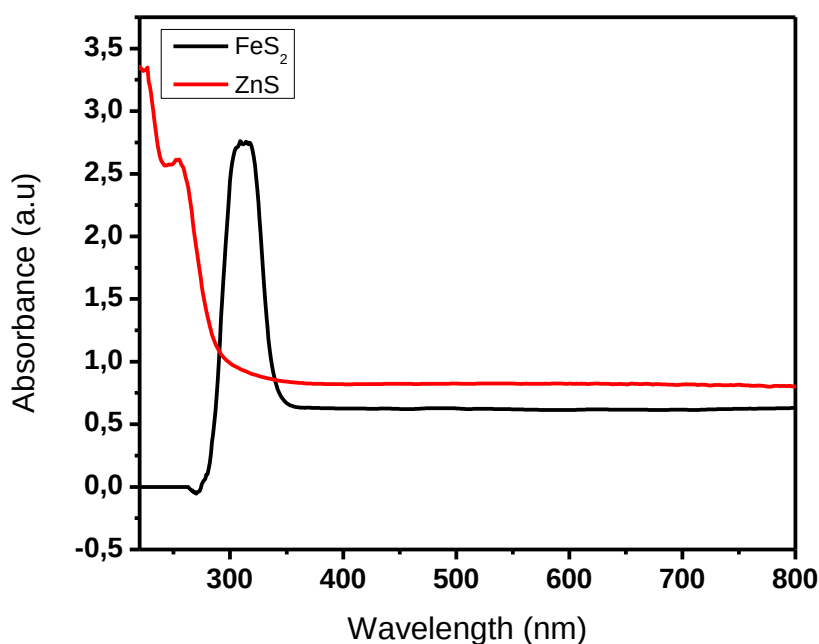


Figure 5.5: UV-VIS spectra of FeS₂ and ZnS NPs.

5.2. Characterisation of FeS₂/Functionalised-MWCNTs based nanocomposites

5.2.1. Morphological Characterisation of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites

The synthesized FeS₂/Functionalised-MWCNTs based nanocomposites were characterised for its morphology, crystallinity, and purity by using TEM, EDX, XRD and Raman studies.

5.2.1.1. TEM-EDX characterisation of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites

TEM analysis was performed on the p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs samples to further investigate their structures and confirm the good distribution of the nanoplatelets on the surface of the MWCNTs. The TEM images of the p-MWCNTs (Figure 5.6 (a)) confirms the presence of tubular structure of the nanotubes. The functionalised (COOH-MWCNTs (Figure 5.6 (b) and NH₂-MWCNTs (Figure 5.6 (c)) present tubes that are long, straight, and uniformly distributed without any damage. In the TEM images of FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs nanocomposites, as shown in {Figure 5.6 (d and e)}, homogeneous dispersion of near-spherical FeS₂ nanoparticles on the surface of the COOH-MWCNTs and NH₂-MWCNTs are clearly displayed. The average diameters for COOH-MWCNTs and NH₂-MWCNTs were found to be 25,14 nm and 32.39 nm respectively. The presence of FeS₂ nanoparticles, as depicted in Figure 5.6 (d and e), is a clear indication and confirmation that the in-situ incorporation of the nanoparticles on the functionalised MWCNTs was successful.

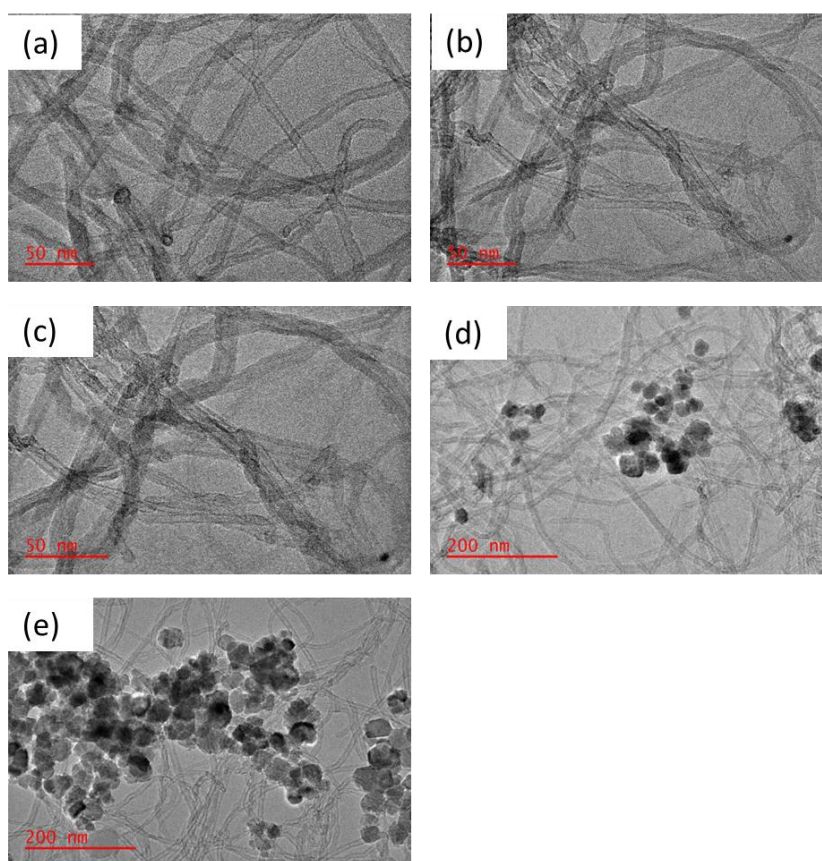


Figure 5.6: TEM images of p-MWCNTs (a), COOH-MWCNTs (b), NH₂-MWCNTs (c), FeS₂/COOH-MWCNTs (d) and FeS₂/NH₂-MWCNTs (e).

Figure 5.7 (a) and (b) illustrates the particle size histograms of FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs nanocomposites with average particle size of 24,15 nm and 32,39 nm.

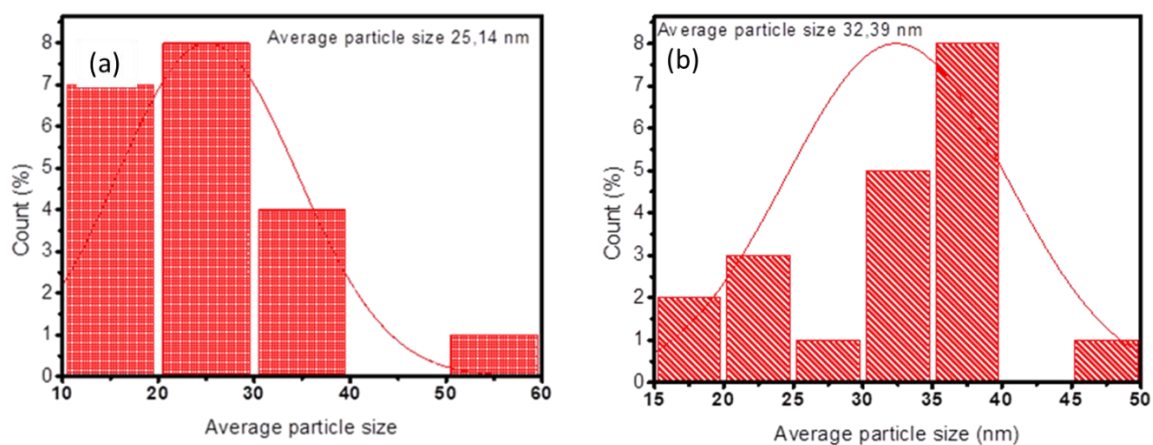


Figure 5.7: Particle size histogram of FeS₂/COOH-MWCNTs (a) and FeS₂/NH₂-MWCNTs (b).

EDX spectra of FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs (Figure 5.8 (a) and (b)) confirmed that C, Fe, S, O and Cu (for FeS₂/COOH-MWCNTs), and C, Fe, S, O, N and Cu (for FeS₂/NH₂-MWCNTs) elements are present in the nanocomposites. The EDX spectra (Figure (a) and (b)) exhibits a strong carbon peak, which agrees well with the high percentage of carbon in the MWCNTs. The EDX spectra further exhibits Fe and S peaks, which confirms the presence of the FeS₂ nanoparticles in the nanocomposites. A strong nitrogen peaks shown in the FeS₂/NH₂-MWCNTs nanocomposite confirms the presence of the amine group. A strong copper peak shown in the spectra originates from the copper grid used for TEM analysis.

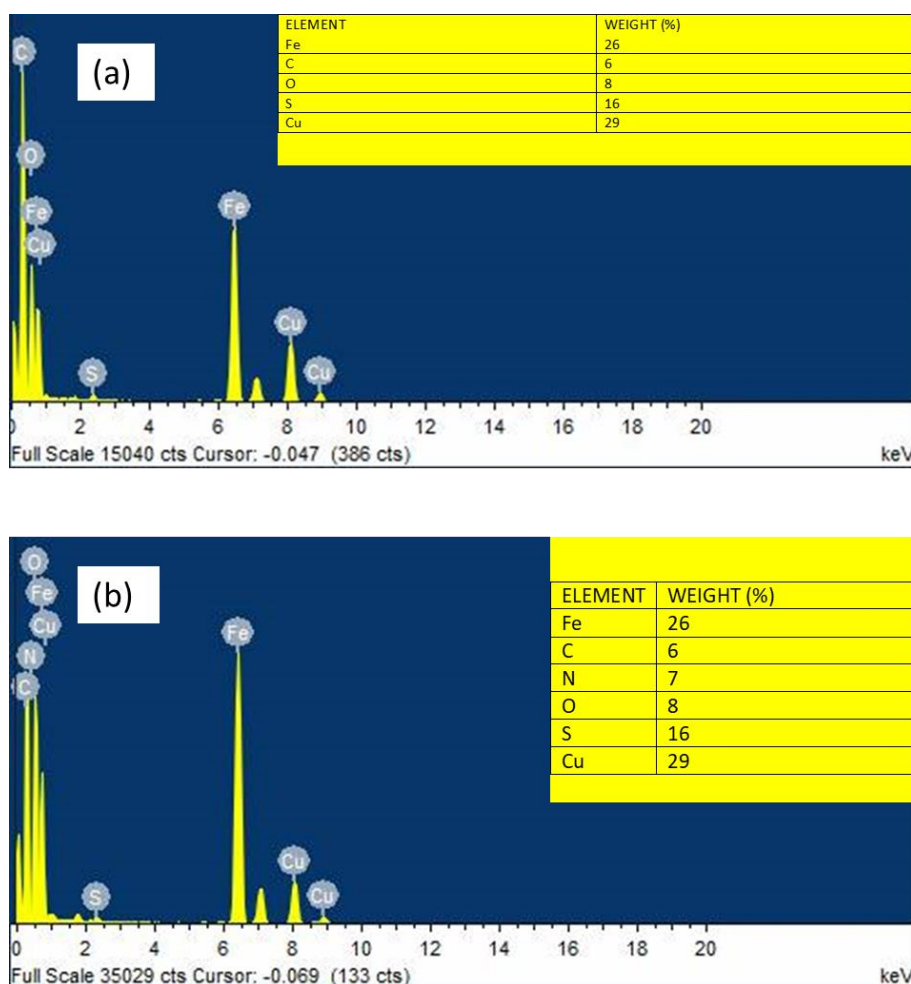


Figure 5.8: EDX spectra of FeS₂/COOH-MWCNTs (a) and FeS₂/NH₂-MWCNTs (b).

