

ELECTROCHEMICAL DETECTION OF SEROTONIN AND EPINEPHRINE USING MULTI -WALLED CARBON NANOTUBE/POLYANILINE NANOCOMPOSITE FILMS DOPED WITH TiO_2 AND RuO_2 NANOPARTICLES

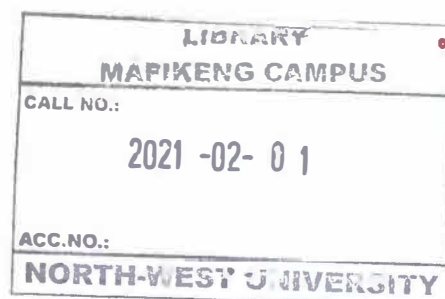
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DECLARATION

I hereby declare that the work presented in this dissertation entitled "Electrochemical detection of serotonin and epinephrine using multi-walled carbon nanotube/polyaniline nanocomposite films doped with TiO_2 and RuO_2 nanoparticles" submitted to the Department of Chemistry, North-West University, Mafikeng Campus in fulfilment of the requirements for the degree of Masters of Science in Chemistry was compiled and written by me under the supervision of Prof. Eno E. Ebenso and Dr. A.S Adekunle has not been included in any other research work submitted previously by any other student at the North-West University or any other University. Sources of my information are acknowledged in the reference pages.

T.P. TSELE.....

Tebogo Palesa Tsele

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ABSTRACT

Electrochemical properties of functionalized multiwalled carbon nanotube MWCNT/polyaniline (PANI) doped with metal oxide (TiO_2 , RuO_2) nanoparticles were explored. Successful synthesis of MWCNT, TiO_2 , RuO_2 , PANI, MWCNT-PANI- TiO_2 , and MWCNT-PANI- RuO_2 nano materials were confirmed using suitable characterization techniques such as fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-vis), high resolution scanning electron microscopy (HRSEM) and x-ray diffraction spectroscopy (XRD). Successful modification of gold (Au) electrode with these nanoparticles was confirmed using electrochemical techniques such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Serotonin (ST) and epinephrine (EP) are biomolecules, which are vital for message transfer in the mammalian central nervous system. Au-MWCNT-PANI- TiO_2 and Au-MWCNT-PANI- RuO_2 gave the best electron transport properties towards the oxidation of EP and ST compared with other electrodes investigated. The electrodes were also characterized with some degree of adsorption attributed to analyte oxidation intermediates products. The Tafel values of 0.448 V and 0.452 V (EP, ST) and 0.422 V and 0.445 V (EP, ST) were obtained for Au-MWCNT-PANI- TiO_2 and Au-MWCNT-PANI- RuO_2 respectively. The stability results had the RSD of (4.4, 10 %) EP and (3.6, 6.6 %) ST on the Au-MWCNT-PANI- TiO_2 and Au-MWCNT-PANI- RuO_2 respectively. The linear calibration plots were obtained over different concentrations ranging from 492 to 63.2 μM (EP) and 14.3-1.5 μM (ST). The limit of detection were calculated to be 0.16, 0.26 μM (EP, ST) and 0.18, 0.32 μM . (EP, ST) for Au-MWCNT-PANI- TiO_2 and Au-MWCNT-PANI- RuO_2 electrodes respectively. The interference study was conducted using differential pulse voltammetry (DPV) and three clear peaks were observed for AA, ST and EP. The concentration of AA was 1000 times higher than that of ST and EP. Therefore, the modified electrodes can selectively detect epinephrine and serotonin without interference from ascorbic acid signal. The performance of the fabricated sensors was evaluated for detection of epinephrine (EP) and serotonin (ST) in a pharmaceutical sample with satisfactory results.

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LIST OF ABBREVIATIONS

NT	Neurotransmitters
NPs	Nanoparticles
EP	Epinephrine
ST	Serotonin
UA	Uric acid
AA	Ascorbic acid
CNTs	Carbon nanotubes
MO	Metal oxide
GO	Graphene oxide
PANI	Polyaniline
CE	Capillary electrophoresis
TiO₂	Titanium dioxide
RuO₂	Ruthenium dioxide
MS	Mass spectrometry
FTIR	Fourier transform infrared spectroscopy
EDX	Energy dispersive X-ray
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
XRD	X-ray diffraction spectroscopy
UV-Vis	UV-visible spectroscopy
CV	Cyclic voltammetry
EIS	Electrochemical impedance spectroscopy
Ag/AgCl	Silver/silver chloride reference electrode
DMF	Dimethylformamide
LoD	Limit of detection

LIST OF SYMBOLS

E_{pa}	Anodic peak potential
E_p	Cathodic peak potential
E	Potential
E°	Standard potential
$E_{1/2}$	Half-wave potential
I_{pa}	Anodic peak current
i_{pc}	Cathodic peak current
Γ	Surface coverage or concentration
Π	Pi bonding
C_{dl}	Double-layer capacitance
CPE	Constant phase electrode
D	Diffusion coefficient
F	Faraday constant
Hz	Hertz
K	Kelvin
n	Number of electron
R	Universal gas constant
R_{ct}	Charge transfer resistance
R_s	Resistance of electrolyte

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CHAPTER ONE

INTRODUCTION

1.1 Nanostructured Materials

Nano-structured functional materials and their applications have attracted a lot of interest owing to many of their exceptional properties. These significant applications are involved in lithium ion battery with high energy, super capacitor, catalysts, solar cells, nanodevices, chemical sensors, biosensors and biomedical fields [1-7]. A chemical sensor has prospective applications in many fields, such as environmental monitoring, detection of explosives, medical diagnoses, and so on [8-10]. It is not compulsory to always stick sensors into the body or to take blood samples in order to retrieve chemical information. Chemical examination of urine, saliva, sweat and exhaled air can give information on the status of the body. Moreover, these quantities do not require such delicate levels of care in the encapsulation of the sensors [11].

1.2 Sensor

Sensors have been accredited as relevant tools for detection and quantification of several biochemical compounds, chemicals, minerals, etc. When associated with various traditional systems, sensors are devices composed of active sensing tools coupled with a signal transducer. These devices transmit the signal from a change in reaction or selective compound and hence produce a signal (such as electrical, thermal or optical output signals) which is changed into digital signals for further processing [12-16]. Future detection systems have to satisfy traditional requirements such as sensitivity, response time, probability of detection, and false-alarm rates, but they could also satisfy other constraining factors such as cost, power consumption, and maintainability [17].

1.2.1 Biosensors

Biosensors are influential analytical tools whose series of applications in medical diagnostics [18], food quality control [19] and environmental monitoring [20] is rapidly expanding. The project of an electrochemical biosensor started with the target analyte and then the selection of appropriate biological element, e.g. L-glutamate detection has been done using glutamate receptor ion channels, glutamate oxidase [21] or glutamate dehydrogenase [22] and finally the subsequent electrochemical processes. The majority of enzyme-based amperometric

biosensors exploit the biocatalytic oxidation of analyte by oxidase enzymes having the prosthetic group, flavin adenine dinucleotide (FAD). In the modification of a biosensor device, the main issue is to increase the selectivity and sensitivity of the sensor by sustaining the movement of the immobilized biomolecules which are affected by pH, temperature, humidity and toxic chemicals. The biosensor performance usually depends on the immobilizing matrices and/or supporting materials, numerous conventional immobilizing matrices such as inert materials such as platinum and gold or carbon-based materials were broadly studied [23]. Another important point shaping the biosensor design is the nature of the planned application matrix. Devices for monitoring the dynamic analyte concentration in vivo should be biocompatible due to the course of the implantation, both in terms of tissue effects on sensor functionality and physiological reaction to the probe [24-25]. The appropriate level of biocompatibility, implantable oxidase-based biosensors should also satisfy the following minimum criteria for reliable analyte monitoring, viz. appropriate size and geometry [26-27].

1.2.2 Chemical sensors

Chemical sensors, with recent substantial developments for detection and quantification of chemical species, are attractive and have a wide range of application such as clinical, industrial, agricultural and military technologies thus resulting in public and economic benefits. A chemical sensor is defined as a small device where in a chemical, relationship occurs between the analyte gas and/or liquid and the sensor device, transforming chemical or biochemical information of a quantitative or qualitative nature into an analytically useful signal.

The sensor signal is a typically electronic in nature, being a current, voltage, or impedance/conductance change produced by electron exchange. These devices have a physical transducer and a chemically sensitive layer or recognition layer. Chemical sensors could be characterized by numerous features such as stability, selectivity, sensitivity, response and recovery time, and saturation [28].

1.3 Neurotransmitters

Neurotransmitters (NTs) are the major chemical messengers that are released from the neuron terminals upon depolarization [29]. These chemicals are formed in pituitary and adrenal glands and are usually found at the axon endings of motor neurons in muscle fibres. Neurotransmitters are produced from simple precursors, such as amino acids, which are freely available from the diet and which need a slight number of biosynthetic steps to convert. There are several types of neurotransmitters, and they are classified as amino acids, peptides and monoamines. Neurochemical measurements have improved our understanding of the relationship between chemistry in the central nervous system (CNS) and the behavioural and moods of an organism. Abnormal neurotransmission is related to extensive range of conditions including depression [30], drug dependence [31], schizophrenia [32] and degenerative diseases [33].

To detect and monitor NTs, numerous methods have been applied. Capillary electrophoresis (CE), microdialysis and liquid chromatography have been used for the separation and fractionation of NTs, whereas laser-induced fluorescence, immunoassay and mass spectrometry (MS) have been applied for their detection [34-36]. Because there is limited information regarding the origin of urinary NTs, studies have focused on determining NTs *in vivo*. Additionally, it has been observed that neurotransmission occurs on the millisecond to minute time scale, which confirms the real-time analysis to be easily achieved [37].

1.4 Metal Oxide

Nanoporous metal oxide nanoparticles such as titanium oxide (TiO_2), cerium oxide (CeO_2), zinc oxide (ZnO), tin oxide (SnO_2), and zirconium oxide (ZrO_2) have lately been used for modification of enzyme-based biosensors. Sol-gel derived nanostructured metal oxides such as TiO_2 [38-39], CeO_2 [40-41], ZnO [42-43], SnO_2 [44] and ZrO_2 [45] because of their fascinating properties such as better thermal stability, low cost, biocompatibility, non-toxicity and low temperature of processing, etc. have provoked much interest for immobilization of desired biomolecules. Among these, TiO_2 nanoparticles has appealed much interest due to their exclusive properties including high mechanical strength, oxygen ion conductivity, wide band gap (3.2 eV), biocompatibility and retention of biological activities [38-39]. Curulli et

al. stated that nanoporous TiO_2 electrodes have been used for electron transfer mechanisms of H_2O_2 , and many other exciting biological molecules, such as 3,4-dihydroxyphenylacetic acid, ascorbic acid, guanine, l-tyrosine and acetaminophen, to assemble a different generation of chemical sensors and biosensors [46].

1.5 Polymer

Polymer–semiconductor nanocomposites produce a different field for the development of advanced materials in science and technology [47]. Nanocomposites have different properties from the constituent materials due to interfacial interactions between nanostructured semiconductors and polymers. The properties of these materials could be effortlessly be tuned to the desired application through the difference of particle size, shape and distribution of the nanoparticles. The main struggle is to synthesize inorganic nanoparticles in the matrix of conducting polymers which are infusible and are not soluble in common solvents. Nanoparticles with high surface energy restrict the preparation of nanostructured composites. The composites of TiO_2 with different polymers such as conducting polyaniline (PANI) [48-50], poly (phenylenevinylene) (PPV) [51] and poly (methylmethacrylate) (PMMA) have been widely studied in the last recent years. The most recently studied conducting polymers in the last 15 years, PANI has absorbed considerable attention for the preparation of its composites with inorganic particles [52], such as conducting PANI– BaTiO_2 composite [53], PANI–molybdenum trisulfide composite [54], conducting PANI–inorganic salt composite [55], and PANI– V_2O_5 composite [56]. The conducting PANI– TiO_2 composites were studied, which show high piezosensitivity being maximum at a certain PANI– TiO_2 composition [49]. Majority of the studies are focused on optical properties of the polymer surface modified TiO_2 nanoparticles. The materials with high dielectric constant are very valuable in integrated electronic circuits such as capacitor and gate oxides.

1.6 Carbon Nanotube

The carbon nanotubes have attracted extensive investigation since their discovery in the early 1990s due to their chemical, physical and mechanical properties [57]. They are considered quasi-one dimensional nanostructures, which are graphite sheets rolled up into cylinders with diameters of sufficient nanometers and up to some millimeters in length. There are three

kinds of nanotubes, which are the single-walled nanotubes (SWCNTs), double-walled nanotubes (DWCNTs) and the multi-walled nanotubes (MWCNTs). The MWCNTs is known to have multiple layers of graphite organized in concentric cylinders. CNTs have drawn considerable attention because of their special structure and high mechanical strength which influenced them to be great candidates for advanced composites. Depending on the helicity and the diameter of the tube, they are either semiconducting, semimetallic or metallic. Based on the structure and shape, they conduct electricity because of their delocalization of the pi bond electrons. Furthermore, it is found that CNTs are efficient adsorbents because of their large specific surface area, heavy and layered structures and the presence of pi bond electrons on the surface [58-59]. The connection between TiO_2 with CNTs has provided a synergistic effect which can improve the overall efficiency of a photocatalytic process. CNTs/ TiO_2 nanocomposites have drawn attention in literature regarding the treatment of contaminated water and air by heterogeneous photocatalysis [60-61].

1.7 Problem statement

Brain chemicals that deal with the transfer of information throughout the brain and the body are known as Neurotransmitters (NTs). They are fundamental chemicals used by the brain to aid heartbeat, breathing action through the lungs and digestion in the stomach. Neurotransmitters are known to cause disturbance in the patterns of the moods, sleep, concentration, weight, and while at abnormal concentration they can cause server severe symptoms [62]. Therefore, it is very important to control the level of neurotransmitters in the body because imbalance in their concentration can cause much disorderliness in the body. Neurotransmitters that assist on creating a balance are known to be inhibitory neurotransmitters. They stabilise the mood and are washed-out when there is intense excitatory neurotransmitters. These electroactive neurotransmitters can be easily oxidised and well determined by voltammetric techniques. The major problem is that, at bare electrode the oxidation response was poor. In order to increase the sensitivity and selectivity in the determination, modification of the working electrode has been proposed.

1.8 Research aim and objectives

The aim of this study is to conduct a comparative study of the electrochemical properties of graphene oxide/polyaniline nanocomposite film doped with metal oxide (TiO_2 and RuO_2)

nanoparticles and also verify their electro activity towards biological analytes such as serotonin and epinephrine.

The objectives of the work are to:

- Synthesize MWCNT, PANI, RuO₂ TiO₂, and MWCNT-PANI-MO nanocomposite using suitable characterization techniques such as Fourier Transformation Infrared (FTIR), Raman, Scanning Electron Microscopy (SEM), Transmission Electron microscopy (TEM), Ultraviolet-visible spectroscopy (UV-vis), X-ray Diffraction (XRD) and Electron Dispersive X-ray Spectroscopy (EDX);
- Confirm successful modification of gold electrode with nanoparticle materials using electrochemical techniques namely cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS);
- Compare the electron transport properties of the synthesized materials using cyclic voltammetry (CV) electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), and differential pulse voltammetry (DPV);
- Compare the electrocatalytic properties of the synthesized materials towards serotonin and epinephrine oxidation using cyclic voltammetry (CV) electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), and differential pulse voltammetry (DPV) and to explore the potential of the MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ nanocomposite towards epinephrine real sample analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Neurotransmitters

In recent decades, neurochemical measurements have steered to improvements of understanding the relationship between chemistry in the central nervous system (CNS) and the behavioral, cognitive, and emotional state of an organism [63]. The key monoamine neurotransmitters are serotonin (ST) and the catecholamines dopamine (DA), norepinephrine (NE), and epinephrine (EP). DA is the most distant of the four monoamine neurotransmitters [64]. Significant dopaminergic pathways are associated with perceiving rewards and regulation of learning and feeding. Abuse of drugs affect the DA system, hence there has been much research focus on DA. NE and EP are both excitatory neurotransmitters and have been associated with the control of the arousal, attention, mood, learning, memory, and stress response [65]. ST is a pacemaker function in numerous regions of the brain during times of alertness, coordinates sensory and motor activity [64], and contributes to good execution of feeding, sleeping, and reproductive behaviours [66].

2.1.1 Serotonin and its Applications

Serotonin (5-hydroxytryptamine or ST) is a monoamine neurotransmitter produced in serotonergic neurons in the central nervous system and plays a vital role in the emotional system collectively with other monoamine transmitters such as regulation of mood, sleep, emesis (vomiting), sexuality and appetite. Small amounts of ST have been associated with several disorders, notably depression, migraine, bipolar disorder and anxiety [67-68]. In addition, neurodegeneration of ST- and DA-containing neurons contributes to neurological diseases, such as Parkinson's and Alzheimer's diseases, and perhaps to normal ageing of the brain [69]. Selective serotonin reuptake inhibitors (SSRIs) are the most approved class of psychotropic medications and used as first-line agents to elevate serotonin levels [70-72]. The management of SSRIs to serotonergic neurons indirectly reduces negative response sensitivity to serotonin release, thus modifying the synthesis and transport of serotonin [73-74]. There is uncertainty whether the effects of 5-HT are of physiological and pathogenic importance in food digestion, mucosal defence against noxious components and in functional intestinal disorders. The enteric nervous system (ENS) takes luminal sensory signalling and monitors epithelia functions including secretions. Secretomotor reflexes are introduced by chemical and mechanical interaction between luminal subjects and the mucosa, which results

in the release of ST and other neuroendocrine substances [75-76]. The main signs of the link between ST and anxiety-related behaviour started from the observation that methysergide and metergoline, later on known as 5-HT antagonists, had an anxiolytic outcome in animal studies. The same anxiolytic effect was observed after inhibition of 5-HT synthesis by parachlorophenylalanine in rats in the Geller-Seifter test. This conflict-reducing effect was stopped by treatment with 5-hydroxytryptophan (5-HTP), the precursor of 5-HT [77]. As a result, an increased activity of the central serotonergic system would be connected with anxiety and vice versa reduced activity with declined anxiety [78]. Effective quantity of 5-HT levels is valuable because of their coexistence within biological systems. Several problems are often encountered in the determination of 5-HT concentrations. One is the interference of ascorbic acid (AA), which contains similar oxidation potential and is frequently present in vivo at 0.2 mM concentrations. These evidences have encouraged chemists to develop faster, simpler, and more sensitive techniques to meet the various demands and, various research experiments have been published describing the amount of serum 5-HT concentrations using chemically modified electrodes [79-80]. Tryptophan is an amino acid that is fundamental to the protein biosynthesis. The reduction of tryptophan has been studied in the clinical and preclinical studies to know the relationship between a lowered serotonin system and cognition [81]. The reduced levels of tryptophan are used as a pathway to investigate the importance of serotonin in neurological disorders. In Parkinson's disease patients, it is evident that the reduction in global cognitive function and verbal recognition through acute tryptophan depletion is observed compared with placebo and control patients confirming an interaction between serotonergic and cholinergic impairment [82]. A typical structure of the building blocks of serotonin is shown in Figure 2.1.

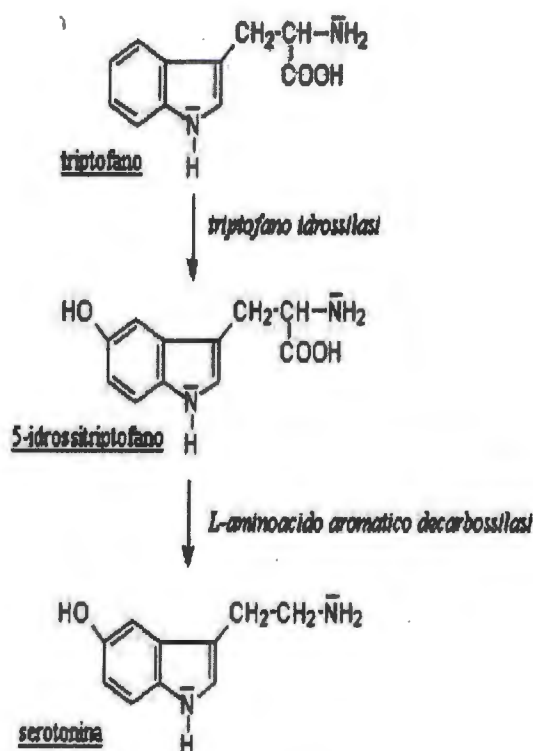


Figure 2.1: Typical Scheme of serotonin synthesis [83].

2.1.2 Epinephrine and its Applications

Epinephrine (EP) known as adrenaline, is a compound that neutrally channel the nerve impulse and is a key hormone produced by the medulla of the adrenal glands [84]. Furthermore, it is recognized as the ‘fight’ or ‘flight’ hormone and released into the blood stream in response to worry or anger and elevates the blood glucose stage [85]. It helps as a chemical mediator for carrying the nerve pulse to different body parts. EP in the medical field is used to stimulate the heartbeat and to manage emphysema, bronchitis, bronchial asthma and other allergic conditions; it is also used in the eye treatment and glaucoma [84]. The alterations in the concentration of EP lead to several diseases, such as, schizophrenia and Parkinsonism [86], therefore it is important to improve quantitative means for epinephrine detection to learn its physiological role and diagnosing certain illnesses in clinical medicine field. The properties of epinephrine in local anesthetics have been well established. Epinephrine is the vital constrictor of blood vessels and blood coagulation accelerator, specifically on the skin or mucous membranes for bleeding control at the procedure site [87-

88]. It decreases the absorption of local anesthetics into the bloodstream, causing reduced systemic toxic side effects, prolonged medical duration of action and decreased surgical blood loss [89-90]. Relating to other neurotransmitters, epinephrine is known for its electroactive groups and the oxidized quinone is widely explored from the electrochemical perspective [91-95]. But so many problems have occurred studying its electrochemical behaviours, due to its electron transfer proportions which are slow hence the adsorbed molecule on the surface of electrode, resulting in a light coat of material. EP detection in biosensors is still encountering problems of the irreversible redox reaction in standard environments, and experiences interference from the coexisting ascorbic acid (AA). Recently, there has been interest in improving electrocatalytic properties of fabricated electrochemical sensor towards the electrode monitoring of EP [96-97]. Humans synthesize tyrosine from the important amino acid phenylalanine (phe), which comes from food this is illustrated on Figure 2.2. The change of phe to tyr is catalyzed by the enzyme phenylalanine hydroxylase, a monooxygenase. This enzyme catalyzes the reaction forming an additional hydroxyl group to the end of the 6-carbon aromatic ring of phenylalanine, hence we get tyrosine. In dopaminergic cells, tyrosine is changed to L-DOPA by the enzyme tyrosine hydroxylase (TH). TH is the rate-limiting enzyme which assists in the synthesis of the neurotransmitter dopamine. Dopamine can be easily changed into catecholamines, such as norepinephrine (noradrenaline) and epinephrine (adrenaline) [98].

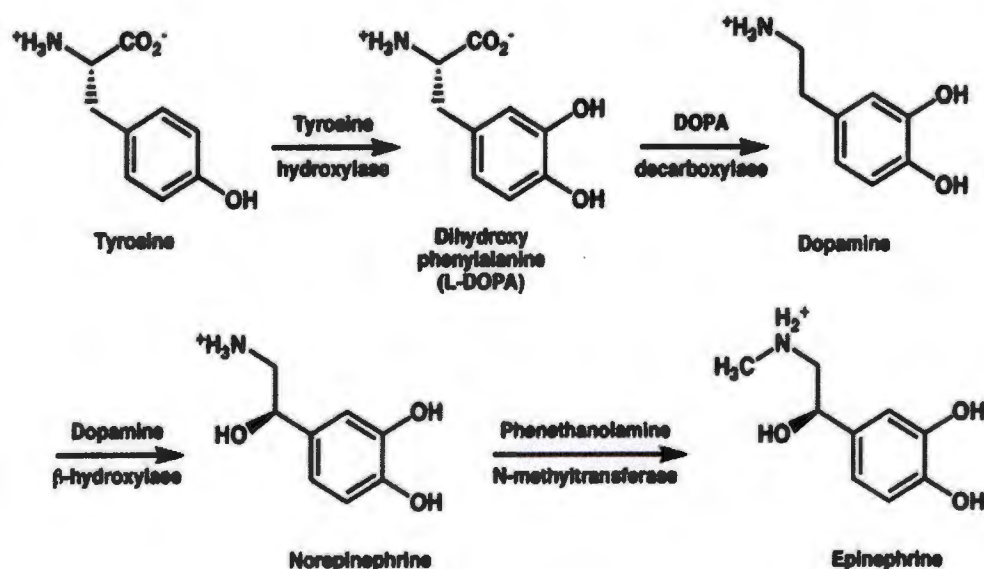


Figure 2.2: Mechanism of conversion of tyrosine to epinephrine [99].

2.2 Ascorbic and their applications

Ascorbic acid, well known as vitamin C is the main water soluble compound present in fruits and vegetables that acts as an antioxidant against a selection of diseases and is essential for life, health, and regular physical activities [100]. The examination of ascorbic acid concentration is crucial for monitoring of food and vegetables quality in daily basis. Ascorbic acid concentration in food, drugs and plants can be determined with different analytical techniques such as indirect spectrophotometric, solid-phase iodine technique and liquid chromatography [101-104]. Interference in the electrochemistry from oxidizable species, such as ascorbic acid and uric acid, in the biological samples impose a threatening problem to apply amperometric biosensors with a working potential of 0.4 V or higher [105]. The modification of the gold nanoparticles on the glassy carbon electrode [106-107] has been tested for determination of EP detection in the presence of AA and UA. Furthermore, the deposition of the over oxidised dopamine on a gold electrode has been effectively tested for selective EP detection in the existence of AA and UA [108].

2.3 Metal Oxide

Metal, semiconductor and magnetic elements performance as functional units for electroanalytical applications has been explored previously [109-112]. Metal nanoparticles offer three significant functions for electroanalysis. These consist of the roughening of the conductive sensing interface, the properties on the catalysis of the nanoparticles permitting their expansion with metals and the improved electrochemical detection of the metal deposits and the conductivity properties of nanoparticles at nanoscale phase that permit the electrical contact of redox-centers in proteins with electrode surfaces [113]. Furthermore, metal and semiconductor nanoparticles offer resourceful labels for improved electroanalysis [114]. Disbanding of the nanoparticles labels and the electrochemical assembly of the dissolved ions on the electrode followed by the removal of the deposited metals present at the usual electroanalytical method. The key functions of nanoparticles were engaged for developing electrochemical gas sensors, electrochemical sensors constructed on a molecular- or polymer functionalized nanoparticles sensing interfaces, and for the assembly of various biosensors as well as immunosensors and DNA sensors [115] and enzyme form of electrodes [116].

2.3.1 Titanium Dioxide and its Application

Titanium dioxide (TiO_2) occurs in nature in three types of polymorphs namely; rutile, anatase and brookite consisting of octahedrally coordinated Ti cations organized in edge sharing chains, but vary in the total shared edges and corners [117]. TiO_2 has certainly become one of the promising *n*-type semiconductors due to its wide band gap (3.2 eV) under ultraviolet light [118]. Its high physical and chemical stability together with its high refractive index makes it one of most researched material [119-120]. Because of its optical and electronic properties, it is widely used in numerous fields such as photocatalyst, solar cells, sensors, self-cleaning, and bactericidal action [121-123]. The required surface properties of TiO_2 make it a promising interface for the immobilization of biomolecules and its use [124] as a food additive [125], in cosmetics [125] and as a possible tool in the treatment of cancer [126]. TiO_2 is usually used in the destruction of toxic organic compounds and microorganisms such as bacteria and viruses and thus used in the purification of polluted air and wastewaters [127-129].

2.3.2 Ruthenium dioxide and its applications

Hydrous ruthenium dioxide, generally expressed as $\text{RuO}_2 \cdot x\text{H}_2\text{O}$ is produced in amorphous or crystalline form [130-131]. From reports, the nano-sized electroactive materials naturally exhibit attractive properties for their electrochemical applications because of the small particle size and high surface/volume ratio, which makes the best out of the electrochemical application of the materials and therefore improve their electrochemical redox performances [132]. RuO_2 has different applications owing to its unique characteristics as well as its high chemical and thermal stability [133-134], good catalytic activity [135], good electrochemical properties [136] and good metallic conductivity [137]. Crystalline RuO_2 electrodes are normally used for Cl_2 development in water [138-139], water splitting into H_2 and O_2 [140], CO oxidation in sensors [141, 135, 142] and reduction of CO_2 in photocatalysis [143]. In contrast, amorphous hydrous RuO_2 ($\text{RuO}_2 \cdot n\text{H}_2\text{O}$) has been studied broadly as an electrode material for supercapacitors [144-145]. The RuO_2 thin films as an enzyme biosensor substrate was studied and offered a low resistivity, high thermal stability, good corrosion resistance, and diffusion barrier properties. Additionally the studied reports show a successful modification of a pH sensor [146-149] by spluttering the thin film as a hydrogen ion sensing

membrane on silicon or PET substrates [150]. The resulting RuO₂-based pH sensor was studied as the bulk of enzyme biosensors for detecting uric acid and glucose

2.4 Polymer

Conducting polymers have been studied as vigorous materials in other optical applications, such as photodetectors [151], optocouplers [152], filled colour image sensors [153] and lasers [154]. It is significant to indicate the use of these materials in others areas of interest outside the optical devices. Conducting polymers also have properties that enable them to be used in batteries [154-156], biosensors [157], drug-releasing agents [158], gas separation membranes [159], electrochemical capacitors [160], electromagnetic radiation shielding [161-162], transistors [163], polymer-polymer rectifying heterojunctions [164] and conductive textiles [165]. The wide applications of conductive polymers using different optical properties upon "doping" or oxidation reduction reactions interests many researchers and these materials have been broadly studied. The possibility of reversible doping/undoping, is followed by broad changes, is the main key for these studies [166].

2.4.1 Polyaniline (PANI) and its Applications

Polyaniline (shown in Figure 2.3) is a conducting polymer with a delocalized conjugated structure, and electrochemical active units of benzenoid and quinonoid, [167-168]. Based on the degree of oxidation PANI exists in different forms such as: leuoemeraldine, emeraldine and perningraniline. The perningraniline base is the completely oxidized constituent of polyaniline shown in Figure 2.3. The main active constituent of polyaniline is emeraldin salt, found by spiking or protonation of emeraldine structure [169]. It has attracted interest due to its electrode material that enhances the sensing sensitivity because of its low cost, simple synthesis, and quite high conductivity [170-172]. It has a large spectrum of adjustable properties emerging from a structure that can easy adapt and direct possible applications in several areas, such as battery electrodes, anticorrosive coatings, gas sensors, energy storage systems, and electrocatalytic devices [173-174]. Furthermore, PANI has the highest environmental stability and is the only known conducting polymer stable in air [175]. PANI is a biosensor interface because it performances as an effective mediator for electron transfer in

redox or enzymatic reactions and it is appropriate medium for the immobilization of biomolecules [176]. The protection of metals and alloys in corrosion using PANI is a very important subject. The inspiration for application of polyaniline in corrosion protection emerges from the environmental constraint for the replacement of toxic layers, mostly chromates, from coating systems [177-178]. It used to be known as aniline blacks were it was used as cotton dyes in textile industry [179].

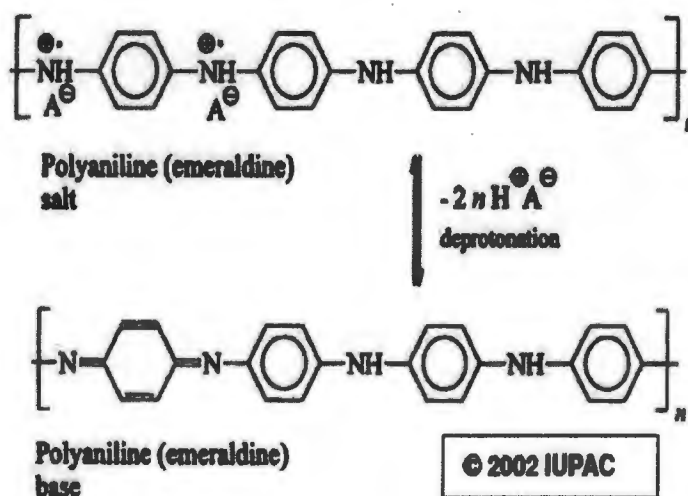


Figure 2.3: Typical schematic diagram of different polyaniline [180]

2.5 Carbon Based Material

An excess of carbon allotropes like diamond, fullerenes, carbon nanotubes and graphene has been studied for use in microelectronics over the years. The application of nanotubes and graphene has been considered extensively because of their low specific resistivity. The real applications for nanotubes and graphene require many layers to be bundled for parallel operation to make their whole resistance comparable or to improve their conventional metallization schemes [181]. A wide spectrum of applications in relation with the environmental protection, energy storage and generation, semiconductors, transparent conducting materials, structural materials, biomaterials, chemical sensors, biosensors, catalysis, and photocatalysis, points out the fields in which the presence of carbon materials play an crucial role in [182-184].

2.5.1 Multiwall Carbon Nanotubes and its Applications

Multiwall carbon nanotubes (MWCNTs) are one of the good supporting materials with photocatalytic properties because of their high mechanical [185] and chemical [186] stability and their mesoporous nature which support the diffusion of the reacting species. Furthermore, MWCNT-based electrodes appear to have high sensitivity with good detection limit [187]. It is known to have unique properties which are capable to change electron transfer reaction when used as modified electrode [188]. Several electrodes based on MWCNT have been studied, such as MWCNT paste electrode [189-190], MWCNT film coated electrode [191], MWCNT powder microelectrode [192], aligned CNT electrode [193-194] and CNT composite electrode [195-196]. There has been an interest on the presence of MWCNT and nano-sized material [197-199]. There has been a review on the function of MWCNT in electroanalytical chemistry particularly in the improvement of new electrochemical sensor and analytical application based on MWCNT-driven electrocatalytic [200]. Nano-sized components constructively support the catalytic sensitivity of MWCNT due to their arrangement of electronic, absorptive, mechanical and thermal properties [201]. For fuel cells, the application of MWCNTs as a catalytic support can possibly decrease Pt usage by 60% compared with carbon black [202], and modified MWCNTs could enable fuel cells that do not need Pt [203-204]. For organic solar cells, continuing efforts influence the properties of MWCNTs to decrease unwanted carrier recombination and improve resistance to photooxidation [205]. MWCNT sensors applications have been seen in toxin detection and gas in the food industry, military, and environmental purpose [206-207].

2.6 Nanocomposite materials

Nanocomposite materials were developed as appropriate alternatives to overcome limitations of microcomposites and monolithics, while encountering problems regarding preparation which determine the elemental composition and stoichiometry in the nanocluster period. They have attracted many researchers in recent year due to their unique design and property combinations that are not found in normal composites. The common knowledge of these properties is yet to be studied [208], although the first interpretation on them was recorded in early 1992 [209]. The surface area/volume ratio of the supporting materials is used in the preparation of nanocomposites and is vital to the understanding of their structure-property

relationships. Additional, sighting of carbon nanotubes (CNTs) in 1991 [210] and their successive use to fabricate composites shows some of the unique CNT related mechanical, thermal and electrical properties [211-213] added an innovative and stimulating aspect to this area. The chance of turning CNTs into multiple products and textiles [214] created a pathway for the processing and applications of CNT-containing nanomaterials. Currently, nanocomposites offer innovative technology and commercial opportunities for all areas of industry, they are also environmentally friendly [215]. Conducting polymer-based composites have attracted interest for the past decade. It is said [216] that the total determination of the whole conducting polymer-based compound system and improve their physical properties (such as electrical conductivity and colloidal steadiness) are yet to be accomplished, Although both their commercial availability in the future and a big rise forward for materials science are expected with their suitable use. In the situation of biodegradable polymer-based nanocomposites, current changes in preparation, characterization and properties, as well as crystallization performance and melt rheology, of both the matrix and the layered (montmorillonite) nanocomposites were debated [217-218]. Likewise, an emphasis on durability and interfacial bonding between CNTs and polymer matrices is critically considered [219] to emphasize the stress transfer from the matrix and the potential of the composites for possible small scale CNT-polymer fabrication. the use of Both synthetic and natural crystalline supports have been applied in Fe and other metal powders, clays, silica, TiO₂ and other metal oxides, whereas clays and layered silicates are regular [220]. This is because of their accessibility of low particle sizes and common intercalation chemistry [221-223], in addition to produce enhanced properties even though they are used at a very low concentration [224]. Most of these supports are prepared by common methods: chemical, mechanical (e.g. ball milling) and vapour deposition.