5.2.1.2. XRD characterisation of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites

The crystalline structure and purity of the p-MWCNTs and the as-synthesised nanocomposites were investigated using XRD powder diffraction (XRD). Figure 5.9 shows the XRD patterns of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, and FeS₂/COOH-MWCNTs. X-ray diffraction pattern of hexagonal MWCNTs depicted two significant peaks at 2 θ values of 26.94° and 43.15°, corresponding to the (002) and (100) crystal plane respectively. Similarly, the XRD patterns of COOH-MWCNTs and NH₂-MWCNTs also represented two peaks appearing at the same 2 θ angles. In the XRD diffraction pattern of FeS/COOH-MWCNTs, characteristic peak at 2 θ values of 29.51°, 36.30°, 46.76°, 57.28° and 62.69° were well attributed to the (111), (210), (221), (311) and (023) planes of cubic pyrite (FeS₂) (JCPDS card no. 42-1340) respectively [7]. In addition to the observed FeS₂/COOH-MWCNTs peaks, a characteristic peak of MWCNTs (002) was also detected, but weak, which might be ascribed to the coverage of COOH-MWCNTs with FeS₂ nanoparticles. The observed diffraction peaks were broad, signifying that the particle size was very small [8]. The peaks of FeS₂/COOH-MWCNTs nanocomposite are well matched with the original material, indicating that the addition of FeS₂ do not have any effect on the crystal structure of the MWCNTs [9].

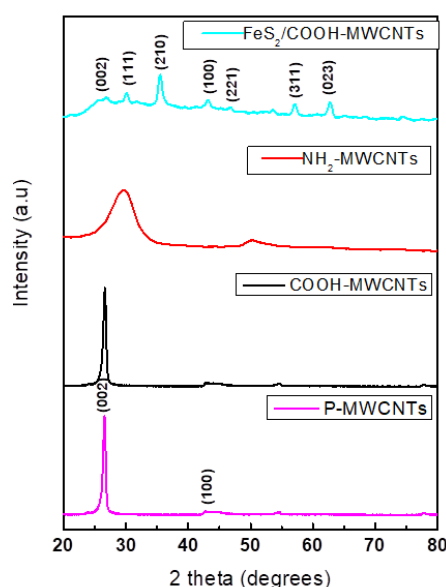


Figure 5.9: XRD patterns of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs and FeS₂/COOH-MWCNTs.

5.2.1.3. Raman characterisation of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites

The p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs were characterised using Raman spectroscopy to understand the graphitic nature of the synthesized MWCNTs (Figure 5.10). Three vibration bands namely, D (~1350 cm⁻¹), G (~1580 cm⁻¹), and G' (~2700 cm⁻¹) [10], are investigated in the MWCNTs' Raman spectra to ascertain their crystalline makeup and quality. The Raman spectra of MWCNTs exhibit a D band, which is indicative of lattice disorders such as broken sp² and sp³ bonds and carbonaceous impurities. The planar C-C stretching, or G band, represents the MWCNTs' graphitic character (crystal structure). Two phonons, which are believed to be the outcome of a second-order dispersion process that produces an inelastic phonon, are responsible for the vibrations that make up the G' band [11]. The extended organisation of the carbon atoms within MWCNTs is indicated by the G' band. The degree of oxidation and functionalisation can be measured using the ratio (I_D/I_G) between the intensities of the D and G bands, which is proportionate to the degree of defect concentration. I_D/I_G ratios for p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs are 1.095, 1.171, 1.289, 1.061 and 0.601, respectively. These numbers imply that whereas sidewall flaws in MWCNTs have been somewhat exacerbated by oxidation and amination, the subsequent FeS₂ deposition steps did not alter the sp³/sp² carbon ratio of the COOH-MWCNTs and NH₂-MWCNTs structure any further.

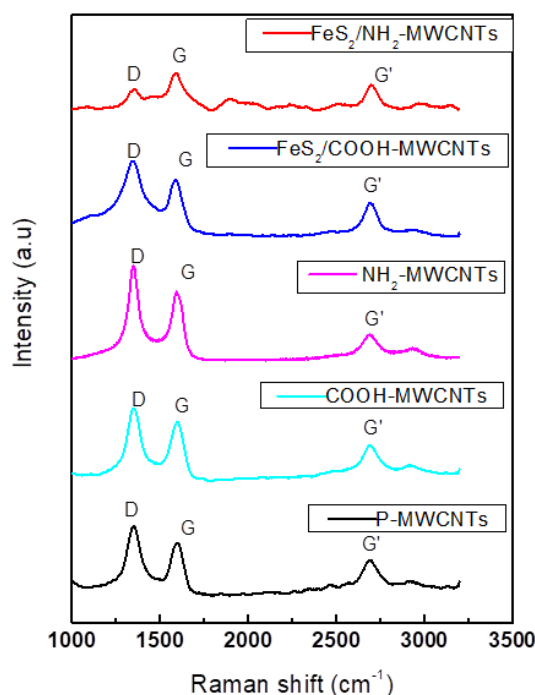


Figure 5.10: The Raman spectra contrast for the p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs.

5.2.2. FT-IR spectra FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites

Fourier transform infrared spectroscopy (FT-IR) was employed to characterise all nanomaterials. The FT-IR spectra of p-MWCNTs, acid-treated MWCNTs (COOH-MWCNTs), amine functionalised-MWCNTs (NH₂-MWCNTs), FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs, are shown in (Figure 5.11). The FT-IR spectra of p-MWCNTs show absorption peaks at 2982 cm⁻¹ and 1450 cm⁻¹, that aligns with the stretching vibrations of C-H. The stretching vibrations of the carboxylic groups' C=O and O-H are represented as absorption peaks in the COOH-MWCNTs' FT-IR spectra at 1600 cm⁻¹ and 3400 cm⁻¹. The characteristic peak that can be detected at 3404 cm⁻¹, 1600 cm⁻¹, and 1047 cm⁻¹ in the FT-IR spectra of NH₂-MWCNTs represents the stretching vibrations of N-H, C=O, and C-N [12]. Peaks at 1398 cm⁻¹ (C-H) and 1047 cm⁻¹ (C-N stretching) were visible in the FT-IR spectra of FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs. The C-H stretching band is located at 2996 cm⁻¹. The FT-IR spectra of FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs displayed a peak at 429 cm⁻¹ and 438 cm⁻¹ corresponding to the formation of FeS₂. This entailed that the FeS₂ bond remained

intact even after in-situ incorporation of the FeS₂ nanoparticles on the surface of MWCNTs.

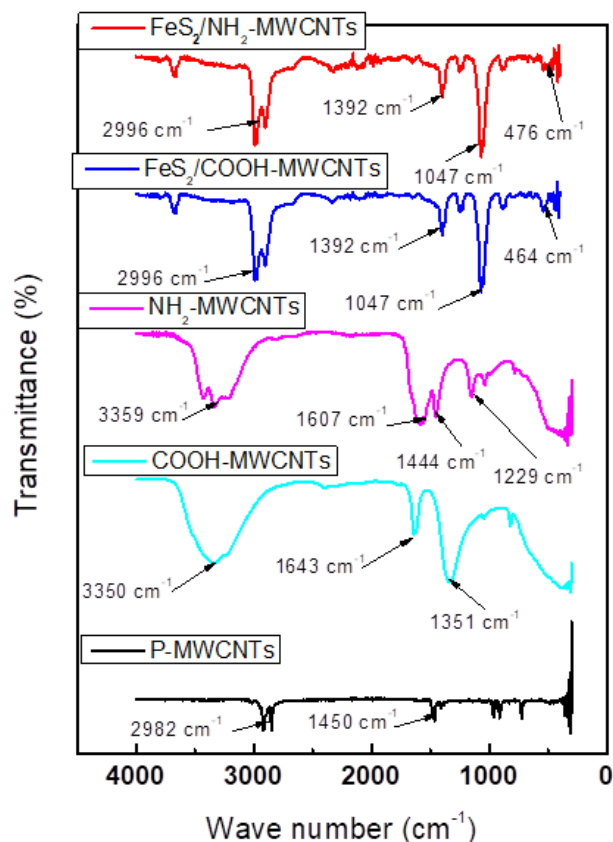


Figure 5.11: FT-IR spectra of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs.

5.2.3. UV-VIS characterisation of FeS₂/Functionalised-MWCNTs based nanocomposites

The UV-vis absorption spectra of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs nanocomposites dispersed in ethanol are presented in Figure 5.12. The characteristic absorption band at 312 nm was seen in the aqueous solution of p-MWCNTs, and it is linked to the MWCNTs' C=C bonds [13]. The characteristic absorption band of MWCNTs in the spectra of COOH-MWCNTs and NH₂-MWCNTs appeared at 308 nm and 310 nm. The presence of COOH and NH₂ groups and their strong interaction with MWCNTs are evidenced by the blue shift observed in the characteristic bands of COOH-MWCNTs and NH₂-MWCNTs. The FeS₂/COOH-MWCNTs nanocomposite exhibited a shoulder band at 220 nm, which is the characteristic band of FeS₂ NPs. On the other hand, the

FeS₂/NH₂-MWCNTs nanocomposite exhibited an absorption tail between 250 and 600 nm [14].

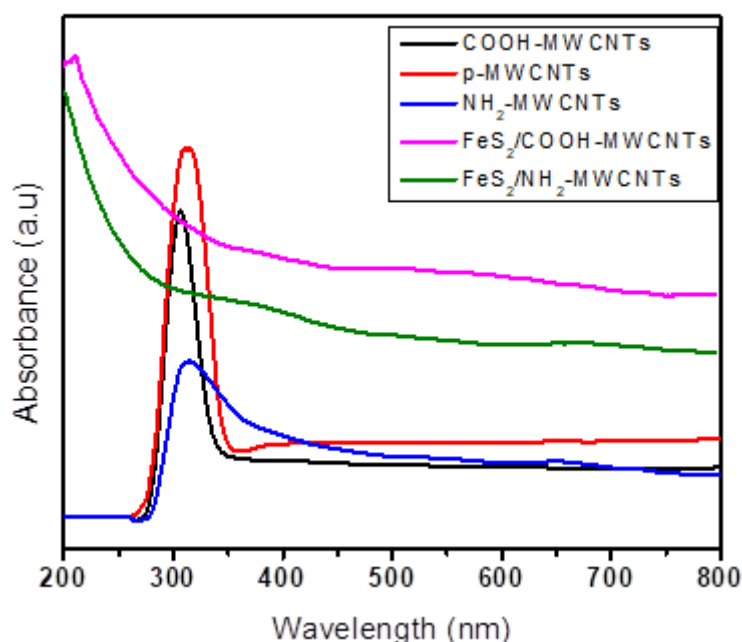


Figure 5.12: UV-VIS spectra of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, FeS₂/COOH-MWCNTs and FeS₂/NH₂-MWCNTs.

5.3. Characterisation of ZnS/Functionalised-MWCNTs based nanocomposites.

5.3.1. Morphological Characterisation of ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites

The synthesized ZnS/Functionalised-MWCNTs based nanocomposites were characterised for its morphology, crystallinity, and purity by using TEM, EDX, XRD and Raman studies.

5.3.1.1. TEM-EDX characterisation of ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites

Figure (5.13) depicts the TEM photographs of pristine MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs. Following the acid treatment, the nanotube's closed end aperture is visible. The pristine MWCNTs (Figure 5.13(a)) frequently have coarse topped tubular sidewalls, "convex concave" walls following the selective oxidation treatment and amination (Figure 5.13(b) and (c)) [15]. The functionalized MWCNTs' specified surface area and pore volume are enhanced

by these disfigurements. [16]. As for the nanocomposites of ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs in Figure 5.13 (d and e), the incorporation of spherical-like ZnS nanoparticle on the surface of the COOH-MWCNTs and NH₂-MWCNTs is clearly displayed. Nanometre-sized nanoparticle with agglomeration due to the nature of ZnS were observed. Agglomeration of the ZnS nanoparticles helped to achieve a balance between specific capacitance and cyclic stability for electrode. The ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs nanocomposites were prepared in-situ. Spherical-like nanoparticles were still observed with an average diameter of 24,11 nm and 35.95 nm for ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs nanocomposites, respectively. A similar trend, where particles average diameters for ZnS/NH₂-MWCNTs nanocomposites was bigger than for ZnS/COOH-MWCNTs, was observed as seen in section 5.1.1.1. The range of particle size formed for ZnS incorporated in functionalised MWCNTs nanocomposites are also depicted by the particle size histogram as shown in Figure 5.14 (a) and (b).

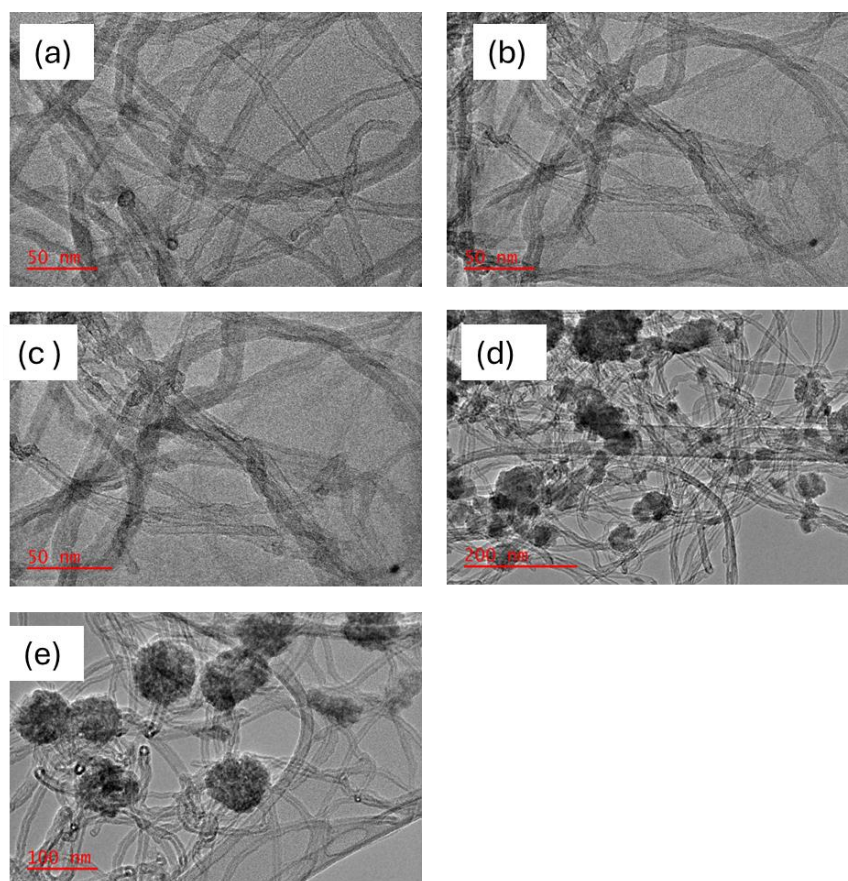


Figure 5.13: TEM images of p-MWCNTs (a), COOH-MWCNTs (b), NH₂-MWCNTs (c), ZnS/COOH-MWCNTs (d) and ZnS/NH₂-MWCNTs (e).

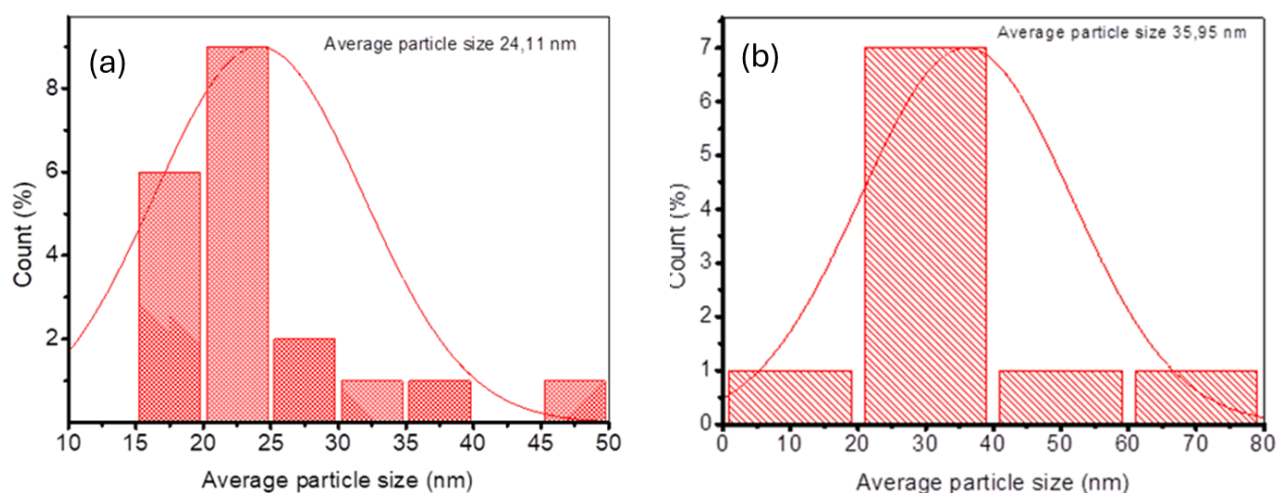


Figure 5.14: Particle size histogram of ZnS/COOH-MWCNTs (a) and ZnS/NH₂-MWCNTs (b).

EDX spectra of ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs (Figure 5.15 (a) and (b)) confirmed that Cu, Zn, O, S and C elements are present in the nanocomposites. However, in addition to the elements mentioned above, N element was observed in the EDX spectrum of ZnS/NH₂-MWCNTs. This is due to the presence of the amine group which was used to functionalise the MWCNTs. As stated before (Section 5.1.1.1), the EDX spectra (Figure 5.15) exhibits a strong carbon peak which agrees with the high percentage of carbon in MWCNTs. The EDX spectra further exhibits Zn and S peaks, which confirms the presence of the ZnS nanoparticles in the nanocomposites. A strong copper peak shown in the spectra originates from the copper grid used for TEM analysis.

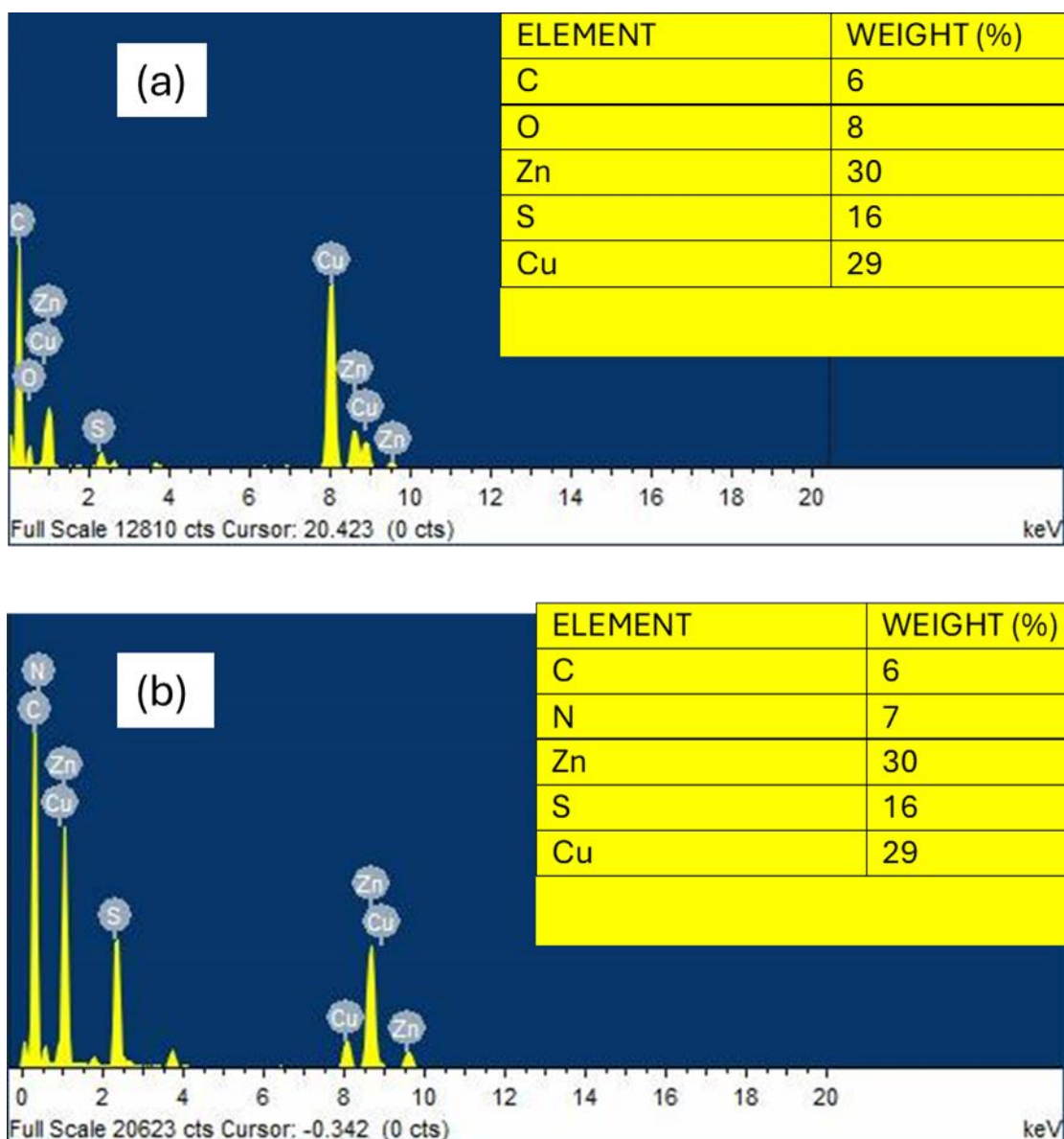


Figure 5.15: EDX spectra for ZnS/COOH-MWCNTs (a) and ZnS/NH₂-MWCNTs (b).

5.2.1.2. XRD characterisation of ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites

The XRD analysis of the p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs and ZnS/COOH-MWCNTs nanocomposites are shown in Figure 5.16. Two peaks were seen in the XRD patterns of p-MWCNTs, COOH-MWCNTs, and NH₂-MWCNTs at 26.94° and 43.15°, respectively, which corresponded to the (002) and (100) crystal planes of the MWCNTs. In the ZnS/COOH-MWCNTs XRD pattern (Figure 5.16), the diffraction peaks at 2θ values of 28.98°, 48.19°, and 58.08° correspond well to the cubic ZnS crystallographic planes (111), (220), and (311), respectively (JCPDS Card No. 65-0309) [17]. The significant broadening of the diffraction peaks attributed to

small ZnS crystallite sizes. The results obtained are consistent with those reported in the literature [18] and validate the formation of ZnS in the cubic (sphalerite) phase with good crystallinity. Although COOH-MWCNTs do not exhibit a distinctive peak point, this indicates that ZnS nanoparticles have effectively coated the MWCNTs' surface.