2.7 Chemically Modified Electrodes (CMEs)

Research has proven that proteins under investigation are actively immobilized on the surface of an electrode. Though, this immobilization process may denaturalize many proteins with the form of change, they also disturb the further analysis of the proteins. Thus, bare electrodes are not the best interfaces to find direct electrochemistry of many proteins; hence, CMEs are established to enhance the situation. CMEs emerged in 1973 when Lane and Hubbard modified different olefin compounds on clean platinum electrode through chemisorption,

which effectively changed the electrochemical response of the electrode [225-226]. Later on the CMEs have been increasing the development of direct electrochemistry of proteins and the mechanisms of redox reactions. The establishment of CMEs is to immobilize particles with definite functions on the regular electrode surface by chemical or physical means. The modification of the CME maintains the biological activities to a certain level hence, electrochemical performance of the electrode is enhanced for the analysis of proteins [227].

Electrodes such as platinum, gold and silver have been broadly used. These electrodes provide favourable electron transfer kinetics and a range of anodic potential. The cathodic potential phase of these electrodes is normally limited due to the low hydrogen overvoltage. Gold and platinum electrodes have stable chemical properties. Thus, these electrodes have turn out to be the most popular electrodes. Silver is decent electrode substrate, which is generally used for the preparation of CMEs in different electrochemical researches [228-231]. Besides noble metal electrodes, there was an opportunity to use other metal as electrode substrates. For example, copper electrode and nickel electrode have been developed for the detection of carbohydrates or amino acids in alkaline media. Associated with platinum or gold electrodes, these two types of electrodes have a stable response for carbohydrates at fixed potentials [232]. Furthermore, alloy electrodes like platinum–ruthenium and nickel–titanium electrodes have been studied, which are regularly used for the preparation of fuel cells, due to their bifunctional catalytic mechanism [233].

2.8 Electrochemistry techniques

Most transducers in electrochemical research are established on potentiometric, amperometric, or conductivity measurements. A sensor is known as a device that measures a physical amount by measuring features of an electrical nature (charge, voltage, or current). The amperometric electrochemical is composed of working (or sensing) electrode (WE), a counter electrode (CE), and a reference electrode (RE). The three electrodes are bounded in the sensor housing which they will be in contact with a liquid electrolyte. An oxidation reaction effects in the flow of electrons from the working electrode to the counter electrode across the external circuit. Equally, a reduction reaction effects in the flow of electrons from the counter electrode to the working electrode. The detection ideologies of the amperometric

biosensor [234] should increase the voltage for the reaction tank and provide enough energy of electrode surface to development the electron transformation [235-236].

2.8.1 Voltammetry Methods

Voltammetry involves different electroanalytical methods in which electrochemical currents are measured as roles of the applied potentials on an operational microelectrode. Depending on the shown current vs potential relationship, the data on the analytes present can be derived. In these case the solution equilibria involves metal ions and metal complexes, voltammetry offers facts about the speciation of the system. Although, in these circumstances the interpretation of voltammetric data is often rather difficult, especially when numerous signals overlap in the experimental voltammograms and their relative sizes and morphologies change during the experiment. The use of voltammetric procedures to determine the metal ions along with biological molecules are of interest as a classical subject from the innovative research on polarography [237-238]. As a result, classical methods such as direct current polarography (DCP) and cyclic voltammetry (CV), at mercury drop electrodes, and other modern ones such as differential pulse voltammetry (DPV), square wave voltammetry (SWV) and linear sweep voltammetry (LSV) have been used for the detection and the clarification of electrochemical mechanisms at rather low concentrations.

2.8.1.1 Cyclic Voltammetry (CV)

Cyclic voltammetry is one of the most resourceful electroanalytical method for the investigation of electroactive species. It is the main characterization in the electrochemical study for biological compounds on the surface of the electrode. CV involves the response of potential of an electrode, which is immersed in an electrolyte solution, and measuring the resulting current [239]. The potential of this working electrode and a reference electrode are controlled by a saturated calomel electrode (SCE) or a silver/silver chloride electrode (Ag/AgCl) respectively. The significant parameters of a cyclic voltammogram are the sizes of the anodic peak current (i_{pa}) and cathodic peak current (i_{pc}), and the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). The procedure of measuring i_p includes extrapolation

of a baseline current. The development of a correct baseline is crucial for the precise measurement of peak currents.

A redox couple that quickly exchange electrons with the working electrode is labelled an electrochemically reversible couple. The official reduction potential (E^0) for a reversible couple is placed between E_{pa} and E_{pc} .

$$E^0 = \frac{E_{pa} + E_{pc}}{2} \quad (1)$$

The total of electrons transferred in the electrode reaction (n) for a reversible couple can be confirmed from the separation between the peak potentials.

$$\Delta E_p = E_{pa} - E_{pc} \cong \frac{0.059}{n} \quad (2)$$

Hence, a one-electron practice such as the reduction of $\text{Fe}^{11}(\text{CN})_6^{3-}$ to $\text{Fe}^{11}(\text{CN})_6^{4-}$ shows a ΔE_p , of 0.059 V. Prolonged electron transfer at the electrode surface, "irreversibility," makes the peak separation to increase. The peak current for a reversible system can be determined by the Randles-Sevcik equation for the forward first cycle.

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

where i_p is peak current (A), n is electron stoichiometry, A is electrode area (cm^2), D is diffusion coefficient (cm^2/s), C is concentration (mol/cm^3), and u is scan rate (V/s). Therefore, i_p increases with $v^{1/2}$ and is relatively proportional to concentration. The concentration relationship is particularly essential in analytical applications and in the research of electrode mechanisms [240]. The Tafel equation can be used for the irreversible-diffusion controlled process [241]

$$E_p = \frac{2.303RT}{2(1-\alpha)n\alpha F} \log v \quad (4)$$

where a is the transfer coefficient, b is the Tafel value, n_α is the number of electrons involved in the rate-determining step. R , T and F are gas constant, temperature and Faraday constant, respectively.

2.8.1.2 Differential Pulse Voltammetry (DPV)

DPV is derived from linear sweep voltammetry and staircase voltammetry, which is exceptionally useful to identify trace levels of organic and inorganic analytes. In this method, there are sequences of consistent voltage pulses superimposed on the potential linear sweep or stair steps. Before each potential transition and late in the pulse life, the currents are documented. The current change is then plotted against the applied potential. In the differential pulse voltammogram, the peak current height can be directly proportional to the concentration of equivalent analytes. The peak potential differs with different analytes, which could be used to differentiate the detected species like the one presented in Figure 2.4. DPV can also help increase the sensitivity of the detection and the resolution of the voltammogram, but also offers records about the chemical form of the analytes, such as oxidation and complexation rank, which is essential for an analysis. Hence, this technology has been extensively used for the electrochemical analysis of proteins and cells [242].

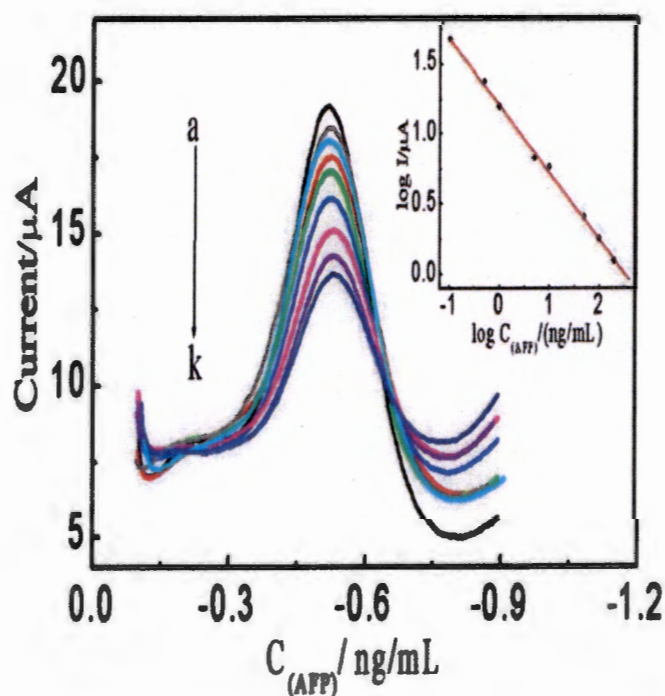


Figure 2.4: Typical differential Pulse Voltammetry plot [243].

2.8.1.3 Square Wave Voltammetry (SWV)

Square wave voltammetry (SWV) (shown in Figure 2.5) has been a primary production of sensitive electrochemical sensors and biosensors. The success of a sensor is directly proportional to how sensitive and selective it connects to its analyte [244]. This may be improved by applying a more sensitive electrochemical method such as SWV. Other procedures for increasing sensitivity comprise the adjustment or improvement of more effective electrodes. Research regarding the outcome of electrodes on sensitivity and detection limits have been applied using boron-doped diamond film electrodes [245], carbon paste electrodes [246], metal oxide based nanowires/ nanotubes [247], and carbon nanotubes [248]. Each of these lessons include the employment of modified or bare electrodes in which SWV was active as the main practice. An analysis of relevant papers dating ten years back in which SWV was used as a technique in sensor study which reveals that the method is increasing in popularity. This technique has affected various fields including diagnostics, environmental analysis, food sciences, enzyme kinetics and pharmaceuticals. This assessment will determine each of these fields derived from the literature over the past five years, but save for pharmaceutical uses, which have been widely recorded [249-254].

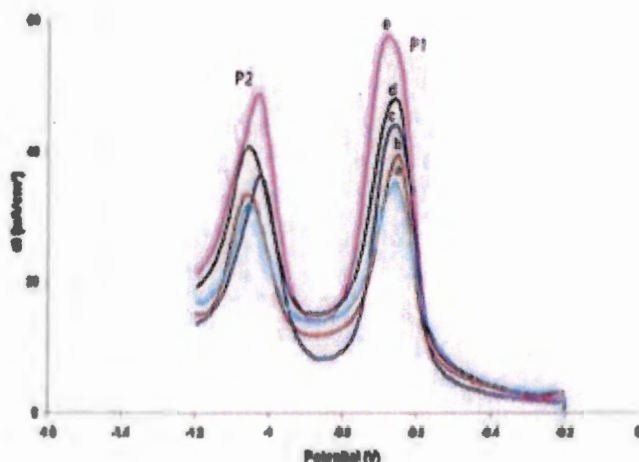


Figure 2.5: Typical square wave voltammetry plot [255]

2.8.2 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) has been used to investigate the electrochemical systems [256], which is involved in studies such as corrosion, electrodeposition [257], batteries [258] and fuel cells [259]. For impedance quantities, a small sinusoidal AC voltage probe (typically 2–10 mV) is used, and the current response is fixed. The current response in-phase controls the real (resistive) component of the impedance, whereas the out-of-phase current response determines the imaginary (capacitive) component. The AC investigation voltage must be small enough so that the system response is linear, enabling easy equivalent circuit analysis. Impedance procedures are powerful, because they are able to characterize physicochemical processes of broadly differing time constants, testing electron transfer at high frequency and mass transfer at low frequency. Impedance results are usually fitted to equivalent circuits of resistors and capacitors, of the Randles circuit presented in Figure 2.6 [260], which is often used to understand simple electrochemical systems.

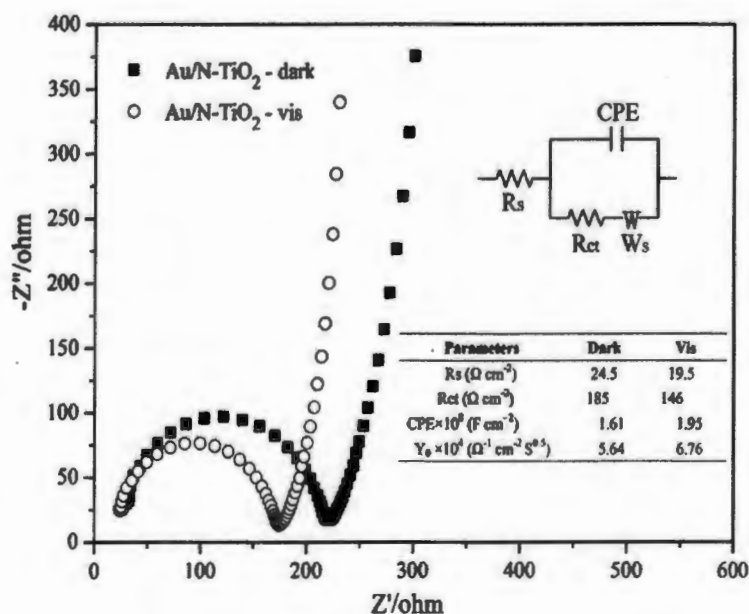


Figure 2.6: Typical electrochemical impedance spectroscopy plot and Randles equivalent circuit [261].

This corresponding circuit produces the Nyquist plot shown in Fig. b, which offers visual understanding of the system's dynamics. R_{ct} is the charge-transfer resistance, which is inversely proportional to the rate of electron transfer; C_d is the double-layer capacitance; R_s is

the solution- phase resistance; and, Z_w is the Warburg impedance, which emerges from mass-transfer boundaries. If an analyte disturbs one or more of these equivalent circuit parameters and these parameters are not disturbed by intrusive species, then impedance procedures can be used for analyte detection. R_s emerges primarily from the electrolyte resistance and it is mostly used in analytical application in conductivity sensors, The Warburg impedance, which is used to measure active diffusion coefficients, is rarely useful for analytical applications. The equivalent circuit components in Figure 2.6 are mostly used in the analyte detection [262].

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials and Reagents

About 2 mm diameter working electrode (Au) was obtained from CH instrument (USA). sulfuric acid (H_2SO_4), potassium permanganate (KMnO_4), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), nitric acid (HNO_3), ethanol absolute ($\text{CH}_3\text{CH}_2\text{OH}$), dimethylformamide (DMF), sodium Nitrate (NaNO_3), potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$), potassium phosphate monobasic (KH_2PO_4), sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) titanium dioxide (TiO_2) precursor powder, ruthenium (111) chloride hydrate ($\text{RuCl}_3 \cdot x\text{H}_2\text{O}$), octanoic acid, pristine multi-walled carbon nanotubes (MWCNT), aniline, ammonium persulfate, serotonin hydrochloride, ($\pm$) epinephrine hydrochloride, ascorbic acid, sodium hydroxide (NaOH) used were of analytical grade and purchased from Sigma-Aldrich chemicals, Merck chemicals and LabChem. Phosphate buffer mixture (PBS, pH 7.0) was composed with suitable amounts of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and the pH monitored with already calibrated pH meter.

3.2 Apparatus and Equipment

Petri dishes, conical flasks, beakers, volumetric flasks, measuring cylinder, Buchner funnel and Buchner flask used were washed in detergent solution and rinsed several times with distilled water. Other apparatus and equipment used include oven, magnetic stirrer, and magnetic bar. Fourier transformed infrared spectrometer (Agilent Technology, Cary 600 series FTIR spectrometer, USA), UV-visible spectrophotometer (Agilent Technology, Cary series UV-vis spectrometer, USA), Transmission electron microscopy (Tecnai G2 spirit FEI, USA), while the high resolution scanning electron microscope uses (Zeiss Ultra Plus 55 HRSEM, Germany) and X-ray diffraction spectrophotometer (Bruker-AXS, Madison, Wisconsin). Electrochemical experiments were performed on an Autolab Potentiostat PGSTAT (Eco Chemie, Utrecht, and The Netherlands) determined by the GPES software version 4.9. Electrochemical impedance spectroscopy (EIS) quantities were achieved with Autolab NOVA software ranging from 100 kHz and 0.1 Hz using a 5 mV rms sinusoidal modulation with the oxidation of the analyte individual peak potential (vs. $\text{Ag}|\text{AgCl}$ in sat'd KCl). A $\text{Ag}|\text{AgCl}$ in saturated KCl and platinum wire were used as reference and counter electrodes respectively. Every experiment was carried out at $25 \pm 1^\circ\text{C}$ whereas the solutions were de-aerated before each electrochemical experiment.

3.3 Synthesis of Titanium dioxide nanoparticles

TiO₂ (2 g) precursor powder (bulk) was mixed in 10 mL NaOH and was treated with hydrothermal at 150 °C for 24 hours in a furnace. The white solid was recovered after the reaction was completed and washed with HCl (0.1 M), accompanied by distilled water. The white solid was dried at 80 °C for 24 hours. Afterward, the powder acquired was constantly heated at 500 °C for 2 hours to produce TiO₂ nanoparticles [263].

3.4 Synthesis of Ruthenium dioxide nanoparticles

Doubled distilled, deionized water was mixed with RuCl₃.xH₂O (0.28 g). 2 mL of octanoic acid was then introduced as a pro-surfactant. About 1.5 M NaOH was gradually added to the solution to produce a precipitate under continuous stirring at a pH 8. The reaction was then heated for an hour at 80 °C. The reaction was chilled and the formed product was washed with distilled water till the product became impartial. It was then again washed with ethanol numerous times. The product was dried for 3 hours at 100 °C and lastly calcinated at 950 °C for 4 hours [264].

3.5 Treatment of MWCNT

MWCNT, 110 mg was treated with a mixture of concentrated H₂SO₄ and HNO₃ acid (120 mL) (v/v 3:1). This mixture was sonicated for 3 hours at 40 °C, and then cooled to room temperature. The solution was slowly added dropwise to 300 mL cold deionised water. It was then filtered and washed with deionised water till pH was neutral. The product was dried at 70 °C for 8 hours [265].

3.6 Preparation of Polyaniline (PANI)

Aniline (0.5 g) was mixed with 40 mL of HCl (1M) and H₂SO₄ solution, stirring at room temperature. The solution was cooled in an ice bath at 5 °C. A mixture of ammonium persulfate (1.22 g), 40 mL of HCl (1 M) and H₂SO₄ solution at 5 °C was slowly added dropwise to the above solution as an oxidant. The mixture took 40 hours to react, and it was then filtered. The filtrate was then washed with deionised water and acetone numerous times. It was then dried at 50 °C for 24 hours [266].

3.7 Electrode Modification Procedure

The surface of the working electrode (Au) was prepared by polishing in aqueous slurry of alumina nanopowder (LabChem) using a SiC-emery paper. The electrode was put through the ultrasonic vibration for 5 minutes in distilled water and absolute ethanol to eliminate the remaining alumina particles that may be caught on the surface. Six suspensions (MWCNT, PANI, TiO₂, RuO₂, MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂) were prepared and in each suspension 5 mg of each synthesized samples (MWCNT, PANI, RuO₂, TiO₂, MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂) were dissolved in 0.5 mL DMF and ultrasonicated for 30 minutes. Au-PANI, Au-MWCNT, Au-RuO₂, Au-TiO₂, Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ were prepared by drop-dry method. Five microliter drops of the PANI, MWCNT, RuO₂ and TiO₂ suspensions were dropped on the bare Au electrode and dried in an oven at 50 °C for 5 minutes.

3.8 Characterization of Synthesized Nano-materials

Successful synthesis of the nano-materials and the nanocomposite were confirmed using techniques such as transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), UV-visible spectroscopy, Raman spectroscopy, x-ray diffraction spectroscopy (XRD) and scanning electron microscopy (SEM). Successful modification of the electrodes was confirmed by using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Electrochemical characterization was carried out in 5 mM ([Fe(CN)₆]^{3-/4-}) redox probe and 0.1 M PBS to study the electron transport properties of the modified electrodes. During electrochemical experiment, bare gold (Au) and Au modified electrodes (Au-PANI, Au-MWCNT, Au-MWCNT-PANI-RuO₂ and Au-MWCNT-PANI-TiO₂) were used as the working electrodes, a platinum wire as the auxiliary electrode, and an Ag/AgCl saturated KCl as the reference electrode respectively. Every experiment was carried out at 25 ± 1 °C, and the solutions were de-aerated before each electrochemical experiment.

3.9 Electrocatalytic Experiment

The electrochemical cell used had three electrodes with separate compartments for the reference (Ag/AgCl) and for the counter electrode. Gold electrode (Au) served as a working electrode. The electrocatalytic experiment was carried out using cyclic voltammetry techniques in 3×10^{-4} M epinephrine and serotonin as analytical probe, prepared in 0.1 M pH 7.0 PBS. Different electrodes were prepared to determine the oxidation of the analytes at

scan rate 25 mVs^{-1} with the potential ranging between 0.2 and 0.8 V. Electrochemical impedance spectroscopy experiment was conducted to study the electron transport performance of electrodes towards epinephrine and serotonin oxidation.

3.10 Concentration Study

Methods such as square wave voltammetry (SWV) and differential pulse voltammetry (DPV) were carried out using Au-MWCNT-PANI-TiO₂ and Au-MWCT-PANI-RuO₂ in 0.1 M pH PBS containing 3×10^{-6} M of EP and ST respectively. The prepared concentrations were used to determine the sensitivity of the electrodes towards the analytes, and the limit of detection (LOD) was calculated.

3.11 Interference Study

The interference study was carried out on Au-MWCNT-PANI-TiO₂ and Au-MWCT-PANI-RuO₂ electrode. The effect of ascorbic acid on the electrocatalysis of 3×10^{-6} M of EP and ST were carried out in 0.1 M PBS pH 7.0. The concentration of AA examined was 1000 times greater than the concentration of EP and ST. The square wave voltammetry was the effective method to determine the increasing concentrations of EP and ST while the ascorbic acid was kept constant.

3.12 Preparation of Real Sample Analysis

The adrenaline injection sample (1 g/mL) was diluted with 0.1 M pH 7.0 PBS. 2 mL of the diluted solution was spiked in six 50 mL volumetric. Five flasks were spiked with another two microliter of different concentration of the standard EP solution, and filled to the mark with 0.1 M PBS pH 7.0. The concentrations were determined using differential pulse voltammetry. The experiment was repeated 3 times.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 FTIR Characterisation

The spectra in Figure 4.1(a) is the FTIR spectrum of TiO_2 , showing a peak around 700 cm^{-1} which agrees with the Ti-O stretching vibration band, suggesting the formation of TiO_2 [267]. The spectra in Figure 4.1(b) is the FTIR spectrum of RuO_2 , showing the distinctive peak at 600 cm^{-1} indicating the Ru-O vibrations band, also confirming the formation of RuO_2 [268]. The spectra in Figure 4.1(c) is the FTIR spectrum of PANI, showing distinctive peaks at 1583 and 1494 cm^{-1} which are related to the C=C vibration bands of quinoid and benzenoid rings in PANI [269], while peaks at 1303 and 1243 cm^{-1} correspond to the C-N and C=N stretching types [270]. Also, the peak at 1150 cm^{-1} is attributed to the in-plane flexibility of C-H [271], and the peak at 826 cm^{-1} is assigned to the out-of-plane flexibility of C-H [272]. Figure 4.1(d) is the spectrum of MWCNT, indicating a broad peak at 1960 cm^{-1} which is associated to the stretch of the carboxylate anion mode. The peak around 2361 cm^{-1} is attributed to the O-H stretching vibration from firmly hydrogen-bonded $-\text{COOH}$ [273-274]. The absorption peak around 3723 cm^{-1} is associated to the hydroxyl (OH) groups [275]. The FTIR spectrum of MWCNT-PANI- TiO_2 in figure 4.1(e) and MWCNT-PANI- RuO_2 in figure 4.1(f) show the characteristics of the metal oxide and MWCNT which confirms the successful formation of the nanocomposites.

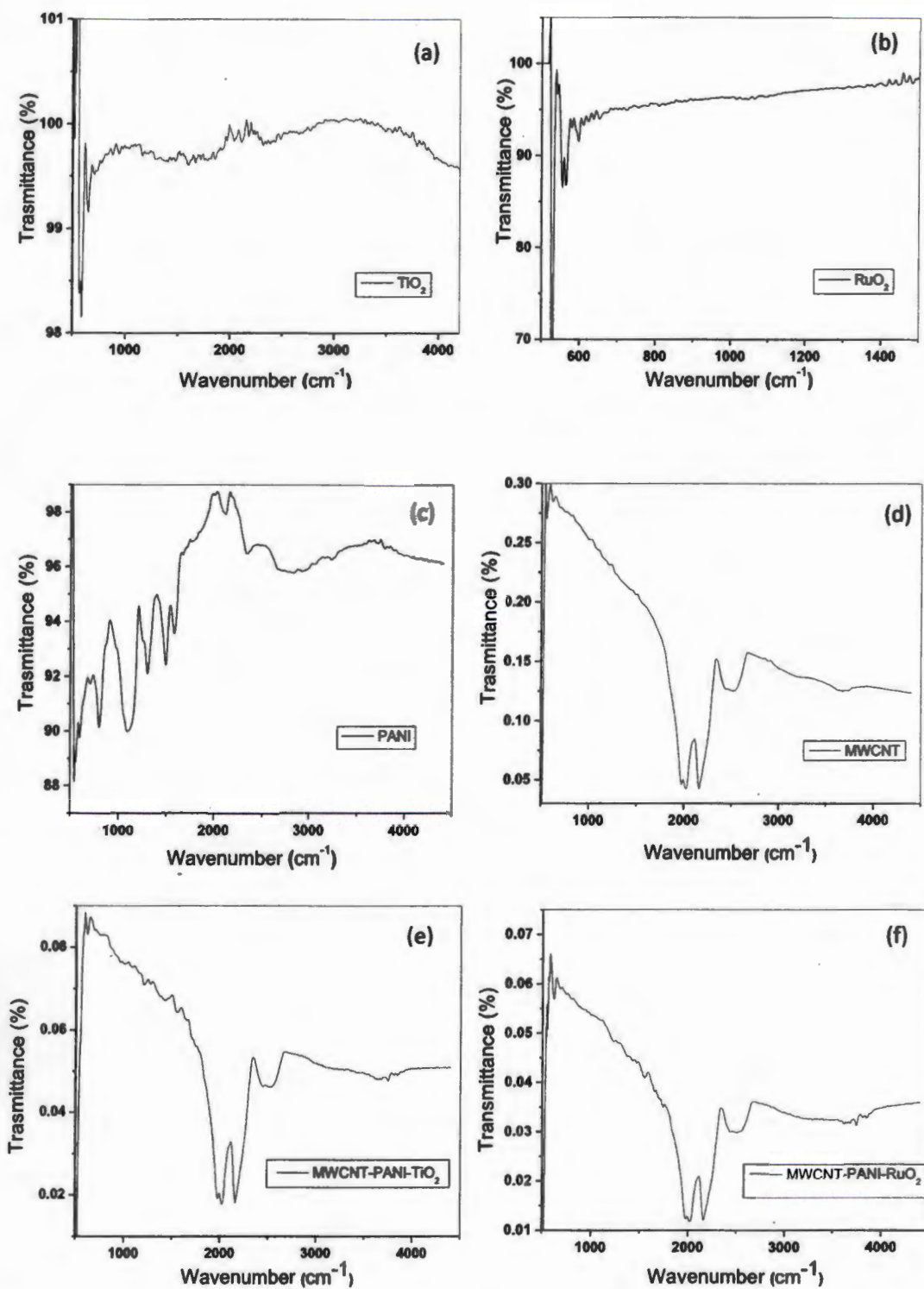


Figure 4.1: FTIR spectra of (a) TiO_2 (b) RuO_2 (c) PANI (d) MWCNT (e) MWCNT-PANI- TiO_2 and (f) MWCNT-PANI- RuO_2 .

4.2 UV-vis Characterization

Figure 4.2(a) is the UV-vis spectra of MWCNT, PANI, TiO_2 and MWCNT-PANI- TiO_2 nanoparticles. It can be seen that the UV-Vis spectrum of PANI shows two clear peaks at 329 and 615 nm. These observed peaks correspond to $\pi-\pi^*$ transitions centered on the benzenoid and quinoid units, respectively [276]. The spectrum of MWCNT-PANI-MO nanocomposites typically contains two broad bands placed around 620 nm and 330–360 nm which indicates $\pi \rightarrow$ polaron, polaron $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ changes in the emeraldine salt of PANI. The shifted blue band relies on the concentration of the metal oxide. The shift shows a rearrangement of polaron density in the PANI emeraldine band gap which is affected by the metal oxide nanoparticles [277]. No observed peaks in the 300–800 nm range for MWCNT sample as reported by Wu et al. [278]. A broad absorption peak was observed for RuO_2 Figure 4.2(b).

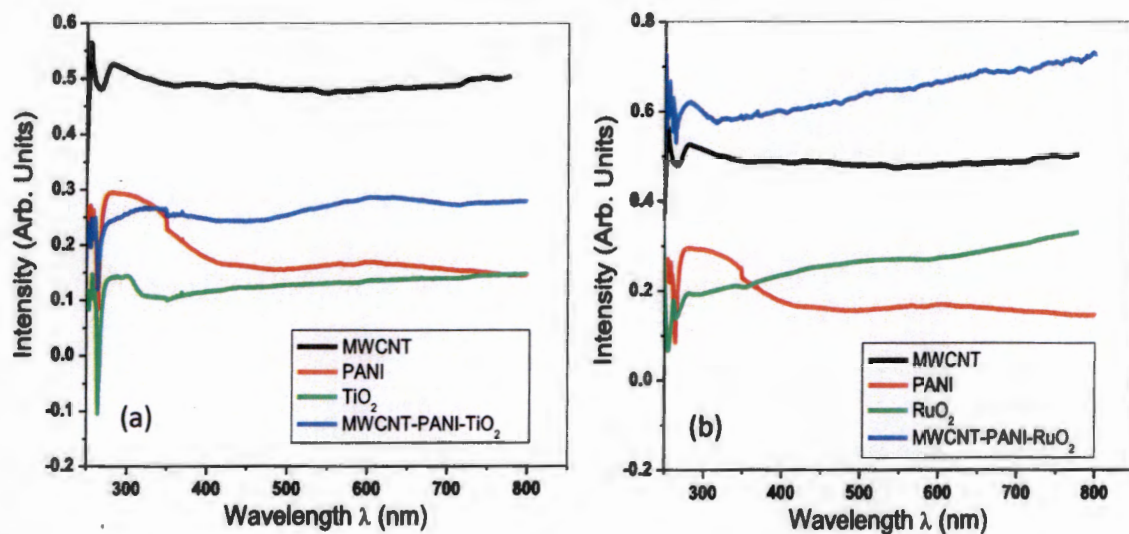


Figure 4.2: UV-Vis spectra of (a) MWCNT, PANI, TiO_2 , MWCNT-PANI- TiO_2 and (b) MWCNT, PANI, RuO_2 , MWCNT-PANI- RuO_2 .

4.3 XRD Characterisation

XRD can offer valuable information on the crystallographic structure of the specimen. Figure 4.3(a), show that no peak patterns were observed in the XRD of TiO_2 , indicating an amorphous structure. XRD spectrum of RuO_2 nanoparticle is presented in Figure 4.3(b). The concentrated peaks validate the crystalline nature of the fabricated RuO_2 nanoparticles. The d-lines patterns indicated for RuO_2 nanoparticles goes as follows (diffraction plan: 110, 101, 200, 211, 220, 002, 310, 112, 301, 202) could be recorded as tetragonal RuO_2 [267]. The pure MWCNTs showed an intense peak centered at 2θ value of 26° which confirm the (002) planes of MWCNTs in Figure 4.3(c). The peaks centered at 53° were because of the (110) and (100) graphitic planes and small amount of the constituent enclosed inside MWCNTs walls [279]. Figure 4.3(d) indicates a broad peak around 25.3° , assigned to the diffraction of (110) plane of the crystalline phase of PANI, confirming the effective synthesis of PANI [280]. When carbon nanotubes were integrated into the PANI medium, the sharp and strong diffraction peak of MWCNT at (26.28°) was observed as an overlap with the PANI peak resulting in a wide peak in the composite as shown in Figure 4.3(e). The information indicates that no other crystalline order was added into the composite [281]. Similarly, Figure 4.3(f) clearly showed the enhanced crystallinity nature of the MWCNT-PANI- RuO_2 composite due to the well-defined peaks of the RuO_2 nanopartilces overlaying the PANI molecules.

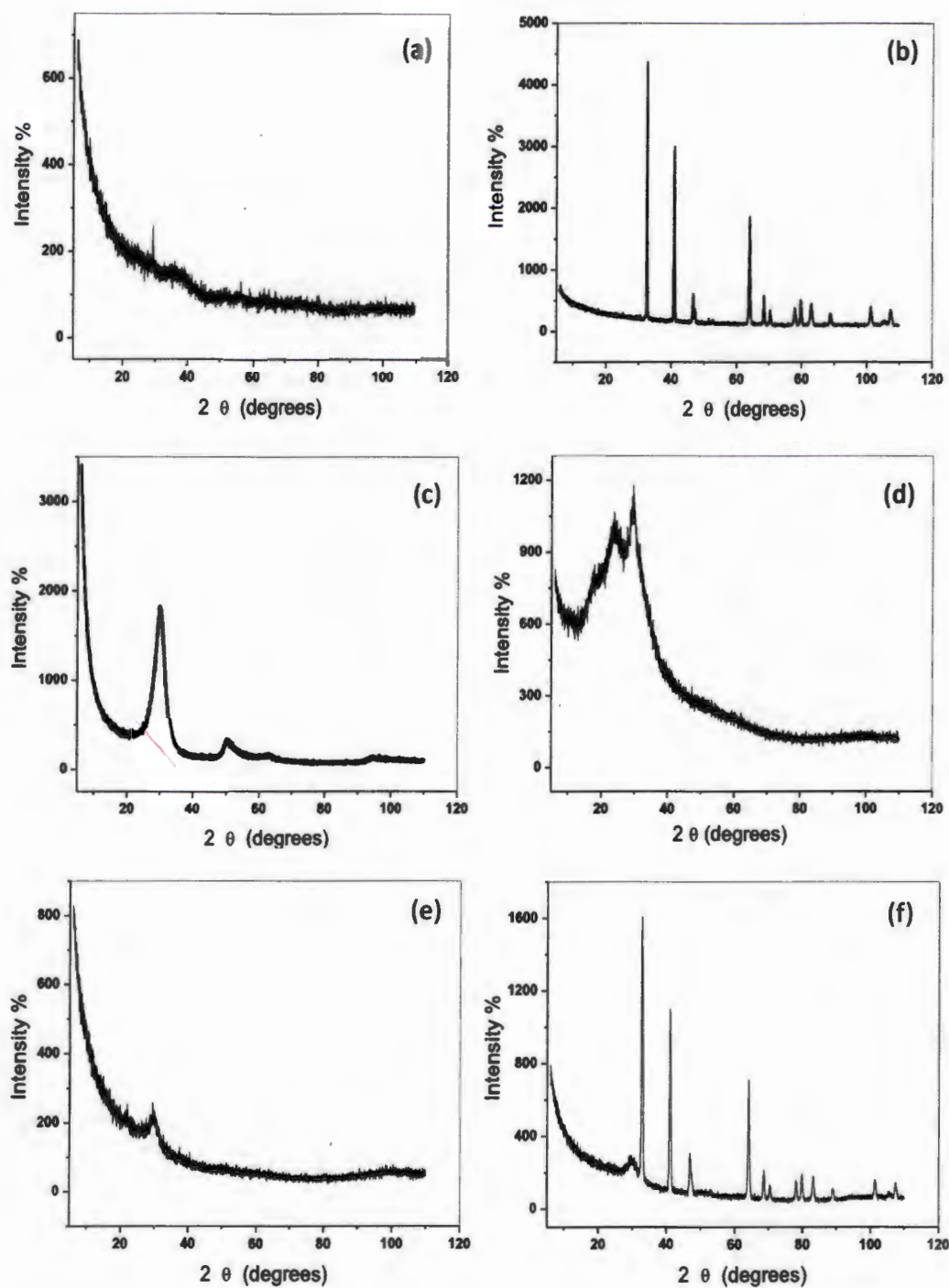


Figure 4.3: XRD spectra of (a) TiO_2 (b) RuO_2 (c) MWCNT (d) PANI and (e) MWCNT-PANI- TiO_2 and (f) MWCNT-PANI- RuO_2 .

4.4 Surface Morphology

The surface morphologies of the prepared nanocomposites were analysed by SEM. The samples were first gold coated to make the surface well conducting. Figure 4.4 (a-f) represent the SEM images of TiO_2 , RuO_2 , MWCNT, PANI, MWCNT-PANI- TiO_2 and MWCNT-PANI- RuO_2 . Figure 4.4(a) shows the morphology of TiO_2 nanoparticles due to heat treatment. The tube-like structure which broke into smaller particles was observed and this was due to dehydration of inter-layered OH groups that destroyed the nanotubes structure after heat treatment at 500 °C for 2 hours [282]. The SEM image of RuO_2 (Figure 4.4(b)) showed uniformly distributed nanoparticles with some aggregation forming spherical shaped particles with larger sizes [283]. Figure 4.4(c) for the MWCNT material appeared to show thin nanofibers which merged to form bundles, entwined together. PANI Figure 4.4(d) shows more of a spherical structure and Figures 4.4(e and f) shows the SEM images of the MWCNT-PANI- TiO_2 and MWCNT-PANI- RuO_2 respectively, indicating the encapsulation of the $\text{TiO}_2/\text{RuO}_2$ nanoparticles inside the PANI/MWCNT fibres resulting in a more porous nanocomposite materials.