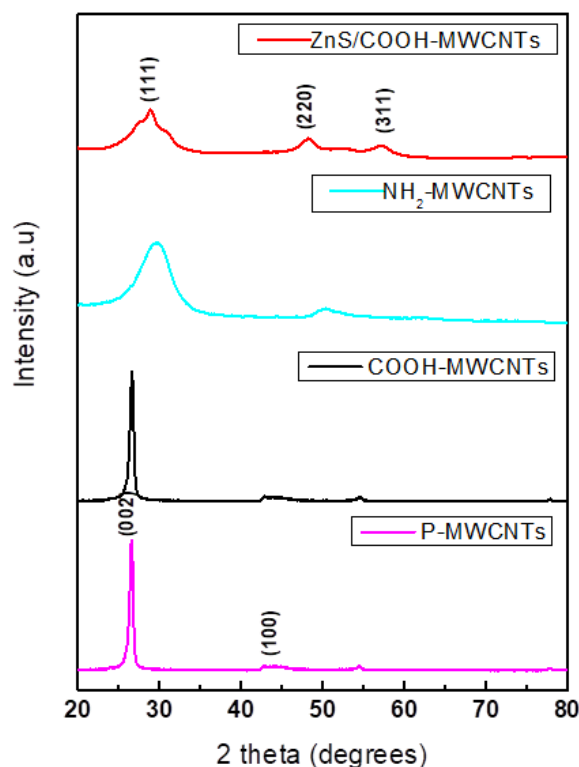


Figure 5.16: XRD patterns of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs and ZnS/COOH-MWCNTs

5.3.1.3. Raman characterisation ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites

Figure 5.17 presents the Raman spectra of p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs. To ascertain the crystalline structure and purity of MWCNTs, their Raman spectra are examined. D (~1350 cm⁻¹), G (~1580 cm⁻¹), and G' (~2700 cm⁻¹) are the three vibration bands that have been detected. The Raman spectra of the ZnS/NH₂-MWCNTs nanocomposite, however, showed a weaker D band. The characteristic peaks of multi walled carbon nanotubes are the D (tangential band) and G (disorder band) peaks, which are situated at 1343 cm⁻¹ and 1585 cm⁻¹, respectively [19]. The G band represents the splitting of graphite's E_{2g} stretching mode, whereas the D band is brought on by a disorganised structure of graphite or the nanotubes' sp³-conjugated carbons [20, 21]. Through measurement of

the intensity ratio of the D and G bands (I_D/I_G), the degree of crystallization or surface flaws density of materials was determined. The I_D/I_G ratio of p-MWCNT (1.095) is lower than that of COOH-MWCNTs (1.171) and NH₂-MWCNTs (1.285), representing the less disordered structure in p-MWCNT. After the functionalisation of p-MWCNT, the intensity ratio was increased due to the attachment of oxygen and amine functional groups on the surface of p-MWCNT. The increase in I_D/I_G ratio of composite compared to that of p-MWCNT suggested the increase of surface defects in functionalised structure. The peak intensity D and G band ratios (I_D/I_G) for ZnS/COOH-MWCNTs (1.064) and ZnS/NH₂-MWCNTs (0.13) are lower than that of COOH-MWCNTs and NH₂-MWCNTs which suggests that the subsequent ZnS deposition steps did not alter the sp³/sp² carbon ratio of the COOH-MWCNTs and NH₂-MWCNTs structure any further.

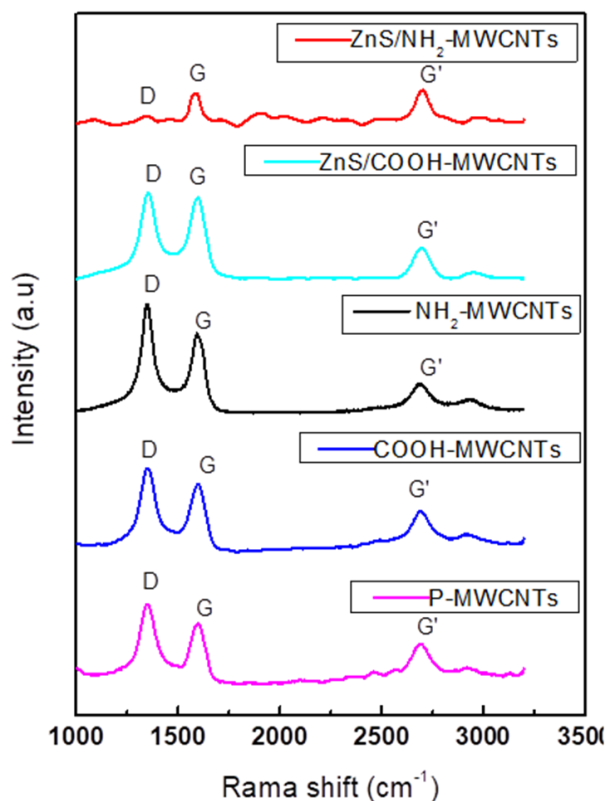


Figure 5.17: Contrast of the Raman spectra for the p-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNT.

5.3.2. FT-IR characterisation of ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites

The FTIR spectroscopic study provides details regarding the functional groups that are found within a molecule. The FT-IR spectra of p-MWCNTs, acid-treated MWCNTs (COOH-MWCNTs), amine functionalised-MWCNTs (NH₂-MWCNTs), ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs, are represented in Figure 5.18. The stretching vibrations of C-H are represented by absorption peaks in the FT-IR spectra of p-MWCNTs at 2982 cm⁻¹ and 1450 cm⁻¹. An extensive peak at 3400 cm⁻¹ indicates the O-H expansion of the hydroxyl group. The signal at 1643 cm⁻¹ indicates that the carboxylate group (–COO–) replaces the –COOH group, which was previously attached to the surface of MWCNT as a result of acid functionalization. This suggests that Zn²⁺ has formed a connection with the MWCNT surface via MWCNT/ZnS synthesis. The distinctive peaks of the N-H, C=O, and C-N stretching vibrations are found at 3404 cm⁻¹, 1600 cm⁻¹, and 1047 cm⁻¹ in the NH₂-MWCNTs spectra. The FT-IR spectra for ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs displayed peaks at 1600 cm (C=O), and at 1398 cm⁻¹ (C-H), and 1047 cm⁻¹ (C-N stretching). At 1628 cm⁻¹, the stretching vibration peak attributed to C-C is also visible in the interim. Consequently, it may be said that the MWCNTs and ZnS nanoparticles in the nanocomposite have chemical bonding roles. The dominant high-frequency shift for f-MWCNTs stabilized ZnS nanoparticles result from the association between the OH and NH groups (from COOH-MWCNTs and NH₂-MWCNTs) and the ZnS nanoparticles [22].

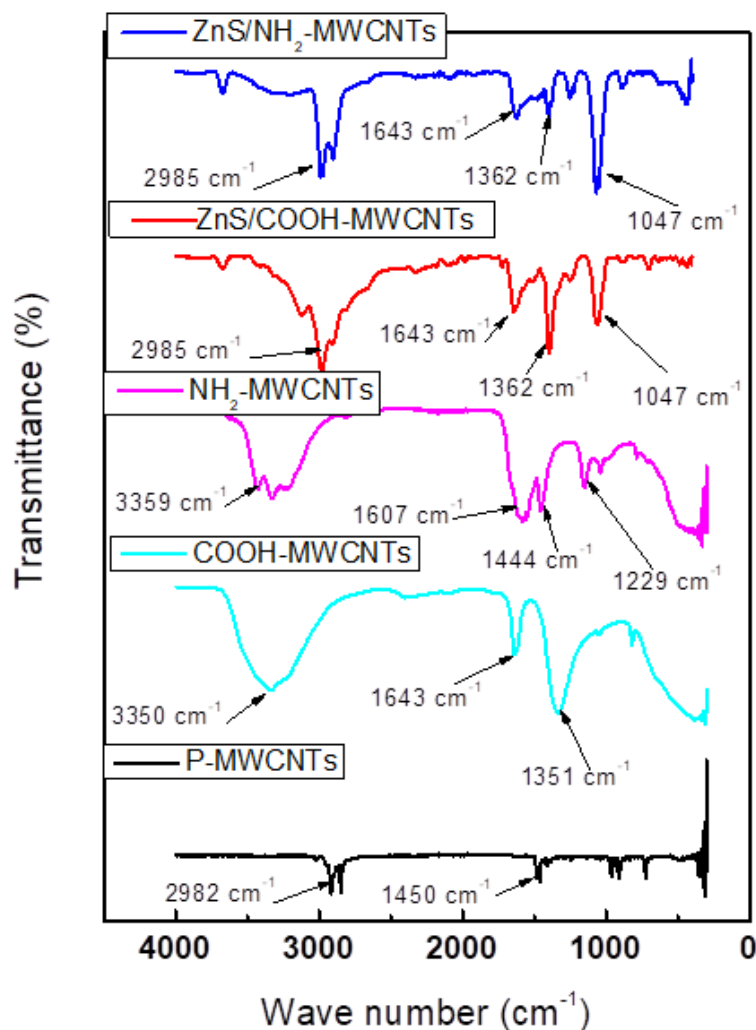


Figure 5.18: FT-IR spectra of P-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs.

5.3.3. UV-VIS characterisation of ZnS/Functionalised-MWCNTs based nanocomposites

The UV-Vis absorption spectra of each sample are displayed in Figure 5.19. The MWCNTs array has a maximum absorbance of 200–300 nm, which spans the visible to ultraviolet spectrum. Around 312 nm is when the absorption peak caused by the π and C-C bond transitions emerges. Particularly noticeable are the absorption peak characteristics in COOH-MWCNTs and NH₂-MWCNTs. The characteristic absorption peaks of MWCNTs in the spectra of COOH-MWCNTs and NH₂-MWCNTs appeared at 308 nm and 310 nm [13]. The ZnS/NH₂-MWCNTs exhibit a distinctive absorption peak at comparable sites, signifying the integration of ZnS nanoparticles into the composite, their surface combination with NH₂-MWCNTs, and the modification of the local electric

field on the particle surface. ZnS/COOH-MWCNTs do not exhibit an absorption peak within the 200–400 nm range, indicating that COOH–MWCNTs influences the variation in the absorption strength of the composites.

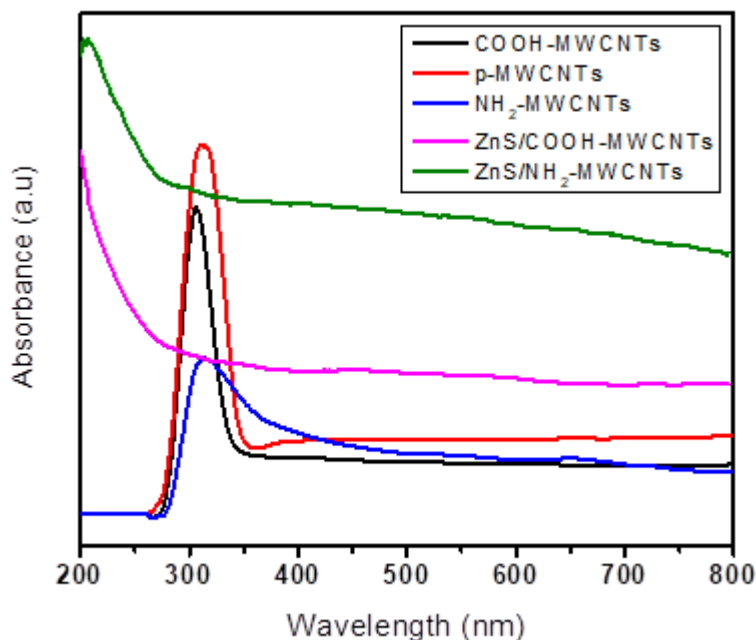


Figure 5.19: UV-VIS of *p*-MWCNTs, COOH-MWCNTs, NH₂-MWCNTs, ZnS/COOH-MWCNTs and ZnS/NH₂-MWCNTs

5.4. Electrochemical characterization of metal sulfides/functionalised-MWCNTs

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were employed for evaluating the electrochemical properties of the metal sulfides/functionalized MWCNTs nanocomposites. Each of the electrochemical investigation was conducted out utilizing 0.1 M KCl as the supporting electrolyte and 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ as the redox probe. CV measurements were conducted at a scan rate of 50 mV/s and a potential from -0.4 to +0.8V. For the quantitative EIS data analysis, equivalent circuits models were simulated and used for fitting the Nyquist plot and bode plots.

5.4.1. Electrochemical characterisation FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites using cyclic voltammetry (CV)

Figure 5.20 displays an analysis of CV measurements using 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ in 0.1 M KCl at a scan rate of 50 mV/s. The outcomes of the BARE-GCE's

CV curves, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, FeS₂-GCE, FeS₂/COOH-MWCNTs-GCE and FeS₂/NH₂-MWCNTs-GCE were obtained by scanning the potential from -0.4 to +0.8 V at a scan rate of 50 mV/s. In the bare GCE, there are two ferricyanide ion redox peaks that are normally reversible. Before alteration, the GCE's curvature was observed, displaying a redox peak with -0.045 V for the cathodic maximum potential and +0.370 V for the anodic maximum potential, and a peak separation of 0.415 V ($\Delta E_p = E_{pc} - E_{pa}$). This result demonstrated that GCE had a high peak-to-peak separation potential value, a high formal potential, and a low charge transfer rate, all of which were considerably increased by the addition of FeS₂ nanoparticles. A redox optimum having cathodic and anodic maximum potentials of +0.148 V and +0.300 V was produced by the addition of p-MWCNTs, with peak separation of 0.152 V. The cyclic voltammogram unequivocally demonstrates that p-MWCNTs increase the electrode's current sensitivity; the high conducting nature and wide surface area of p-MWCNTs are responsible for this increase in current and decrease in overpotential [23,24]. Employing COOH-MWCNTs to modify the GCE's surface increased the redox peak, which had peak separation of 0.411 V and maximal potentials for the cathodic and anodic periods of 0.000 V and +0.411 V, accordingly. The COOH-MWCNTs-GCE CV curve was clearly shaped like a rectangle and had some evident tiny reduction peaks. These reduction peaks are the result of a redox connection among the oxygen-rich groups on the outermost layer of COOH-MWCNTs. [25]. A redox maximum with 0.100 V and 0.280 V cathodic and anodic potentials, with peak separation of 0.180 V, was produced by modifying the GCE using NH₂-MWCNTs. The NH₂-MWCNTs-GCE CV curve showed a sigmoidal-like shape with distinct minor decrease peaks. The redox connection that occurs among nitrogen-containing groups on the NH₂-MWCNTs' surface is what causes these reduction peaks. Functionalised-MWCNTs (both COOH and NH₂) demonstrated low oxidation potential and overpotential oxidation due to their poor direct electron transfer capabilities. Modifying the GCE with FeS₂ resulted in redox maximum with cathodic and anodic peak potentials of +0.150 V and +0.280 V, with peak separation of 0.130 V. It has been demonstrated that the presence of negatively charged sulfur atoms enhances the materials' electrochemical characteristics; in this instance, the sulfur atoms are a result of the FeS₂ nanoparticles [26]. Surface modification of GCE with FeS₂/COOH-MWCNTs resulted in the redox maximum with cathodic and anodic peak potentials of +0.011 V and +0.380 V, with peak separation of 0.369 V. FeS₂/NH₂-MWCNT coating of the GCE

produced a redox maximum having cathodic and anodic peak potentials of +0.014 V and +0.360 V, accordingly with peak separation of 0.346 V. Additionally, it is noted that following the modification of the GCE with FeS₂/F-MWCNTs nanocomposites, the reduction peak potential (E_{pc}) switched to negative values, suggesting that the forward (reduction) process needs more activation energy than analogous reactions on the other modified electrodes. The higher ΔE_p seen for the altered electrodes' CV scans with FeS₂/F-MWCNTs nanocomposites [27,28] further supports this finding. Figure 5.20 voltammogram shows the GCE's comparative current responses with the changed electrodes in descending order of FeS-GCE > p-MWCNTs-GCE > BARE-GCE > FeS₂/NH₂-MWCNTs-GCE > COOH-MWCNTs-GCE > FeS₂/COOH-MWCNTs-GCE > NH₂-MWCNTs-GCE. For all FeS₂/F-MWCNTs nanocomposites and FeS₂ modified electrode, the CV response has a narrow peak to peak potential separation (ΔE_p) and a conventional sigmoidal shape. This is a sign of rapid electron transfer kinetics, which is explained by the FeS₂ and its documented fast electron transport characteristics, as well as the large surface area that the 1D MWCNTs system presents [29, 30]. Table 5.1 shows the current ratio between the matching reduction and oxidation maxima of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites. These results were caused by reversible voltammograms ($I_{pa}/I_{pc}=1$) or reversible redox processes that happened at the electrode surface because all the ratios were very near to 1.

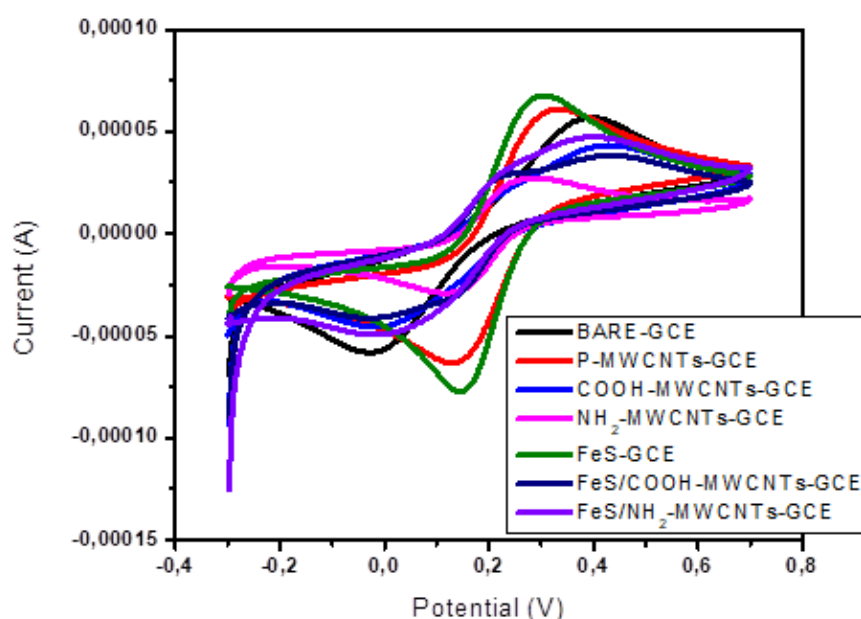


Figure 5.20: CV curves of BARE-GCE, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, FeS-GCE, FeS/COOH-MWCNTs-GCE and FeS/NH₂-MWCNTs-GCE in 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe and 0.1 M KCl at a scan rate of 50mV/s.

Table 5.1: The current ratio between the matching reduction and oxidation maxima of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites.

electrode	$I_{pc}(A)$	$I_{pa}(A)$	I_{pa}/I_{pc}
Bare-GCE	0.000059	0.000056	0.95
p-MWCNTs-GCE	0.000062	0.000058	0.94
COOH-MWCNTs-GCE	0.000050	0.000040	0.80
NH ₂ -MWCNTs-GCE	0.000038	0.000030	0.78
FeS ₂ /COOH-MWCNTs-GCE	0.000040	0.000035	0.88
FeS ₂ /NH ₂ -MWCNTS-GCE	0.000045	0.000048	1.07
FeS ₂ -GCE	0.000078	0.000070	0.90

5.4.2. Electrochemical characterization FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites electrochemical impedance spectroscopy (EIS)

The electrochemical properties of the modified electrodes were taken into account during the EIS by using a 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe and 0.1 M KCl as supporting electrolyte. It is well known that polymers, nanomaterials, and semiconducting materials can be used to adjust the electrode's double layer capacitance (Cdl) and electro transfer resistance [31]. It is crucial to swap out the constant phase element (CPE) in the analogous circuit for the Cdl to properly fit the

Nyquist plots. Both the Cdl and the bulk electrolyte solution resistance exhibit an uneven distribution because of the tiny roughness caused by the presence of the CPE [32]. The diffusion-controlled impedance process is represented by the linear portion of the semicircle in the Nyquist plot of the EIS, while the entire semicircle, also referred to as the electron transfer resistance (R_{ct}), is an active control barrier that works to limit the flow of electrons at the electrode contact. Solution resistance (R_s), constant phase element (CPE), and frequency-dependent Warburg impedance (W) were all visible by circuit equivalent fitting [33]. At higher frequencies, the semicircular part's diameter is equal to the electron transfer resistance (R_{ct}), and the specimen's electrochemical activity is inversely correlated with the R_{ct} value. Figure 5.21 (a) shows the fitted Nyquist plots of bare and modified GCE electrode in 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ redox probe, with 0.1 M KCl as supporting electrolyte. The ohmic R_{ct} , for the p-MWCNTs-GC modified electrode, was calculated to be 943 Ω , which is less as compared with that of the bare GC electrode (4443.19 Ω). The high conductivity of the p-MWCNTs, acting as a good electron conductor between $K_3Fe(CN)_6/K_4Fe(CN)_6$ and the electrode surface, accounts for the p-MWCNTs-GCE fast electron transport [34]. Ohmic R_{ct} values for the GC modified electrodes of COOH-MWCNTs and NH_2 -MWCNTs were calculated and found to be 5388.2 Ω and 3829.6 Ω , respectively. These values are greater than those of the p-MWCNTs-GC electrode. This indicates that the presence of the amine (NH) group in NH_2 -MWCNTs and the electronegative oxygenated groups (C=O and COO-) in COOH-MWCNTs generates resistance to the redox couple's ability to transport electrons at the interface [35]. The R_{ct} value for FeS_2 /COOH-MWCNTs-GCE decreased marginally to 111.08 Ω when the layered matrix was doped with FeS NPs (R_{ct} equals to 636 Ω), whereas the R_{ct} value for FeS_2 / NH_2 -MWCNTs-GCE increased to 4955.7 Ω , indicating the role of FeS_2 NPs in improving electron transfer kinetics at the interface [36,37]. The fitted impedance data are summarized and their chi-square X^2 values are displayed in Table 2. It appears from the low values that the data fit was successful. Because of the ease at that ions diffuse across and between the electrode/solution interface, the N values of all electrodes fall between 0.5 and 0.8.

The Nyquist plots' intrinsic drawback is that they are unable to display the precise frequencies at which impedance values are measured [38]. Bode charts, which show the frequency-specific impedance behaviour of the changed electrodes, are used to

get around this restriction. Figure 5.21 represents the Bode phase angle(b) and Bode magnitude (c). The phase angle values are as follows: BARE-GCE (56.81°), p-MWCNT-GCE (23.08°), COOH-MWCNTs-GCE (33.12°), and NH₂-MWCNTs-GCE (34.26°), FeS₂-GCE (57.69°), FeS₂/COOH-MWCNTs-GCE (35.22°) and FeS₂/NH₂-MWCNTs-GCE (34.26°). All of the electrodes' phase angle values are smaller than 90°, which supports the electrodes' pseudo-capacitive behaviour [39]. Moreover, the fact that the modified electrodes' phase angle frequencies differ from those of the bare GCE suggests that the reactions are now taking place at the modifiers rather than the bare GCE electrode surface [40, 41]. Three frequency-dependent segments were clearly visible in the bode impedance, as demonstrated by the Bode plots (Figure 5.21(c)). In the high frequency range, the $|Z|$ value for each electrode is nearly the same and is not affected by frequency; this indicates the solution resistance (R_s) in the system. Indicative of pseudocapacitive behaviour, the Bode plots' slopes (log Z vs. log f , Figure 5.21 (c)) are roughly equal at the midfrequency region. The nearly zero slopes at high frequencies are a sign of resistive behaviour at these high frequencies.

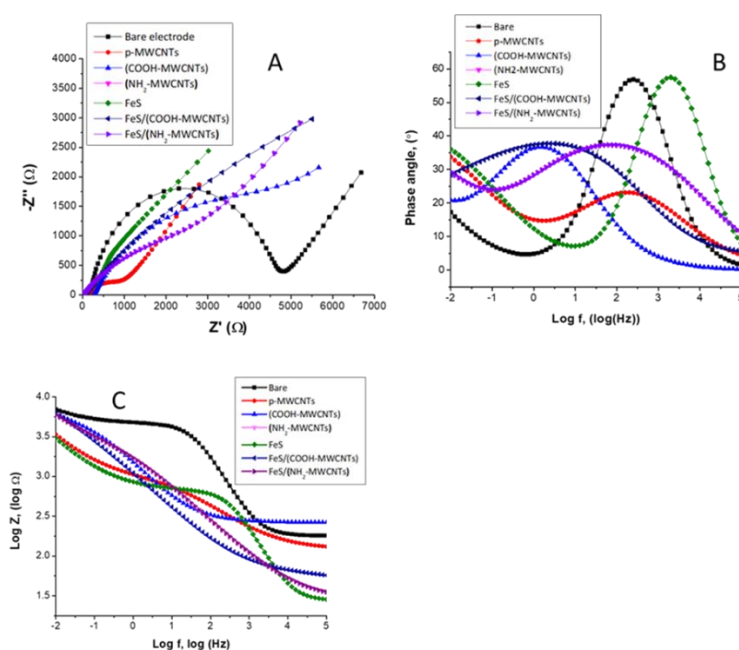


Figure 5.21: Nyquist plot (a) and Bode plots of (b) phase angle and (c) total impedance of BARE-GCE, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, FeS₂-GCE, FeS₂/COOH-MWCNTs-GCE and FeS₂/NH₂-MWCNTs-GCE in 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe in 0.1 M KCl supporting electrolyte.