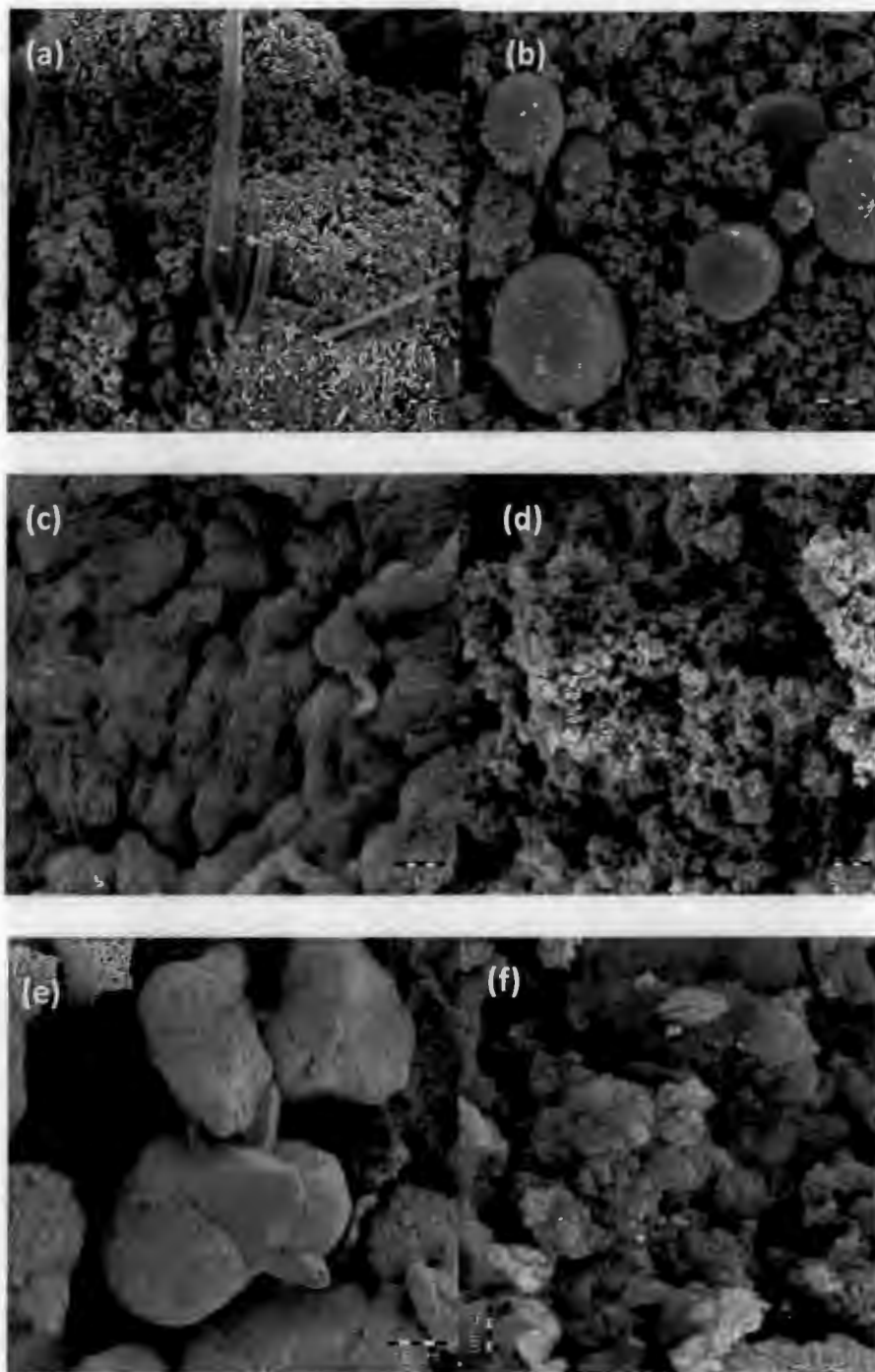


Figure 4.4: SEM images of (a) TiO_2 , (b) RuO_2 , (c) MWCNT, (d) PANI, (e) MWCNT-PANI- TiO_2 and (f) MWCNT-PANI- RuO_2 .

4.5 Electrochemical Characterization

Comparative cyclic voltammetric evolutions of modified electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ preparation in PBS pH 7.0 is shown in Figures 4.5 for different electrodes. The significance of this study was to explore the best electrode with enhanced electron transport properties. In Figure 4.5(b), the redox peaks observed around 0.2 V is ascribed to the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox process while the oxidation peak around 1.0 V observed on the modified Au-MWCNT-PANI-RuO₂ is attributed to Au to Au³⁺ or Au⁴⁺ [284], when MO (TiO₂, RuO₂) nanoparticles were incorporated into MWCNT/PANI in MWCNT-PANI-MO composite modified Au electrode as compared with that of MWCNT modified Au. The redox couple of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ appears rather reversible at the Au-MWCNT-PANI-TiO₂. It is because the peak currents acquired are well-defined with $I_{pa}/I_{pc}=1.01$, and a peak separation of 154 mV as its $E_{pa} = +276$ mV and $E_{pc} = +122$ mV vs. Ag/AgCl. On the other hand, Au-MWCNT-PANI-RuO₂ appeared to be quasireversible due to the different intensity of the anodic and the cathodic currents. It can be seen that peak current of the composites modified electrodes were higher than other electrodes, which are ascribed to the more electroactive sites of PANI which supported the MWCNT-MO fast rate electron transfer due to the synergic effect of MWCNT-MO. The results show that MWCNT contributes to significant improvement of the increase in electrochemical response observed on MWCNT-PANI-MO modified electrodes. Therefore, the comparative cyclic voltammetry studies reveal that the best electrodes are Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ due to their improved current responses [285-286]. Changes observed throughout the CV during the modification processes are another considerable proof for the successful immobilization of PANI, MWCNT, TiO₂ and RuO₂ on the electrode surface. The results showed that MWCNT-PANI-MO nanocomposite modified electrodes demonstrated enhanced electrochemical response suggesting that they could be potential electrode materials for electrochemical sensors applications [287].

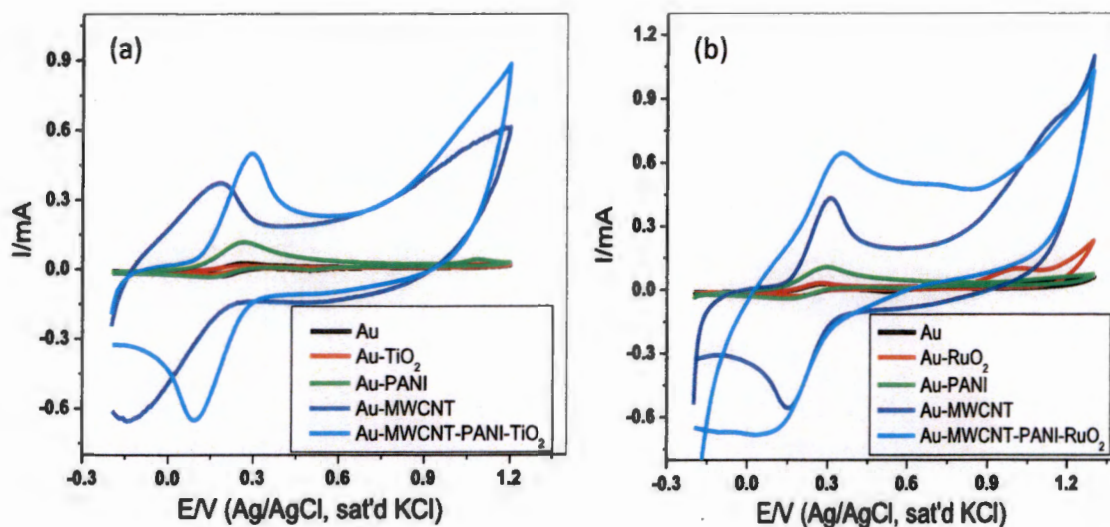


Figure 4.5: Cyclic voltammetric evolutions of the modified electrodes in 5 mM [Fe(CN)₆]⁴⁻/[Fe(CN)₆]³⁻ (a) Au, TiO₂, PANI, MWCNT and MWCNT-PANI-TiO₂ modified electrodes (b) Au, RuO₂, PANI, MWCNT, and MWCNT-PANI-RuO₂ modified electrodes.

4.6 Electrochemical Impedance Studies

Electrochemical impedance spectroscopic studies (EIS) was used to investigate the electrochemical behaviors (electron transfer properties) of various modified electrodes in the presence of [Fe(CN)₆]^{3-/4-} redox probe. The Nyquist plots are shown for different modified electrodes in Figure 4.6(a and b). This platform examines the surface status and the difficulties of modified electrodes due to electron transfer amongst the electrolyte and the electrode channeling through the drawbacks or through defects in their limitation [288]. Figure 4.6(c) denotes the circuit model used in the fitting and the fitting parameters obtained are presented in Table 4.1. In the circuit model, R_s is the solution resistance, Q is the constant phase angle, and R_{ct} is the charge transfer. The observed impedance spectra showed a linear portion and a semicircle portion. The linear part at lesser frequencies reflects the diffusion procedure, and the electron transfer resistance (R_{ct}), is symbolized by the semicircle at a high frequency, which monitors the electron-transfer kinetics at the electrode/electrolyte interface in the redox probe. Therefore, R_{ct} is used to determine the interfacial properties of the electrode. For the bare gold electrode, the probe can effortlessly transfer an electron to the surface of the electrode. On the other hand, redox process of the [Fe(CN)₆]^{3-/4-} probe on MWCNT-PANI-TiO₂ ($R_{ct} = 0.977 \text{ k}\Omega$) and MWCNT-PANI-RuO₂ ($R_{ct} = 0.989 \text{ k}\Omega$) modified

electrodes show an electron-transfer resistance that is considerably lower than that of the bare gold electrode ($R_{ct} = 193 \text{ k}\Omega$). The results showed enhanced electron transport properties and successful modification of the gold electrode with the MO nanocomposite materials. Therefore, the Au-MWCNT-PANI-MO electrodes showed exceptional electron conducting transport properties. This can be ascribed to the negative $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ions which were attracted by the abundant positive charges contained on the Au-MWCNT-PANI-MO electrode surface.

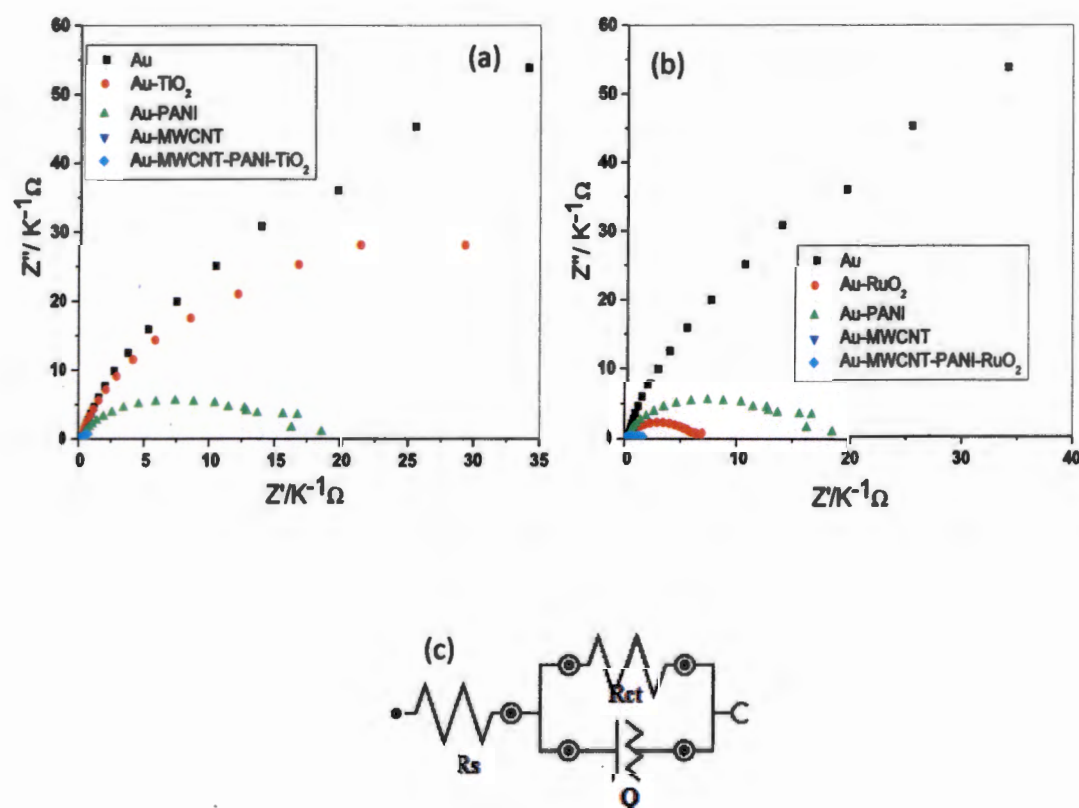


Figure 4.6: Nyquist plots obtained for (a) Au, TiO₂, PANI, MWCNT and MWCNT-PANI-TiO₂ modified electrodes (b) Au, RuO₂, PANI, MWCNT and RuO₂ modified electrodes in 5mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ at a fixed potential of 1.0 V (vs Ag|AgCl, sat'd KCl). (c) Represents the circuit used in the fitting of the EIS data for bare and modified electrodes.

Table 4.1: Impedance data acquired for bare and modified electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ at a fixed potential of 1.0 V (vs Ag|AgCl, sat'd KCl). Values in parenthesis are percentage errors of the data fitting.

Electrode	R_s (Ω)	Q (μF)	R_{ct} ($\text{k}\Omega$)
Au	281 (3.91)	5.99 (13.59)	193 (6.51)
Au-TiO ₂	231 (4.74)	6.10 (9.61)	7.72 (10.21)
Au-RuO ₂	225 (4.28)	7.82 (16.10)	6.12 (6.68)
Au-PANI	268 (4.33)	6.74 (12.2)	15.4 (7.47)
Au-MWCNT	85.2 (4.57)	582 (20.23)	2.73 (12.84)
Au-MWCNT-PANI-TiO ₂	94.8 (4.28)	1220 (12.79)	0.977 (17.78)
Au-MWCNT-PANI-RuO ₂	188 (4.00)	1.46 (18.67)	0.989 (14.16)

4.7 Effects of Scan Rate

The effect of the scan rate ($25 - 1000 \text{ mVs}^{-1}$) on the anodic peak current was investigated for the Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrodes respectively in 0.1 M PBS (pH = 7) containing 5 mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ solution. The study was conducted using cyclic voltammetry method and the plot of peak current (I_p) versus the square root of scan rate ($v^{1/2}$), are shown in Figure 4.7. The anodic oxidation peak current is proportional to the square root of scan rate. This plot was found to be linear in Figure 4.7(b and d), signifying that, at appropriate over potential, the process was diffusion-controlled [289] with a correlation coefficient of 0.9709 and 0.9994 for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrode respectively. The potential shift due to the oxidation increased with the scan rate, signifying the quasi-reversible character of the electrode reaction [290], which indicates that there was some level of adsorption on the electrode surface due to oxidation product. A linear plot for E_{pa} vs. the $\log v$ was obtained for Au-MWCNT-PANI-TiO₂ (Figure 4.8a) and Au-MWCNT-PANI-RuO₂ (Figure 4.8b) modified electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ respectively. From the slope of the Tafel plots (E_p vs. $\log v$), (as shown in equation 5) the Tafel values 'b' was calculated to be

0.8052 and 0.6038 V for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrodes respectively. The Tafel values calculated were greater than the notional 0.118 V dec⁻¹. This is due to the adsorption of electrolyte or its intermediates on the surface of the electrode, suggesting that electrode porosity could be the contributing factor [291].

$$E_p = K + \frac{b}{2} \log v \quad (5)$$

The kinetic parameter α_a (anodic transfer coefficients) was calculated using the equation $2.303RT/(1-\alpha)nF$. The slope shows a one electron transfer process as a limiting step with a transfer coefficient (α), of 0.98 for both electrodes.

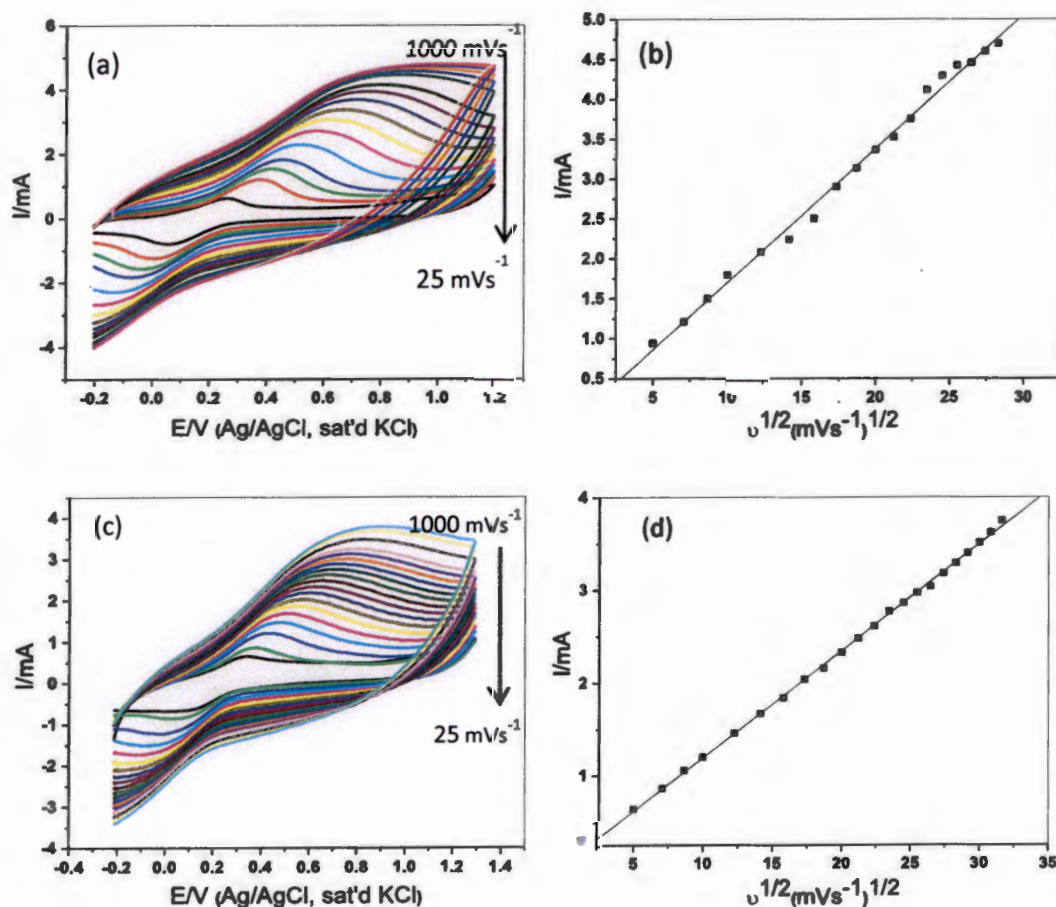


Figure 4.7: Cyclic voltammetric evolutions of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ electrode obtained in 5 mM [Fe(CN)₆]⁴⁻/[Fe(CN)₆]³⁻ (scan rate range 25 – 1000 mVs⁻¹; inner to outer).