Table 5.2: Summary of fitted EIS results of FeS₂/functionalised-multi walled carbon nanotubes- based nanocomposites.

Electrode	R _s (Ω)	CPE (μF)	R _{ct} (Ω)	W (F)	N	X ²
BARE-GCE	179	2.00	4443.19	0.0013662	0.864	0.30644
GCE-p-MWCNTs	121	93.1	943	0.0014238	0.494	0.22647
GCE-COOH-MWCNTs	264	213	5388.2	0.0017665	0.601	0.22117
GCE-NH ₂ -MWCNTs	27.5	159	3829.6	0.0008623	0.484	0.65294
GCE-FeS ₂	27.6	1.94	636	0.0015863	0.880	0.07519
GCE-FeS ₂ /COOH-MWCNTs	-42.2	143	111.08	0.0002958	0.166	0.07645
GCE-FeS ₂ /NH ₂ -MWCNTs	296	190	4955.7	0.0012998	0.582	0.19622

5.4.3. Electrochemical characterization ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites using cyclic voltammetry (CV)

The results for CV measurements using 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe in 0.1 M KCl supporting electrolyte at a scan rate of 50 mV/s are displayed in figure 5.22. The outcomes of the CV curves of the BARE-GCE, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, ZnS-GCE, ZnS/COOH-MWCNTs-GCE and ZnS/NH₂-MWCNTs-GCE were obtained by scanning the potential from -0.4 to +0.8 V at the scan rate of 50 mV/s. Cyclic voltammetry is a useful method for figuring out the

electron transport characteristics of electrochemical materials. As demonstrated by the cyclic voltammograms (Figure 5.22), BARE-GCE, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, ZnS-GCE, ZnS/COOH-MWCNTs-GCE and ZnS/NH₂-MWCNTs-GCE. The discussion of the cathodic peak current (I_{pc}) and anodic peak currents is summarized in table (5.3). The redox mechanism underlies the reported electroconductivity, as evidenced by the two symmetric and well-separated redox peaks observed in GCE electrodes [42–45]. Individual peak separation (ΔE_p) values for each electrode were obtained for the BARE-GCE (0.152 V), p-MWCNTs-GCE (0.415 V), COOH-MWCNTs-GCE (0.411 V), NH₂-MWCNTs-GCE (0.180 V), ZnS-GCE (0.385 V), ZnS/COOH-MWCNTs-GCE (0.070 V) and ZnS/NH₂-MWCNTs-GCE (0.405 V). The comparative current responses of the GCE with the altered electrodes are shown in the voltammograms in Figure 5.22 in decreasing order of ZnS/COOH-MWCNTs-GCE > p-MWCNTs-GCE > BARE-GCE > ZnS/NH₂-MWCNTs-GCE > ZnS-GCE > COOH-MWCNTs-GCE > NH₂-MWCNTs-GCE. The ZnS/COOH-MWCNTs-GCE modified electrode showed an anodic peak current (I_{pa}) of 0.000130 A, which is almost two times larger than that of the BARE-GCE electrode. This suggests that out of all the electrodes, the ZnS/COOH-MWCNTs-GCE electrode has the fastest electron transport. Because of the larger surface area of COOH-MWCNTs as well as strong electrical conductivity [46], and the good electrocatalytic activity of ZnS, the combined cooperative effect between ZnS and COOH-MWCNTs is responsible for the enhanced electron transport of ZnS/COOH-MWCNTs-GCE. Consequently, the ZnS/COOH-MWCNTs nanocomposite showed better electroactivity and great potential to be employed for glucose detection. The quasi-reversible nature of the process at the electrodes is confirmed by the ZnS/functionalized-MWCNTs electrodes' CV curves, which showed values greater than the predicted 0.059 V for a quick one-electron transfer [47, 48]. The current ratio of the matching reduction peaks to the oxidation peaks is displayed in Table 5.3. Reversible voltammograms ($I_{pa}/I_{pc}=1$) were the cause of these results because all the ratios were nearly equal to 1.

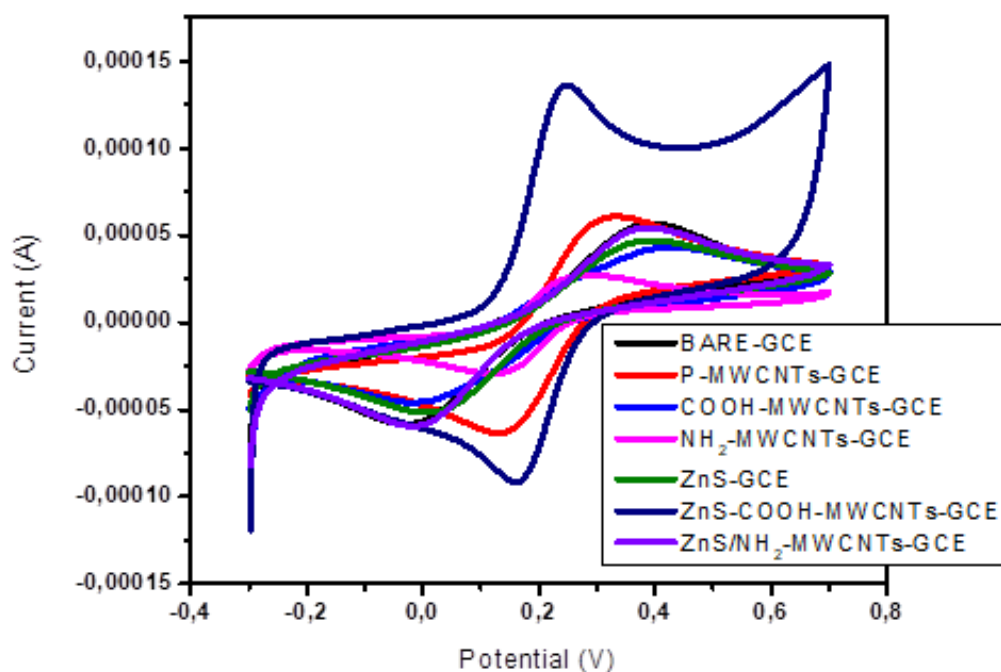


Figure 5.22: CV curves of BARE-GCE, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, ZnS-GCE, ZnS/COOH-MWCNTs-GCE and ZnS/NH₂-MWCNTs-GCE in 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe and 0.1 M KCl supporting electrolyte at the scanning rate of 50mV/s.

Table 5.3: the current ratio of the oxidation peaks to the corresponding reduction peaks of ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites.

electrode	$I_{pc}(A)$	$I_{pa}(A)$	I_{pa}/I_{pc}
Bare-GCE	0.000059	0.000056	0.95
p-MWCNTs-GCE	0.000062	0.000058	0.94
COOH-MWCNTs-GCE	0.000050	0.000040	0.80
NH ₂ -MWCNTs-GCE	0.000038	0.000030	0.78
ZnS-MWCNTs-GCE	0.000056	0.000047	0.84
ZnS/COOH-MWCNTs-GCE	0.000090	0.000130	1.40
ZnS/NH ₂ -MWCNTs-GCE	0.000060	0.000055	0.92

5.4.4. Electrochemical characterisation ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites using electrochemical impedance spectroscopy (EIS)

The R_{ct} values recorded for the electrodes, as presented in Table 5.4, were BARE-GCE (4443.19 Ω), p-MWCNTs-GCE (943 Ω), COOH-MWCNTs-GCE (5388.3 Ω), NH₂-MWCNTs-GCE (3829.6 Ω), ZnS-GCE (2169 Ω), ZNS/COOH-MWCNTs-GCE(33.67 Ω) and ZnS/NH₂-MWCNTs-GCE (2249 Ω). ZnS/COOH-MWCNTs-GCE exhibited the lowest R_{ct} among the electrodes, suggesting its superior capacity to transfer electrons quickly. The great conductivity of the p-MWCNTs, which functions as an excellent electron conductor between the K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe and the electrode surface, contributes for the p-MWCNTs-GCE swift electron transfer. The ohmic R_{ct} for

the p-MWCNTs-GC modified electrode is lower than that of the BARE GC electrode. This was in agreement with the cyclic voltammetry analysis where the ZnS/COOH-MWCNTs showed better electro-activity compared to other modified electrodes. The presence of the amine (NH) group in NH₂-MWCNTs and the incorporation of the negatively charged oxygenated groups (C=O and COO-) in COOH-MWCNTs results in an opposition to electron transfer among the redox couple at the point of contact, as indicated by the higher ohmic R_{ct} for these GC modified electrodes than for the p-MWCNTs-GC electrode. Compared to the single semicircle shown for bare-GCE, the analogous Nyquist plots in Figure 5.23 (a) show two semicircles for electrodes coated with ZnS. It consists of a high-frequency semicircle whose diameter corresponds to the charge transfer resistance and a linear portion with a phase angle of 45° (Warburg impedance) at low frequencies [49]. The ZnS/NH₂-MWCNTs-modified electrode showed the highest R_{ct} value (2249.1 Ω) compared to the ZnS/COOH-MWCNTs-GCE (33.61 Ω) interface in the presence of the K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe while ZnS/COOH-MWCNTs encourage strong electron transfer and increase the electrode's conductivity, ZnS/NH₂-MWCNTs' high R_{ct} suggests that it has low conductivity [50,51]. Table 5.4's fitted impedance data indicates that the GCE-ZnS/COOH-MWCNTs electrode has the lowest R_{ct} value of 33 Ω among the electrodes. This suggests that the electrode transfers electrons more quickly than the other electrodes, which is consistent with the CV results. The high CPE value of the GCE-ZnS/COOH-MWCNTs electrode also plays a part in this. A brief overview of the fitted impedance data and associated chi-square X² values are shown in Table (5.4.). The low values suggest that the data fit was successful. The ease with which ions diffuse to and from the electrode/solution interface may be the reason for all electrodes' N values, which range from 0.5 to 0.8.

Figure 5.23 represents the Bode phase angle(b) and Bode magnitude (c). The phase angle values are as follows (Figure 37 (b)): bare GCE (56.81°), GCE-P-MWCNT (23.08°), GCE-COOH-MWCNTs (33.12°), and GCE-NH₂-MWCNTs (34.26°), ZnS-GCE (45.95°), GCE-ZnS/COOH-MWCNTs (44.96°) and GCE-ZnS/NH₂-MWCNTs (48,08°). The other Bode plots display phase angles that are less than 60°, which is smaller than the 90° predicted by an ideal capacitive behaviour. The Bode charts (Figure 37(c)) clearly showed three frequency-dependent regions in the bode impedance. In the high frequency range, the |Z| value that represents the solution

resistance (R_s) in the system is nearly the same for every electrode and is independent of frequency. Bode phase angle plots showed a definite peak, and bode magnitude plots showed negative slopes, all of which were accompanied by a noticeable increase in the $|Z|$ value in the middle region. This area relates to the electrode/electrolyte interface's capacitive behaviour when the whole resistance including R_s and R_{ct} is considered. In the lower frequency range, the $|Z|$ value steadily rises with noticeable variations in phase angles and slopes. In this area, the electrode's resistive behaviour correlates with a capacitive behaviour [50].