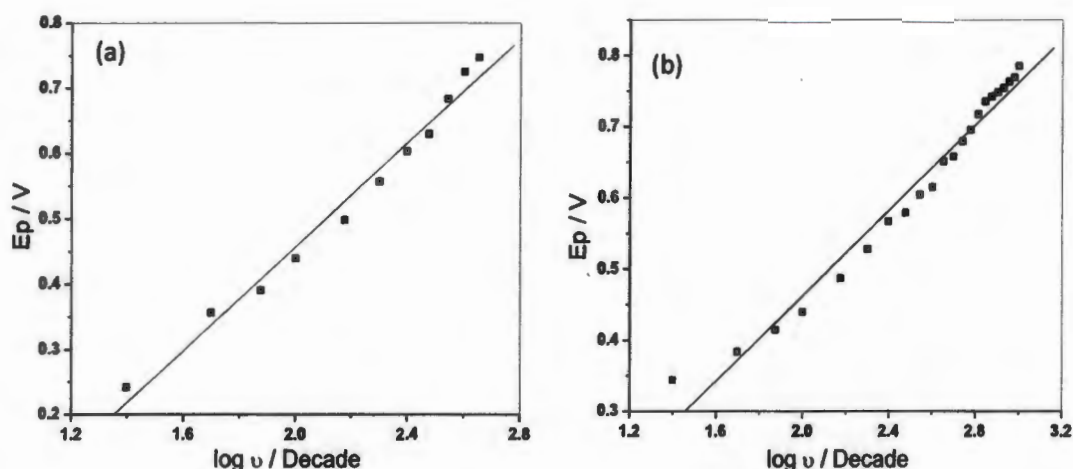


Figure 4.8: Plots of peak potential (E_p) versus $\log v$ for (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ in 5 mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$.

ELECTROCATALYTIC OXIDATION OF EPINEPHRINE

4.8 Electrocatalytic Oxidation of Epinephrine

Figure 4.9 shows the cyclic voltammograms for the electrocatalytic behaviour of bare Au and Au modified electrodes towards 3×10^{-4} M EP in 0.1 M PBS solution (pH = 7) at a scan rate of 25 mVs^{-1} . An enhanced EP oxidation current was observed at Au-MWCNT-PANI-MO modified electrode compared with bare Au and other electrodes investigated, signifying that the Au-MWCNT-PANI-MO modified electrode possessed faster electron transfer kinetics [292]. The electrochemical data due to EP oxidation at the electrodes are presented in Table 4.2. The anodic peak at 0.24 V is attributed to the epinephrine oxidation of the open-chain quinone, and the peak at -0.17 and -1.12 V respectively are attributed to the reduction of adrenochrome to leucoadrenochrome [293]. The modified electrode of the EP oxidation current was almost three times greater than that of the bare electrode. The higher current response observed at the modified electrode evidently shows that MWCNT-PANI-MO modified electrode successfully enhanced electrocatalysis of EP probably due to the presence of porous MWCNT and PANI nanoparticles, leading to increased surface area of the electrode, and the electrical conductive nature of the metal oxide nanoparticles [294].

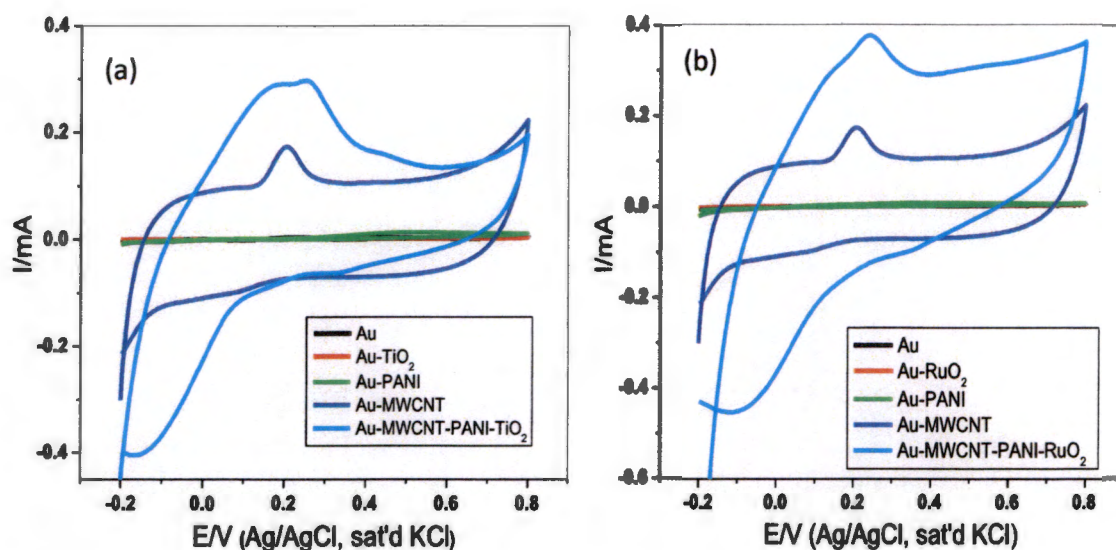


Figure 4.9: Current response of (a) Au, TiO₂, PANI, MWCNT, MWCNT-PANI-TiO₂ and (b) Au, RuO₂, PANI, MWCNT, MWCNT-PANI-RuO₂ modified electrodes in 0.1 pH 7.0 PBS containing 3×10^{-4} M EP.

Table 4.2: Cyclic voltammetric data acquired for bare and the modified electrodes in EP 3×10^{-4} M in pH 7.0 PBS.

Electrode	I_{pa} (mA)	E_{pa}
Au	0.003	0.231
Au-TiO ₂	0.002	0.318
Au-RuO ₂	0.003	0.275
Au-PANI	0.013	0.509
Au-MWCNT	0.17	0.205
Au MWCNT-PANI-TiO ₂	0.29	0.211
Au-MWCNT-PANI-RuO ₂	0.40	0.241

4.9 Electrochemical impedance spectroscopy

The impedance spectral information recorded for bare Au, Au-TiO₂, Au-RuO₂, Au-PANI, Au-MWCNT, Au-MWCNT-PANI-MO (in the form of Nyquist plots) in 0.1 M PBS containing 3×10^{-4} M EP at potential of 0.22 V with the range from 100 KHz to 0.1 Hz are presented in Figure 4.10. The Nyquist plot comprised of a semi-circular portion and a linear portion. The semicircular section relates to the charge transfer limited process and its diameter is equivalent to the charge transfer resistance (R_{ct}), which determines the electron transfer kinetics, whereas, linear part of the plot at lower frequencies relates to the mass diffusion limited process [295]. The value broadly differs based on the modification of the electrode surfaces. To determine the R_{ct} values, the impedance values were fitted to Randles equivalent circuit due to the features of the obtained impedance spectrum. The modification of MWCNT on the Au electrode, responded with a higher diameter of semicircle ($R_{ct} = 11.0 \text{ K}\Omega$) due to the surface fouling activity. The R_{ct} value for the Au-MWCNT-PANI-TiO₂ electrode (3.27 K Ω) and Au-MWCNT-PANI-RuO₂ electrode (5.20 K Ω) in EP are lower compared with other investigated electrodes as reported in Table 4.3. Therefore, Au-MWCNT-PANI-TiO₂ electrode proved to have a lower charge-transfer resistance, indicating remarkable enhancement of the catalytic reaction and electron transfer [296].

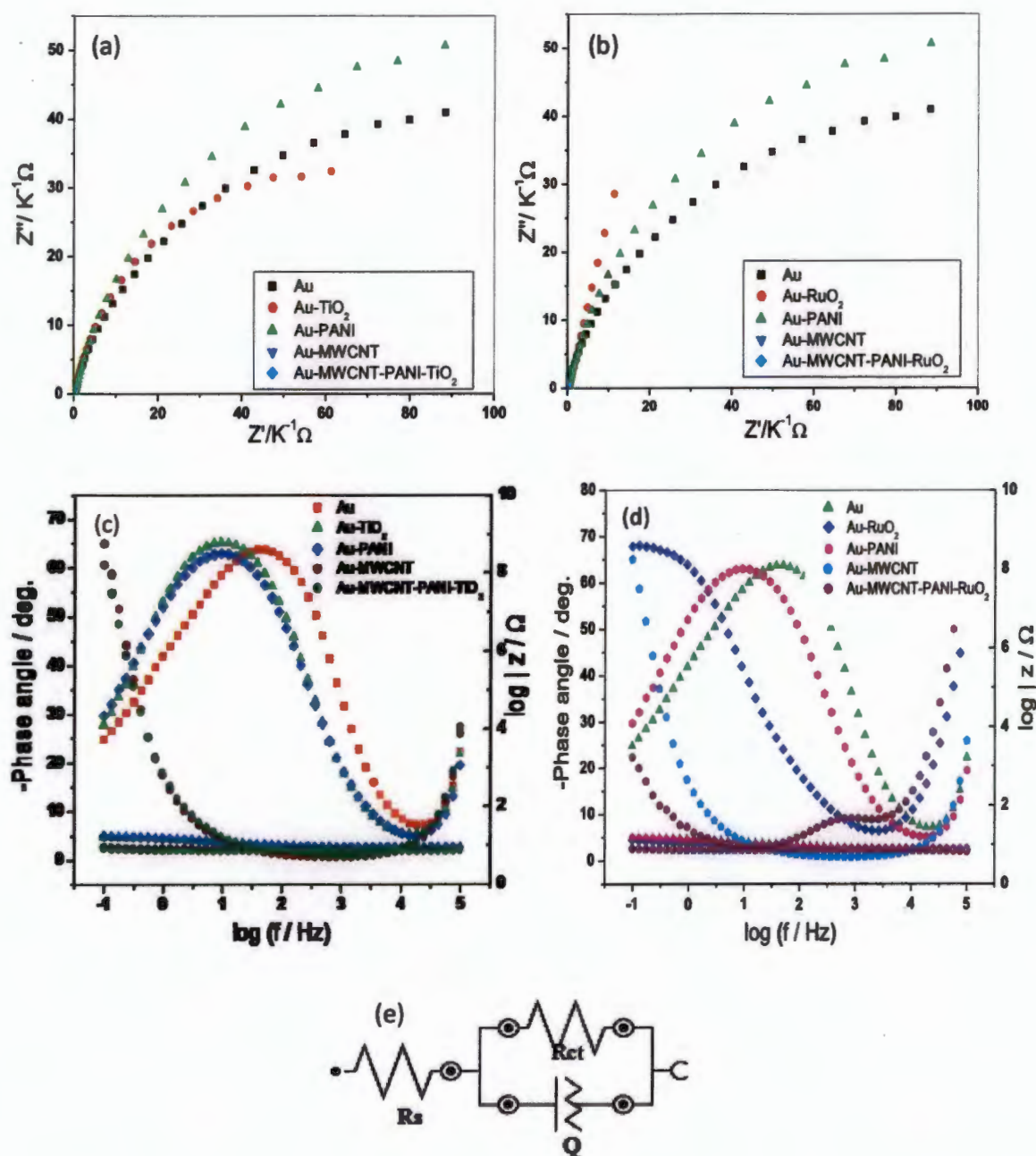


Figure 4.10: Nyquist plots obtained for (a) Au, TiO₂, PANI, MWCNT, MWCNT-PANI-TiO₂ and (b) Au, RuO₂, PANI, MWCNT, MWCNT-PANI-RuO₂ in 0.1M pH 7.0 PBS containing 3×10^{-4} M of EP solutions at a fixed potential of 0.22 V (vs Ag|AgCl, sat'd KCl). (c) Au, TiO₂, PANI, MWCNT, MWCNT-PANI-TiO₂ and (d) Au, RuO₂, PANI, MWCNT, MWCNT-PANI-RuO₂ respectively are the Bodes plots obtained in EP showing the plots of -phase angle / deg. vs $\log(f/\text{Hz})$, and the plot of $\log |Z|/\Omega$ vs $\log(f/\text{Hz})$ (e) denotes the circuit used in the fitting of the EIS data in (a) and (b).

From the Bode plots of -phase angle vs. $\log(f / \text{Hz})$ for the electrodes (Figs. 10 c and d), except for Au-MWCNT, Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂, all the electrodes presented a phase angle greater than -60° , but less than -90° expected for ideal capacitive behaviour indicating pseudocapacitive behaviour of the electrodes towards EP electrocatalytic oxidation. Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes demonstrated much lower phase angles (less than 10°) in EP. This showed that the two composite modified electrodes are less capacitive, and transported EP oxidation current faster than other electrodes in the analyte. This result agreed with high current recorded under the CV experiment, and the low R_{ct} values. The lower capacitance, enhanced electron transport and catalytic behaviour of this electrode can be ascribed to the conductive nature of the MWCNT and RuO₂ and the synergic effect contributed by the two nanoparticles. Since Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes exhibited the best performance in this study, further studies were carried out using the two electrodes.

Table 4.3: EIS data acquired for bare and modified electrodes in EP 3×10^{-4} M in pH 7.0 PBS. Values in parenthesis are percentage errors of the data fitting.

Electrode	R_s (k Ω)	Q (μ F)	R_{ct} (k Ω)
Au	0.30 (3.16)	4.49 (5.33)	102 (5.86)
Au-TiO ₂	0.28 (1.97)	9.05 (3.62)	85.9 (5.44)
Au-RuO ₂	0.36 (7.53)	62.5 (15.94)	110 (6.56)
Au-PANI	4.38 (1.79)	6.72 (5.42)	134 (5.42)
Au-MWCNT	0.16 (1.79)	38.2 (3.26)	11.0 (14.63)
Au-MWCNT-PANI-TiO ₂	3.10 (1.91)	0.136 (6.64)	3.27 (20.78)
Au-MWCNT-PANI-RuO ₂	4.87 (10.53)	0.545 (20.40)	5.20 (8.28)

4.10 Stability Study

The stability of MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ sensor towards EP determination was observed by measuring the current response (20 scans) at constant concentration of EP (3×10^{-4} M) in pH 7.0 PBS (Figure 4.11). After 20 scans, Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes comparatively showed a low drop in current from the first scan to the second scan and this slight decrease was obtained with the relative standard deviation (R.S.D.) of 4.4 % and 3.6 % respectively, indicating that the modified electrodes possesses efficient stability for the determination of EP. This further confirms that the modified electrodes have efficient reproducibility and strong ability to avoid fouling of the electrode due to the oxidation product. The adsorptive nature of the electrodes towards EP can be assigned to the porous MWCNT in the composite [297]. The Au-MWCNT-PANI-RuO₂ electrode performs better toward the analyte compared with Au-MWCNT-PANI-TiO₂ electrode. After experiment, the modified electrodes were kept at 4 °C in the refrigerator. The EP oxidation peak current reduced by 6.8 %, and 6.0 % for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes respectively after three days. The above results indicate that the stability of the sensor was satisfactory for the determination of EP.

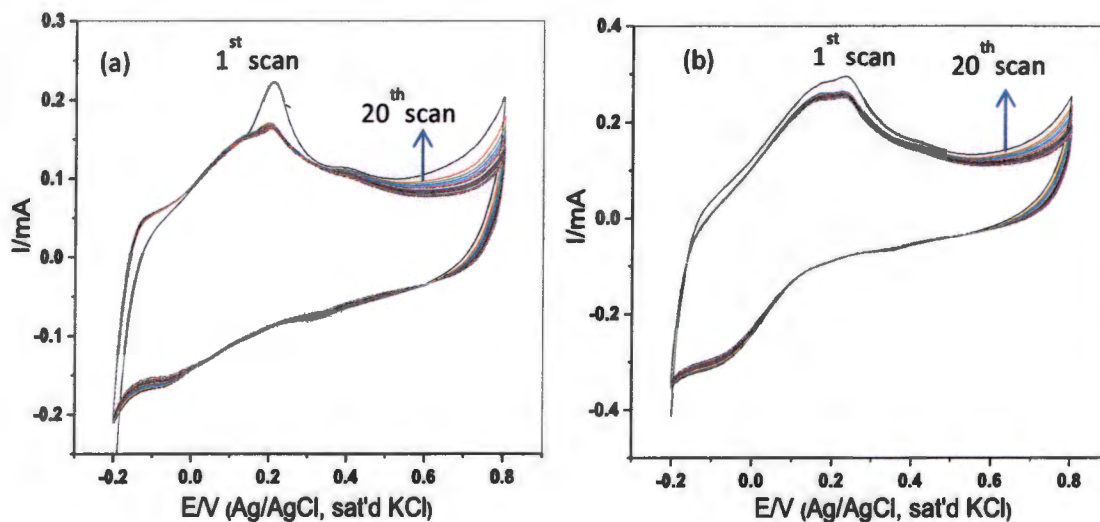


Figure 4.11: Current response (20 scans) of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ in pH 7.0 PBS containing 3×10^{-4} M of EP at scan rate of 25 mVs^{-1} .