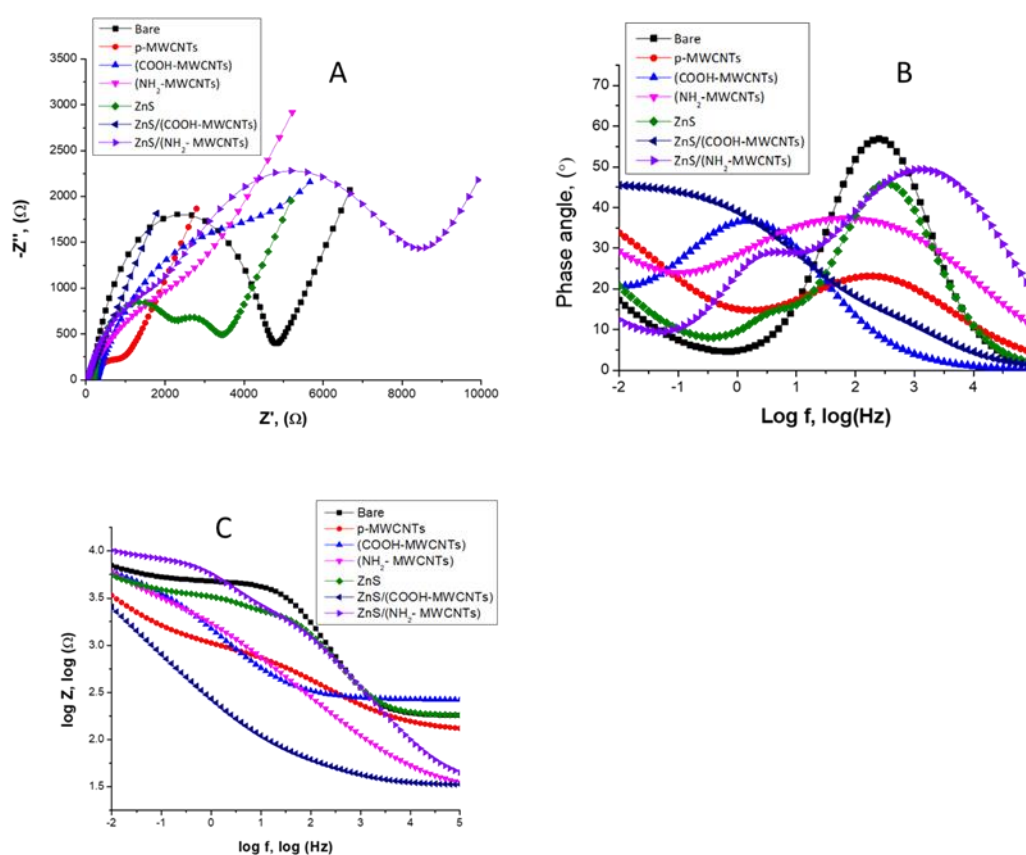


Figure 5.23: Nyquist (a) and Bode plots, phase angle (b) and total impedance (c) of BARE-GCE, p-MWCNTs-GCE, COOH-MWCNTs-GCE, NH₂-MWCNTs-GCE, ZnS-GCE, ZnS/COOH-MWCNTs-GCE and ZnS/NH₂-MWCNTs-GCE in 5mM K₃Fe(CN)₆/K₄Fe(CN)₆ redox probe in 0.1 M KCl supporting electrolyte.

Table 5.4: summary of fitted EIS results of ZnS/functionalised-multi walled carbon nanotubes- based nanocomposites.

Electrode	R_s (Ω)	CPE (μF)	R_{ct} (Ω)	W (F)	N	X^2
GCE-Bare	179	2.00	4443.19	0.0013662	0.864	0.30644
GCE-p-MWCNTs	121	93.1	943	0.0014238	0.494	0.22647
GCE-COOH-MWCNTs	264	213	5388.2	0.0017665	0.601	0.22117
GCE-NH ₂ -MWCNTs	27.5	159	3829.6	0.0008623	0.484	0.65294
GCE-ZnS	180	3.31	2169.2	0.0014553	0.810	0.05690
GCE-ZnS/COOH-MWCNTs	32.8	293	33.627	0.0013832	0.615	0.13938
GCE-ZnS/NH ₂ -MWCNTs	33.2	9.75	2249.1	0.0014198	0.654	0.12515

5.4. Optimisation of experimental variables

The study focused on how the developed glucose biosensor responded to different experimental conditions. The impact of stability, pH, and scan speeds on the electroactivity of ZnS/COOH-MWCNTs modified GC electrode were conducted, in order to establish the best conditions for the optimal detection of glucose

5.4.1. The study of the effect of pH

The pH change in the test solution can have an impact on a biosensor's analytical performance. The effect of the pH of the electrolyte solution pH on the electrochemical performance of ZnS/COOH-MWCNTs-GCE was studied in 0.01 M PBS in the presence of 1 M NaOH/1 M HCl at a varying pH value (3.0, 6.1, 7.3, 8.8, 12.1, 13.6) using cyclic voltammetry (Figure 5.24 (a)). Achieving the optimum oxidation peak resolution and sensitivity for glucose detection was the primary goal of this study. The anodic peak potentials shifted both negatively and positively with an increase in the electrolyte's pH, indicating that proton transfer at the modified nanocomposite electrodes is a necessary component of the electrocatalytic activity. Figure 5.24 (b) shows the linear relationship between pH and the current response. Over a range of 3.0 to 13.6, it was discovered that the pH and current had a linear relationship with regression of $R^2=0.928$ for ZnS/COOH-MWCNTs. The pH between 12.1 and 13.6 provided the highest current amplification, hence the optimal electrochemical activity and was selected as the optimal pH for the detection of glucose using the ZnS/COOH-MWCNTs nanocomposite.

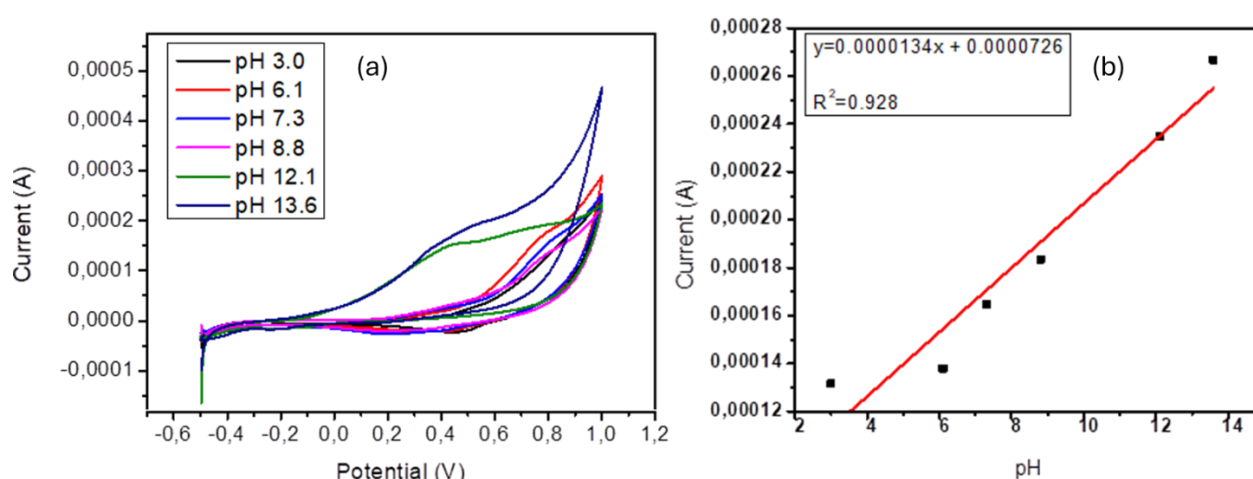


Figure 5.24: pH response for ZnS/COOH-MWCNTs-GCE at varied pH (3.0-13.6) in 0.01M PBS in the presence of 1 M NaOH, (b) linear plot of anodic peak current I_{pa} against pH.

5.4.2. The study of the effect of scan rate

CV scans were performed in the absence of glucose at a range of 0.005-0.06 V/s in order to examine the impact of the possible scan rate at ZnS/COOH-MWCNTs-GCE.

This experiment aims to monitor variations in peak currents in 0.01 M PBS containing 1 M NaOH at different scanning rates. The investigation can be used to describe the type of redox process taking place at the electrode surface. In Figure 5.25 (a), the relevant CV voltammograms are displayed. The anodic peak showed a linear increase with increasing scan rate, suggesting that ZnS/COOH-MWCNTs-GCE electrodeposition is a diffusion-controlled process. In this instance, the high electrode surface area for the redox reaction at the ZnS/COOH-MWCNTs-GCE surface was provided by functionalized carbon nanotubes. The interrelation between the oxidation peak currents (I_p) and (1) scan rate (v) and (2) square root of scan rate ($v^{1/2}$) are demonstrated in Figure 2.25 (b) and (c), respectively. The obtained plots resulted in linear graphs with equations, $y = 5.2 \times 10^{-3} x + 3.57 \times 10^{-5}$ ($R^2 = 0.991$) and $y = 1.68 \times 10^{-3} x + 8.12 \times 10^{-5}$ ($R^2 = 0.955$). This is indicative that the redox process occurring at the electrode surface was both diffusion and adsorption controlled.

In this study, the diffusion coefficient, D , was estimated using the anodic peak current's dependence on scan rate. Plotting the anodic peak current values against the square root of the scan rate for a diffusion-controlled process yields a linear or straight-line relationship with a regression coefficient of ≥ 0.85 . The Randles Sevcik equation (6) [52] should yield an estimate of the value of D based on the slope of these lines.

$$I_p = 2.69 \times 10^5 n^{3/2} A C D^{1/2} v^{1/2} \dots\dots\dots (6)$$

where A is the geometrical electrode area (0.071 cm^2), C is the electrolyte concentration (0.01 M), D is the diffusion coefficient of the electroactive species ($\text{cm}^2 \cdot \text{s}^{-1}$), v is the scan rate (V s^{-1}), and I_p is the peak current density (A). At $T=298\text{K}$, n is the number of electrons transferred. The plot of square root of scan rate vs peak current has a slope of $0.0168 \text{ A/V} \cdot \text{s}^{-1}$ and the calculated diffusion coefficient was $9.4 \times 10^{-3} \text{ cm}^2 \cdot \text{s}^{-1}$, indicating quite fast electrode kinetics or exchange of electrons between the electrolyte and the ZnS/COOH-MWCNTs-GCE modified electrode. Moreover, using the slope of the plot of scan rate vs peak current, the calculated surface coverage (Γ) of the electroactive species was $7.96 \times 10^{-3} \text{ mol} \cdot \text{cm}^{-2}$.

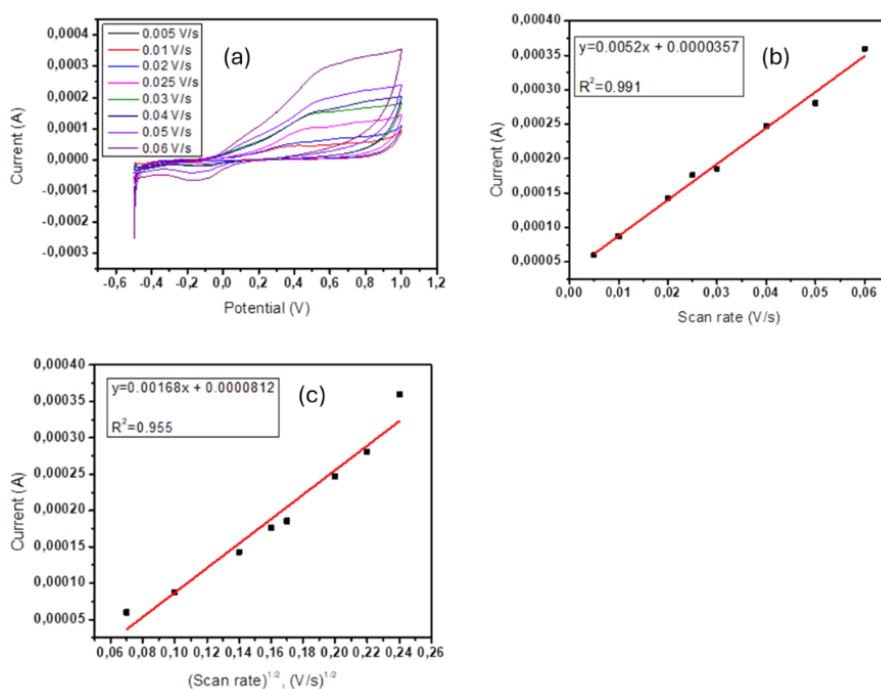


Figure 5.25: Cyclic voltammogram obtained for ZnS/COOH-MWCNTs at varied scan rate (0.005-0.06 V/s) in 0.01 M PBS in the presence of 1 M NaOH (a), linear plot of anodic peak current (I_{pa}) against scan rate (v) (b), and linear plot of anodic peak current (I_{pa}) against square root of scan rate ($v^{1/2}$).