4.11 The Effect of Scan Rate

The effect of scan rate on the peak current due to oxidation of EP was explored at the surface of MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ electrodes at a constant concentration of 3×10^{-4} M EP using cyclic voltammetry in Figure 4.12 (a-d). The anodic current for both electrode composites increased with the increasing scan rate (25 to 300 mVs⁻¹). Figure 4.12(b and d) showed linear graphs of peak current versus square root of scan rate (I_p vs $v^{1/2}$), with a correlation factor $R^2 = 0.9872$, and $R^2 = 0.9986$ for MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ electrodes respectively. The linear plot between peak currents and square root of scan rates symbolise that the oxidation currents were diffusion controlled, which clarifies the quantitative measurements. These occurrences might be due to the rates of adsorption of E_p on the electrode surface being slower than the diffusion rates in that scan rate range [298]. The plot of I_p vs $v^{1/2}$ was used to estimate the surface coverage of the electrode by adopting the method used by Sharp et al [299]. Therefore there must be a relationship between the peak current and the surface concentration of electroactive species, Γ , using the following equation (6) below;

$$I_p = \frac{n^2 f^2 A \Gamma v}{4RT} \quad (6)$$

where the number of electron transfer is n , the geometric surface area of the electrode (A) is (0.0629 cm²), Γ the surface coverage (mol cm⁻²), v is the scan rate, and T , R , and F are the absolute temperature, molar gas constant, and Faraday constant, respectively. The calculated surface concentration of MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ in EP were 6.41×10^{-5} mol cm⁻² and 7.11×10^{-5} mol cm⁻² respectively. The Tafel values were obtained using equation (5), where E_p was plotted against $\log v$ in Figure 4.13, the Tafel 'b' values were estimated to be 0.448 V and 0.422 V. This is due to the adsorption of EP or its intermediates on the surface of the electrode, suggesting that electrode porosity could be the contributing factor [291]. The slope shows a one electron transfer process as a limiting step assuming a transfer coefficient (α), of 0.99 for both electrodes.

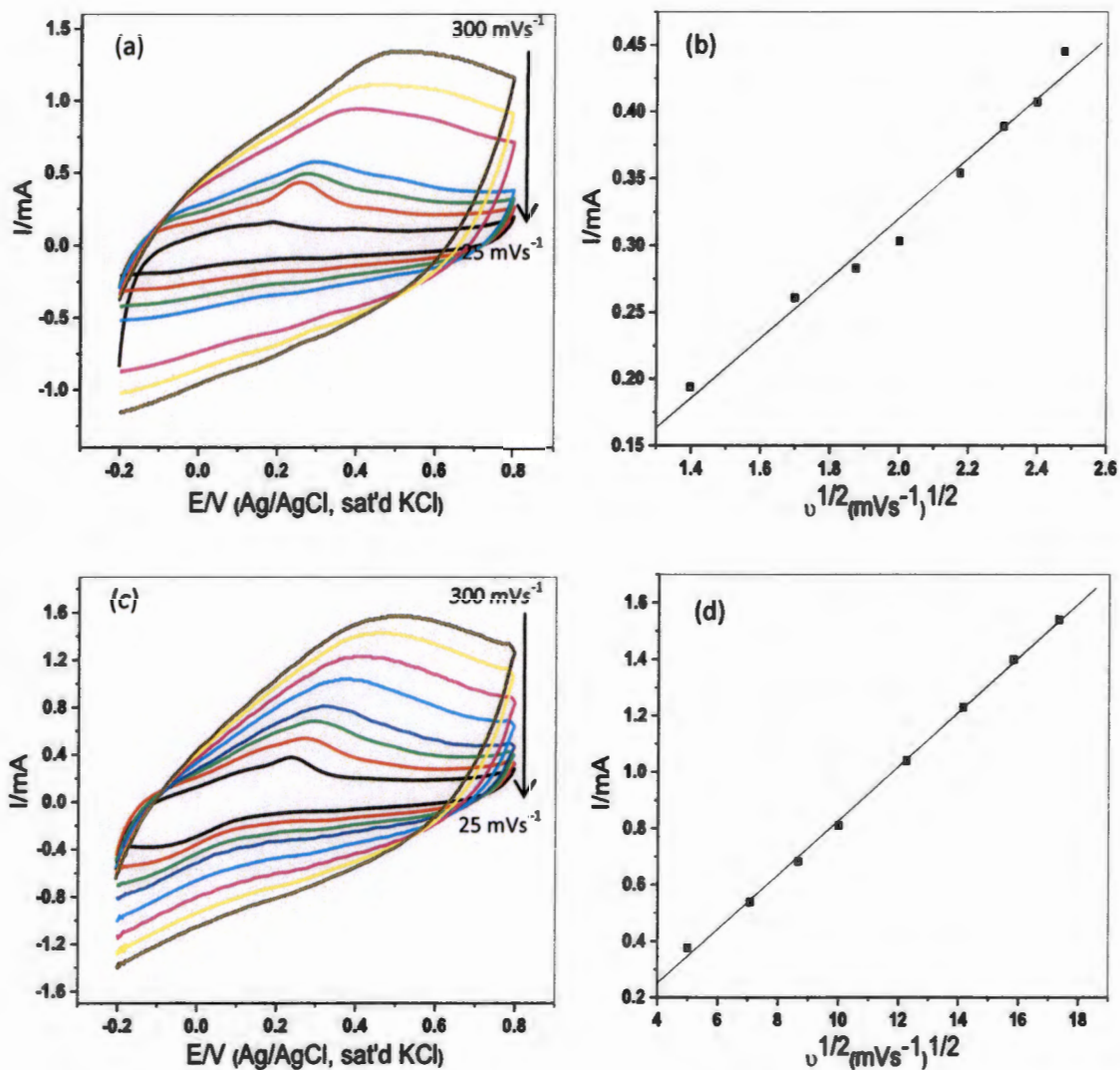


Figure 4.12: Cyclic voltammetric evolutions of (a) Au-MWCNT-PANI-TiO₂ and (c) Au-MWCNT-PANI-RuO₂ modified electrodes at scan rate (25 – 300 mVs^{-1}) respectively in pH 7.0 PBS containing 3×10^{-4} M of EP. (b,d) are peak current vs. square root of scan rate plots for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂.

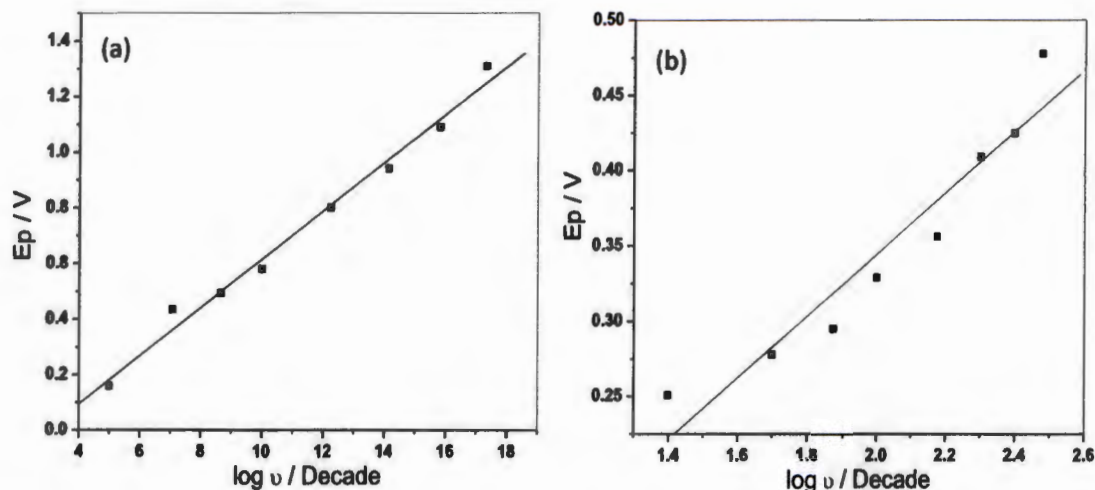


Figure 4.13: Plots of peak potential (E_p) versus $\log \nu$ of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ in pH 7.0 PBS containing 3×10^{-4} M of EP.

4.12 Concentration Study

Figure 4.14(a) illustrates the DPV results at different concentrations of EP at the Au-MWCNT-PANI-TiO₂. It can be seen that the modified gold electrodes improve the sensitivity towards EP detection. The EP concentration range from 4.9-76.9 μ M. The results obtained indicated that the oxidation peak currents increased with the increase of the concentrations of EP. This shows the proportionality of the EP current with EP concentration, with the linear regression equation as: $I_p (\mu A) = 79.122 [EP] (\mu M) + 122.58$. The plots showed good linearity, with a correlation coefficient of 0.969 and the detection limit of 0.16 μ M. The LOD was calculated using the following equation, $LOD = 3.3 S/M$ where, S is the standard deviation and M is the slope (sensitivity) obtained from the calibration curves [300].

It is well known that peak current depends on the concentration of analyte; hence, DPV was recorded for various concentration of EP in phosphate buffer of pH 7.0 using Au-MWCNT-PANI-RuO₂ electrode is shown in Figure 4.14(b). A linear increase in peak current was observed with increase in concentration of EP in the range 4.9-76.9 μ M. The oxidation current of EP was proportional to the concentration of EP, following the linear regression equation given in graph below. The plots showed good linearity, with a correlation coefficient of 0.9758, and the detection limit of 0.18 μ M.

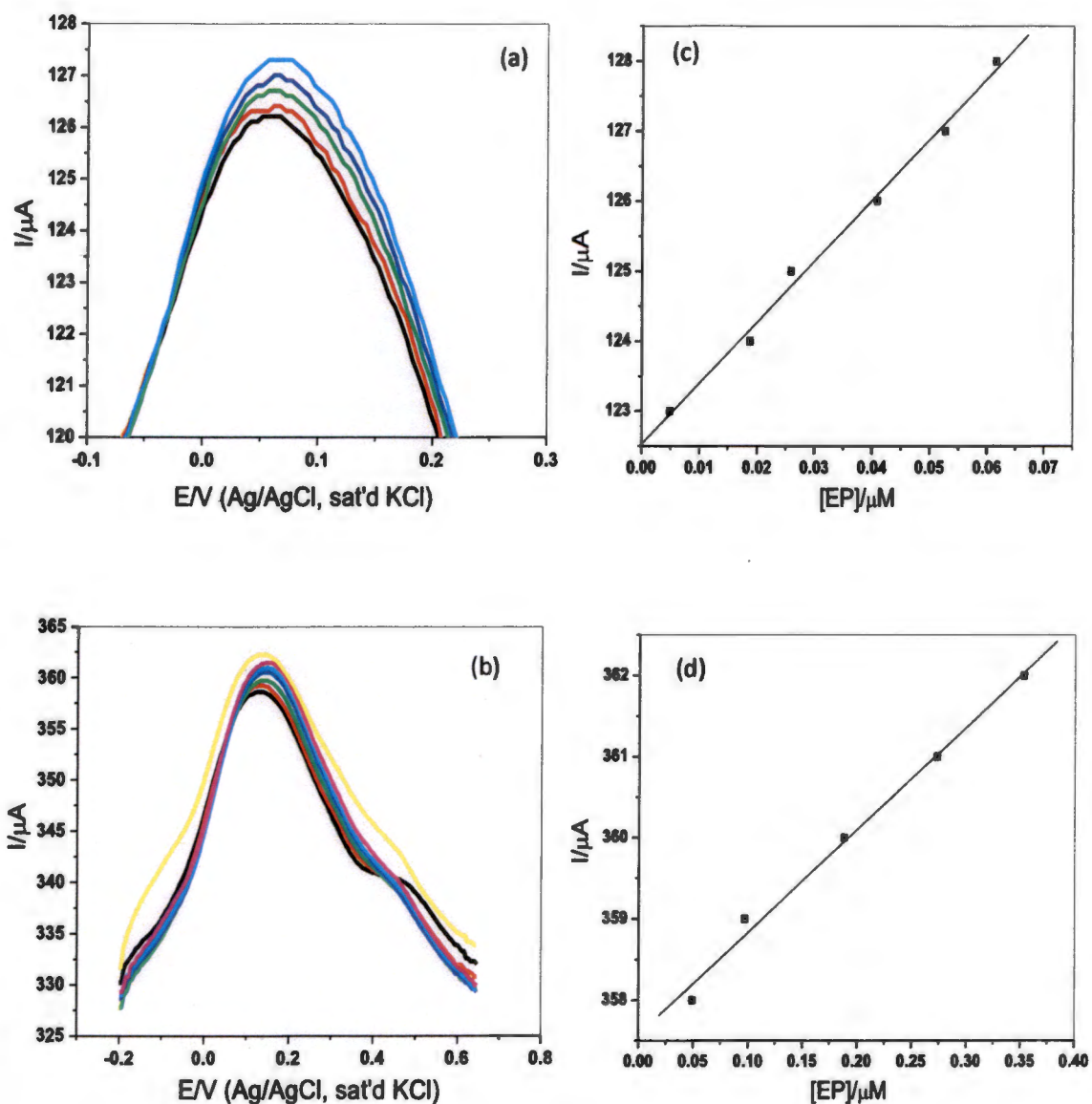


Figure 4.14: Differential pulse voltammogram (DPV) of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ electrode in (a) pH 7.0 PBS containing different concentrations of EP (4.9, 18.7, 27.3, 50.0, 56.8, 63.2, 76.9 μM ; from inner to outer) and (b,d) are peak current vs. concentration of EP plots using MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ electrodes respectively.

4.13 Interference Study: Determination of EP in the Presence of AA

The main purpose of this study was to establish a modified electrode that is suitable to separate the electrochemical response of EP and ST under the influence of foreign species such as AA. Thus, square wave voltammetry was used to determine EP (3×10^{-6} M), ST (3×10^{-6} M), and AA (1×10^{-1} M) simultaneously. The experiment was carried out at different concentrations of EP and ST in the presence of constant concentration of AA in PBS pH 7.0 as shown in Figure 4.15. The use of Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes for the determination of EP, ST, and AA simultaneously was concurrently. Figure 4.15(a) showed three distinctive anodic peaks at potentials of 0.002, 0.222, 0.568 V relating to AA, EP and ST respectively for Au-MWCNT-PANI-TiO₂ electrode, while in Figure 4.15(b) the observed three peaks for Au-MWCNT-PANI-RuO₂ at potentials of -0.071, 0.227 and 0.521 V for AA, EP and ST respectively, confirming the successful simultaneous determination of the biomolecules on the electrode. In the biological fluids normally AA concentration is greater than other biological constituents. Hence, we have studied the sensitivity of the developed nanocomposite modified electrode towards EP and ST in the presence of large amounts of AA. The three analytes peaks were separately visible without any signal interference from one another. Thus, Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ nanocomposite electrodes could potentially be used for electrochemical sensing of these biomolecules in samples.

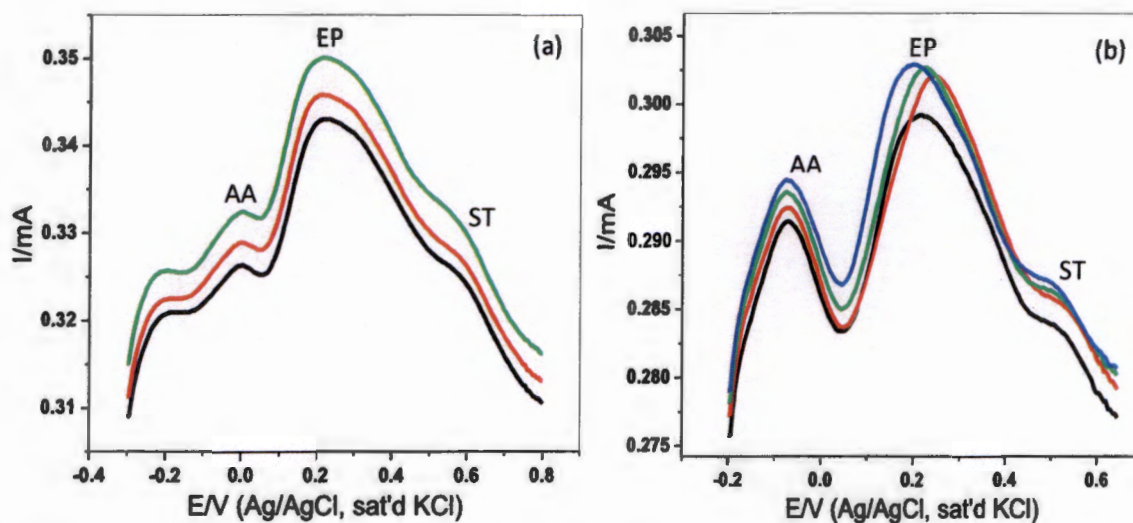


Figure 4.15: Square Wave Voltammetry of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ in pH 7.0 PBS containing AA 1×10^{-1} M and (EP and ST) 3×10^{-6} M solutions respectively.

4.14 Real Sample Analysis

Analytical performance of this sensor was investigated for the determination of EP in epinephrine injection. One microliter epinephrine injection solution was diluted 250 times with 0.1 M PBS at pH 7.0 without any other pretreatment. Table 4.4, shows the results of the percentage recovery test and relative standard deviation. The EP concentration in the injection solution was found to be 4.02, and 4.13 mg/mL ($n = 3$) and recovery was more than 99 % for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrode respectively.

Table 4.4: Results of detection of EP in epinephrine injection ($n = 3$)

Electrode	Sample	Added (mg/ml)	Detected (mg/ml)	Recovery %	RSD %
Au-MWCNT-PANI-TiO ₂	A	4	3.98	99.5	0.25
	B	4	4.02	100.4	0.13
	C	4	4.05	101.3	0.14
Au-MWCNT-PANI-RuO ₂	A	4	4.10	102.5	0.24
	B	4	4.13	103.3	0.17
	C	4	4.13	103.3	0.14

ELECTROCATALYTIC OXIDATION OF SEROTONIN

4.15 Electrochemical Characterisation

Figure 4.16 shows the CVs of the electrocatalytic behaviour of bare Au and Au modified electrodes towards Serotonin (ST) oxidation in 0.1 M PBS solution ($\text{pH} = 7.0$) at a scan rate of 25 mVs^{-1} , while Table 4.5 presents the electrochemical data obtained from the CVs. The ST oxidation current of the modified electrode was almost three times higher than the bare Au electrode. It can be seen that the shift at the high current response for the oxidation of ST at the modified electrode proves that MWCNT-PANI-MO modified electrode successfully electrocatalyzes the oxidation of ST. Figure 4.16(a and b) showed a broad oxidation peak and ill-defined reduction peaks at the Au-MCNT-PANI-MO. Such reflection is due to the distinctive structure of MWCNT with enhanced surface area and conductivity. The MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ electrodes shows typical two pairs of redox peaks (A_1/B_1 and A_2/B_2) as indicated in Figure 4.16. The first pair of peaks A_1/B_1 is attributed to the redox transition from the leucoemeraldine form to polaronic emeraldine of PANI. The second pair of peaks A_2/B_2 resembles the Faradaic transformation of emeraldine/ pernigraniline characteristics of PANI [301-302]. The CV plot of the MWCNT-PANI-MO electrode have a different shape from PANI and MWCNT with a larger bounded area, which shows that MWCNT-PANI-MO electrode displays an important synergistic effect which occur between the PANI layer and MWCNT-MO network [303]. After background current subtraction as shown Figure 4.16(c and d), ST oxidation current at the electrodes follow the order: Au-MWCNT-PANI-RuO₂ (0.118 mA) > Au-MWCNT-PANI-TiO₂ (0.132 mA) > Au-MWCNT (0.051 mA) > Au-PANI (0.0036 mA) > Au-TiO₂ (0.0038 mA) > Au-RuO₂ (0.0045 mA) > Au (0.011 mA) respectively which is approximately three times their oxidation current at the bare Au electrode signifying the importance of chemically modified electrodes in electrocatalysis. Similar factors such as presence of porous MWCNT and PANI, increase electrode surface area and the electrical conductive nature of the metal oxide nanoparticles could be responsible for the improved response of the analyte at the electrode [304].