5.4.3. Stability study

The stability of ZnS/COOH-MWCNTs-GCE was investigated via CV with repeated scans in 0.01 M PBS in the presence of 1 M NaOH. Ten total number of cyclic voltammetry scans were monitored. As demonstrated in Figure 5.26, the ZnS/COOH-MWCNTs-GCE experienced a significant oxidative current drop with increasing number of scans indicating that the ZnS/COOH-MWCNTs electrode modified electrode could be employed only once for glucose measurements. Hence, the ZnS/COOH-MWCNTs-GCE based glucose sensor could be easily configured as a once-off use detection script for accurate and reliable measurements. In the consecutive study, each concentration of glucose was measured using a freshly prepared ZnS/COOH-MWCNTs modified carbon electrode.

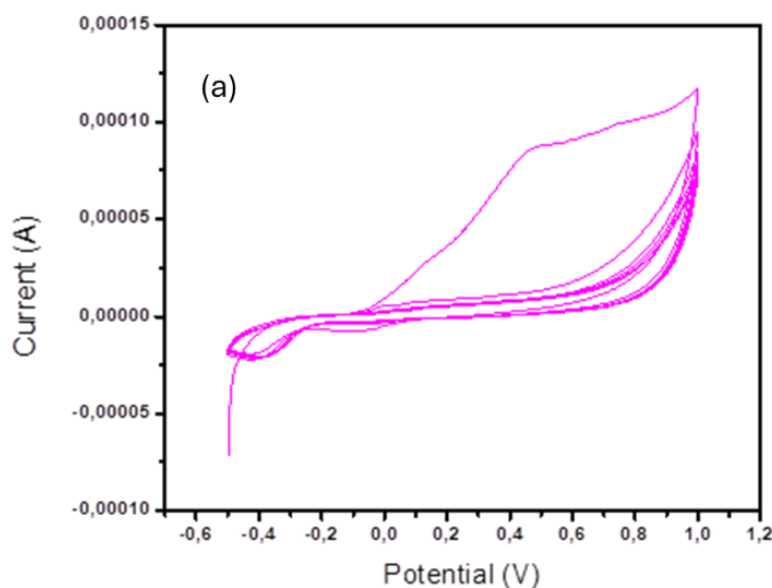


Figure 5.26: Cyclic voltammograms of continuous scans of GCE-ZnS/COOH-MWCNTs-GCE in 0.01 M of PBS in the presence of 0.1 M NaOH.

5.5. Square wave voltammetry response of ZnS/COOH-MWCNTs-GCE biosensor to the detection of glucose

Using square wave voltammetry (SWV), the electrocatalytic oxidation of glucose at ZnS/COOH-MWCNTs-GCE was studied. Because square wave voltammetry has a higher sensitivity than CV, it was utilized to detect glucose. The experiment involved detecting varying glucose concentrations using 0.01 M PBS in the presence of 1 M NaOH. Each concentration was produced in PBS at a different level. Between -1 V and 1 V, the SWV response to various glucose concentrations was observed. Figure 5.27 (a) depicts the SWV responses of ZnS/COOH-MWCNTs modified electrodes, in the presence of 1-9 mM glucose. The peak current proportionally increased with the increasing glucose concentrations. The electrocatalytic mechanism involved in glucose oxidation is supported by the increase in anodic current intensity following increments in glucose concentration, as has been extensively described [53–55]. The linear association between glucose concentration and current responsiveness is depicted in Figure 5.27 (b). Over a concentration range of 1 to 9 mM, it was discovered that the connection between the glucose concentration and the current was linear, with linear regression equation $y = 7.2 \times 10^{-6} x + 1.1 \times 10^{-4}$ ($R^2 = 0.992$) for ZnS/COOH-MWCNTs based sensor. The detection limit ($S/N = 3$), where the S is the standard

deviation of the intercept, the N is the slope of the calibration curve for the non-enzymatic glucose sensors based on ZnS/COOH-MWCNTs-GCE was found to be 0.83 mM. The sensor also offers a very high sensitivity of $7.2 \times 10^{-6} \text{ A.mM}^{-1}$. In Table 5, the performance of the ZnS/COOH-MWCNTs electrode is contrasted with other non-enzymatic glucose sensors that have been documented in academic publications. It is evident that the ZnS/COOH-MWCNTs sensor performed on par with other sensors that have been documented in the literature. The ZnS/COOH-MWCNTs nanocomposite presents very high sensitivity that is desired for electrochemical sensors. The detection performance such as linear range and detection limit for glucose oxidation at the ZnS/COOH-MWCNTs was compatible with most of the previously reported non-enzymatic glucose sensors.

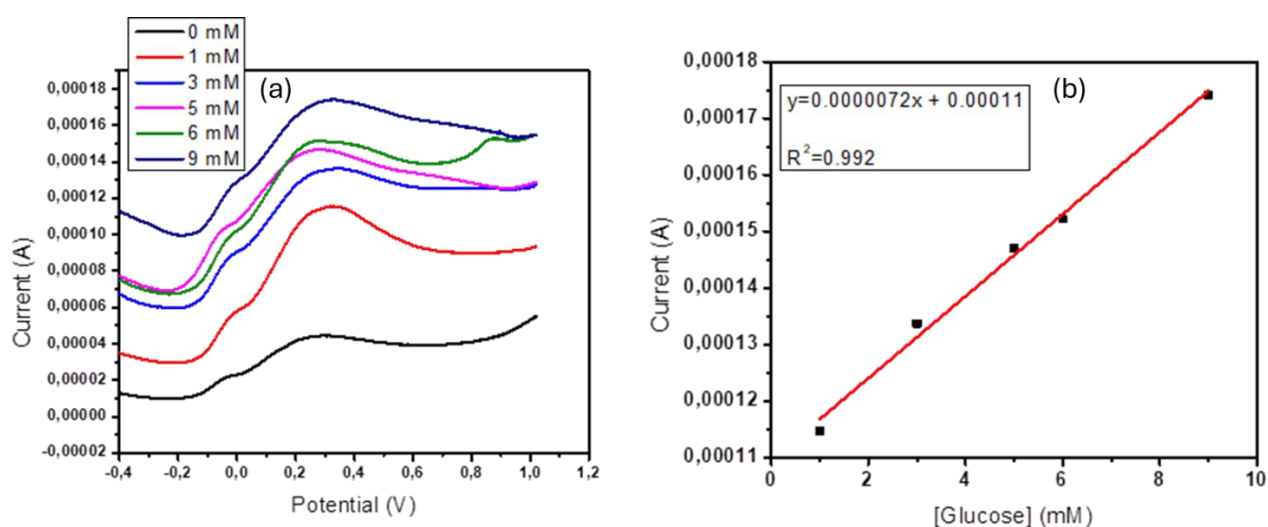


Figure 5.27: Square wave voltammograms of ZnS/COOH-MWCNTs-GCE over glucose concentration range of 1-9 mM at pH 12.12 (a). plot of current versus concentration (b).

Table 5.5: Comparison of the sensing performance of the ZnS/COOH-MWCNTs-GC modified electrode with previously reported sensor electrodes for non-enzymatic glucose detection.

electrode	Detection technique	Detection limit	sensitivity	Linear range	references
Cu ₂ ZnSnS ₂ quantum dots	CV	15.9 nM	1561 μ A. $\text{mM}^{-1}\text{cm}^{-2}$	0.5–1000 μ M	[56]
GO/MWCNT/ Au@Pt /GCE	DPV	0.042 μ M	330 μ A. $\text{mM}^{-1}\text{cm}^{-2}$	0.00005– 0.1 and 0.1–2.5 mM	[57]
PCA@MWCNT- CuO	CV	0.43 μ M	2412 μ A. $\text{mM}^{-1}\text{cm}^{-2}$	0.002–9 mM	[58]
CuS/XGCNFs	CV	0.019 μ M	23.69 μ A. $\text{Mm}^{-1}\text{cm}^{-2}$	0.0001– 0.125 and 0.158– 1.221 mM	[59]
ZnS/COOH- MWCNTs	SWV	0.83 μ M	7.2×10^{-6} A.mM^{-1}	1-9 mM	This work

5.6. References

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CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The metal sulfide-decorated multi-walled carbon nanotube modified non-enzymatic glucose detecting sensor was studied. Through acid treatment, the p-MWCNTs were refined and functionalized to create COOH-MWCNTs. Mintek provided the amine functionalized MWCNTs (NH₂-MWCNTs). For comparison in the electrochemical investigations, metal sulfides nanoparticles (FeS₂ and ZnS) were synthesized using a hydrothermal technique in the same experimental conditions as the metal sulfides/f-MWCNTs nanocomposites. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to analyse the electrochemical characteristics of the metal sulfides/functionalized MWCNTs nanocomposites. Every electrochemical experiment was carried out using 0.1 M KCl as the supporting electrolyte and 5 mM K₃Fe(CN)₆/K₄Fe(CN)₆ as the redox probe. The resultant nanomaterials had redox active groups that produced a signal on the electrode surface, and these groups could be used to build electrochemical sensors and biosensors.

The CV and SWV studies revealed that the nanocomposite sensor demonstrated fast electron transport and better electroactivity when compared with the other fabricated sensors (BARE-GCE, p-MWCNTs-GCE, COOH-MWCTNs-GCE, NH₂-MWCNTs-GCE, FeS₂-GCE, ZnS-GCE, FeS₂/COOH-MWCNTs-GCE, FeS₂/NH₂-MWCNTs-GCE and ZnS/NH₂-MWCNTs-GCE). The study also showed that the nanocomposite electrodes exhibited rapid electron transfer ability.

The study explored the electrochemical square wave voltammetry measurement of glucose using zinc sulfide/carboxylic acid functionalised multiwalled carbon nanotubes (ZnS/COOH-MWCNTs-GCE) modified glassy carbon electrodes. The ZnS/COOH-MWCNTs-GCE exhibit a lower LoD, a wider LDR and a higher sensitivity. The sensor has demonstrated that it can detect glucose at a milimolar level. The mechanism of glucose at the interface of the modified electrode surface of ZnS/COOH-MWCNTs-GCE was found to be diffusion controlled.

The developed sensors exhibited a very little stability. The ZnS/COOH-MWCNTs-GCE experienced a significant oxidative current drop with increasing number of scans indicating that the ZnS/COOH-MWCNTs electrode modified electrode could be employed only once for glucose measurements. Hence, the ZnS/COOH-MWCNTs-

GCE based glucose sensor could be easily configured as a once-off use detection script for accurate and reliable measurements.

6.2. Future recommendations

Based on the results of the findings, while using GCE-ZnS/COOH-MWCNTs for non-enzymatic glucose detection, the following recommendations should be considered for future glucose detection.

1. The proposed hydrothermal method of nanoparticles preparation has shown to be effective. To mitigate the effect of aggregation and clustering of ZnS and FeS nanoparticles in the nanocomposites, ex-situ method of nanocomposite synthesis is recommended.
2. A broader scope of the developed sensors application for interference and reproducibility studies should be explored.
3. Future research should consider the utilisation of different nano catalysts.
4. Printed electrodes and precious metals (gold and platinum) should be coupled with the as-synthesised nanocomposites in sensor fabrication to create more accurate and sensitive sensor devices.