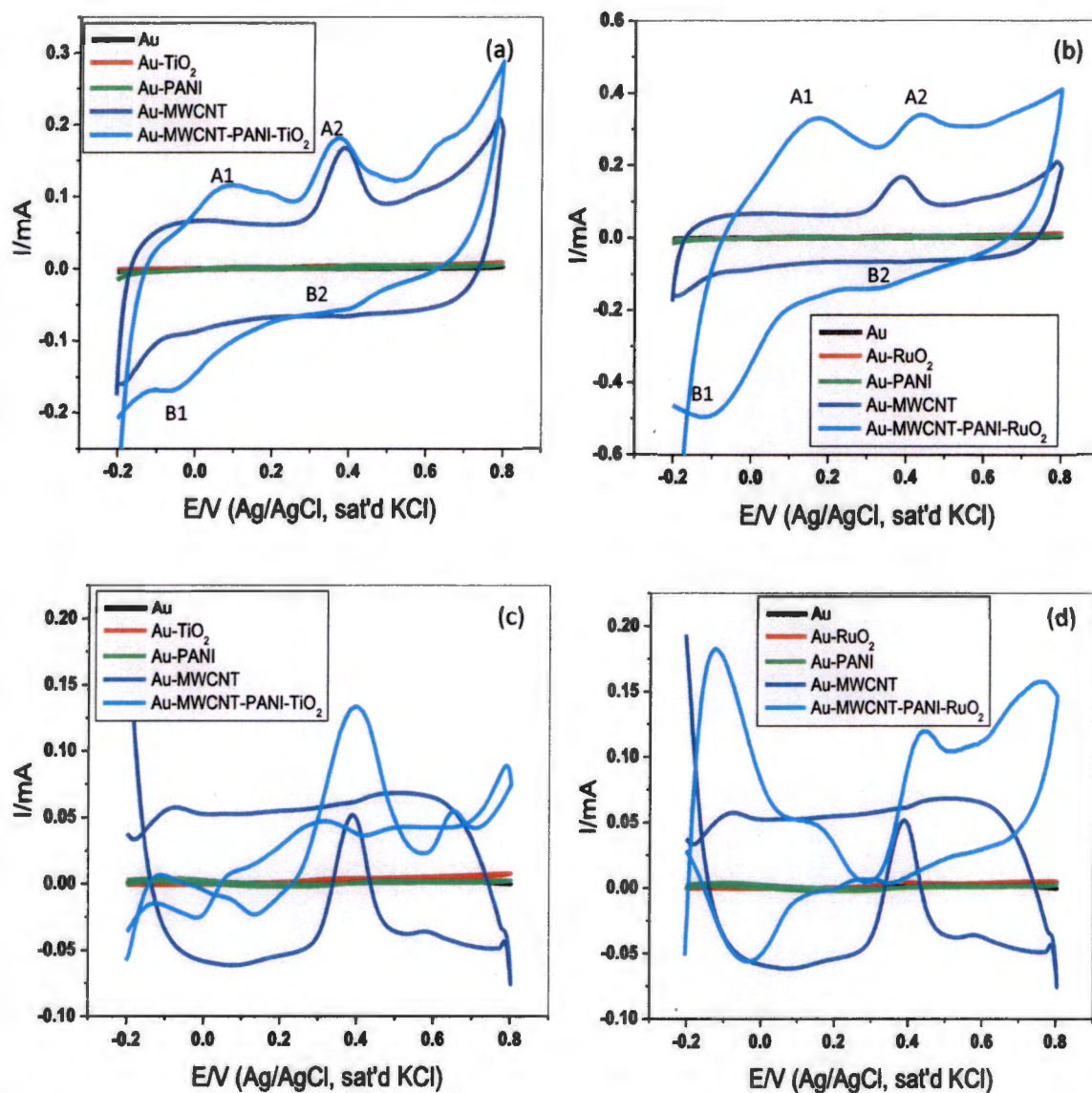


Figure 4.16: Current responses of (a) Au, TiO₂, PANI, MWCNT, MWCNT-PANI-TiO₂ and (b) Au, RuO₂, PANI, MWCNT, MWCNT-PANI-RuO₂ modified electrodes in 3×10^{-4} M ST in pH 7.0 PBS (scan rate = 25 mVs⁻¹); (c) and (d) are the current responses of MWCNT-PANI-MO modified electrodes in 3×10^{-4} M ST (after background current).

Table 4.5: Cyclic voltammetric data obtained for bare and the modified electrodes in ST 3×10^{-4} M in pH 7.0 PBS.

Electrode	I_{pa} (mA)	E_{pa}
Au	0.002	0378
Au-TiO ₂	0.004	0.338
Au-RuO ₂	0.005	0.338
Au-PANI	0.005	0.432
Au-MWCNT	0.165	0.387
Au MWCNT-PANI-TiO ₂	0.177	0.378
Au-MWCNT-PANI-RuO ₂	0.33	0.442

4.16 Electrochemical impedance spectroscopy

Figure 4.17 shows the impedance spectra for bare Au, Au-TiO₂, Au-RuO₂, Au-PANI, Au-MWCNT, Au-MWCNT-PANI-MO (in the form of Nyquist plots) in 0.1 PBS containing 3×10^{-4} M ST as a redox probe at the initial potential of 0.38 V with the frequency range from 100 KHz to 0.1 Hz while the circuit model used in the fitting of the impedance data is represented in Figure 4.17(e). The Nyquist plot consist of semicircle diameters which reflect the electron transfer resistance (R_{ct}), which is associated with the electron transfer kinetics of the redox probe at the surface of the electrode. From the plot and Table 4.6, it can be seen that the values of R_{ct} on Au-MWCNT-PANI-MO are smaller than other electrodes observed, due to the outstanding electrical property of MWCNT that formed a high electron conduction pathway between the electrode and electroactive indicator. The impedance interface indicates that metal oxide integrated with MWCNTs could successfully increase the electron transfer rate between the electrode surface and ST due to the good conductivity of MWCNT-MO. The electron transfer resistances of Au-MWCNT-PANI-TiO₂ (2.19 k Ω) and Au-MWCNT-PANI-RuO₂ (0.17 k Ω) modified electrodes are much lower than that of bare electrode, due to the materials-modified on the surface of the electrode partially block the electron transfer of ST solution to the electrode [305]. Therefore, Au-MWCNT-PANI-RuO₂ electrode proved to

have a lower charge-transfer resistance, indicating exceptional improvement of the catalytic reaction and electron transfer [296] thus, the higher ST oxidation current recorded for its CV in Table 4.5. Figure 4.17(c and d) show the Bode plots charge transfer resistance towards the modified electrodes which possess a phase angle lower than -90° . Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrodes showed a much lower phase angle of less than 20° compared with other electrodes studied. Furthermore the results confirm the R_{ct} values which clarifies that there was fast charge transfer development at Au-MWCNT-PANI-MO electrode. Hence the higher the conductive or charge transfer properties of the sensor [306].

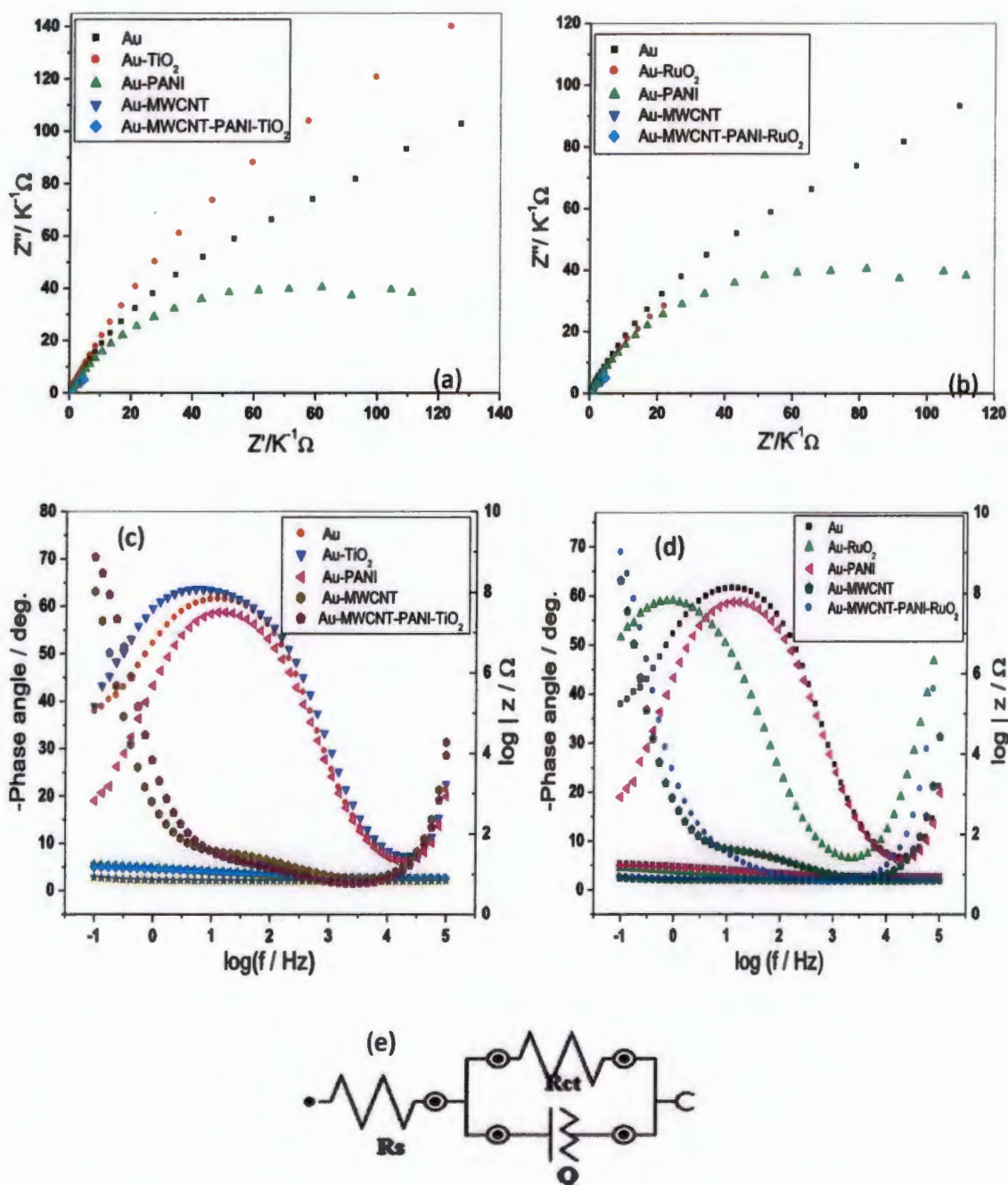


Figure 4.17: Nyquist plots obtained for (a) Au, TiO₂, PANI, MWCNT and MWCNT-PANI-TiO₂ (b) Au, RuO₂, PANI, MWCNT and MWCNT-PANI-RuO₂ in 0.1 M PBS pH 7.0 containing 3×10^{-4} M of ST solutions at a fixed potential of 0.8 V (vs Ag|AgCl, sat'd KCl). (c) and (d) are the Bode plots obtained for Au-MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ in ST respectively showing the plots of -phase angle / deg. vs $\log(f/\text{Hz})$, and the plot of $\log |Z/\Omega|$ vs $\log(f/\text{Hz})$ (e) represents the circuit used in the fitting for the EIS data in (a) and (b).

Table 4.6: EIS data obtained for bare and modified electrodes in ST 3×10^{-4} M in pH 7.0 PBS. Values in parenthesis are percentage errors of the data fitting.

Electrode	R_s (k Ω)	Q (μ F)	R_{ct} (k Ω)
Au	0.45 (2.23)	4.31 (3.49)	275 (7.29)
Au-TiO ₂	0.41 (2.30)	3.48 (3.24)	555 (10.75)
Au-RuO ₂	426 (8.30)	134 (14.52)	11.9 (18.4)
Au-PANI	0.45 (2.20)	4.47 (3.24)	128 (10.73)
Au-MWCNT	0.064 (3.30)	8800 (10.98)	3.21 (29.38)
Au-MWCNT-PANI-TiO ₂	1.49 (2.56)	289 (6.89)	2.19 (14.01)
Au-MWCNT-PANI-RuO ₂	0.055 (8.54)	1590 (23.29)	0.17 (16.54)

4.17 Stability Study

Figure 4.18 shows the stability of the Au-MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ modified electrodes towards ST by running the electrode (20 scans) in 3×10^{-4} M ST solution at a scan rate of 25 mVs⁻¹. Both electrodes displayed measurable oxidation peaks in supporting electrolyte. Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes moderately displayed a low drop from the first scan to the second scan and this minimal decrease was observed with the relative standard deviation (R.S.D.) of 10 % and 6.6 % for modified electrodes Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ respectively, indicating that the modified sensors hold excellent stability for the determination of ST. The modified electrodes possess outstanding reproducibility and robust ability to prevent the electrode from fouling by the oxidation product. The electrode's adsorption nature towards the ST can be ascribed to the porous MWCNT in the composite [297]. This study shows that Au-MWCNT-PANI-RuO₂ electrode performs better toward the analyte compared with Au-MWCNT-PANI-TiO₂ electrode. After measurements, the modified electrode was stored at 4 °C in the freezer. After three days, the respective decrease in peak current intensity of 12.4 %, 10.2 % and 11.5 % for Au-MWCNT-PANI-RuO₂, Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI electrodes respectively, indicating that the modified electrodes possess excellent stability for the determination of ST.

and 8.2 % for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes respectively. The above results showed that the stability of the sensor was good and appreciable for the determination of ST.

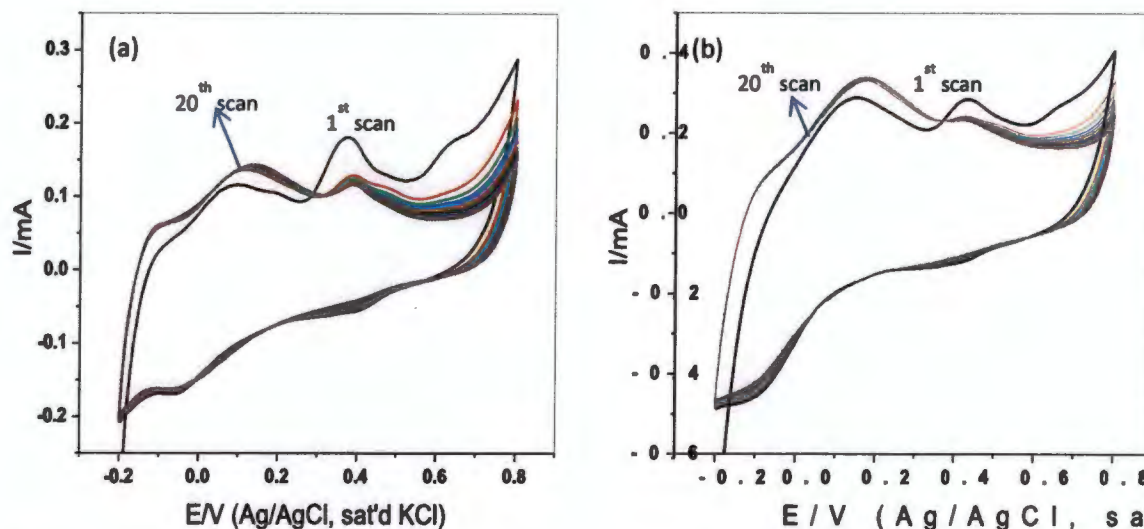


Figure 4.18: Current response (20 scans) of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ in pH 7.0 PBS containing 3×10^{-4} M of ST at scan rate of 25 mVs^{-1} .

4.18 The Effect of Scan Rate

The effect of scan rate on the anodic peak current of ST was studied on the surface of electrodes modified with Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ at a constant concentration of ST (3×10^{-4} M) as shown in Figure 4.19 (a,d) using cyclic voltammetry. The anodic current for both composites increased with the increasing scan rate (25 to 300 mVs^{-1}) for Au-MWCNT-PANI-TiO₂ and (25 - 200 mVs^{-1}) for Au-MWCNT-PANI-RuO₂. Figure 4.19 (b,d) shows a linear graph with a correlation factor of $R^2 = 0.9883$ and $R^2 = 0.9719$ for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ respectively acquired for peak current versus square root of scan rate. These indicate that peak currents are proportional to the square root of scan rates, symbolising that the oxidation currents were diffusion controlled [298]. The plot of I_p vs $v^{1/2}$ was used to estimate the surface coverage of the electrode by adopting the method used by Sharp et al [299]. Therefore, the relationship between the peak current and the surface concentration of electroactive species, Γ , is

expressed by Equation (6). The calculated surface concentration of MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ in ST were $5.04 \times 10^{-5} \text{ mol cm}^{-2}$ and $8.16 \times 10^{-5} \text{ mol cm}^{-2}$ respectively. This provides evidence from the value calculated for the modified electrodes surface concentration of electroactive species. The Au-MWCNT-PANI-RuO₂ electrode has a value which is higher than the Au-MWCNT-PANI-TiO₂ electrode. This further confirms the higher anodic current for the Au-MWCNT-PANI-RuO₂ as shown in Figure 4.19b and Table 4.5. A positive shift was observed with increasing scan rate, signifying that the electron transfer is irreversible. The Tafel 'b' values were calculated to be 0.452 V and 0.445 V from the slope for E_p vs log v for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrodes respectively as shown in Figure 4.20. High Tafel values can be associated with electrode fouling by oxidation product. Therefore, the porosity of the electrode affected the adsorption nature towards ST [297]. The slope shows a one electron transfer process as a limiting step assuming a transfer coefficient α , of 0.99 for both electrodes.

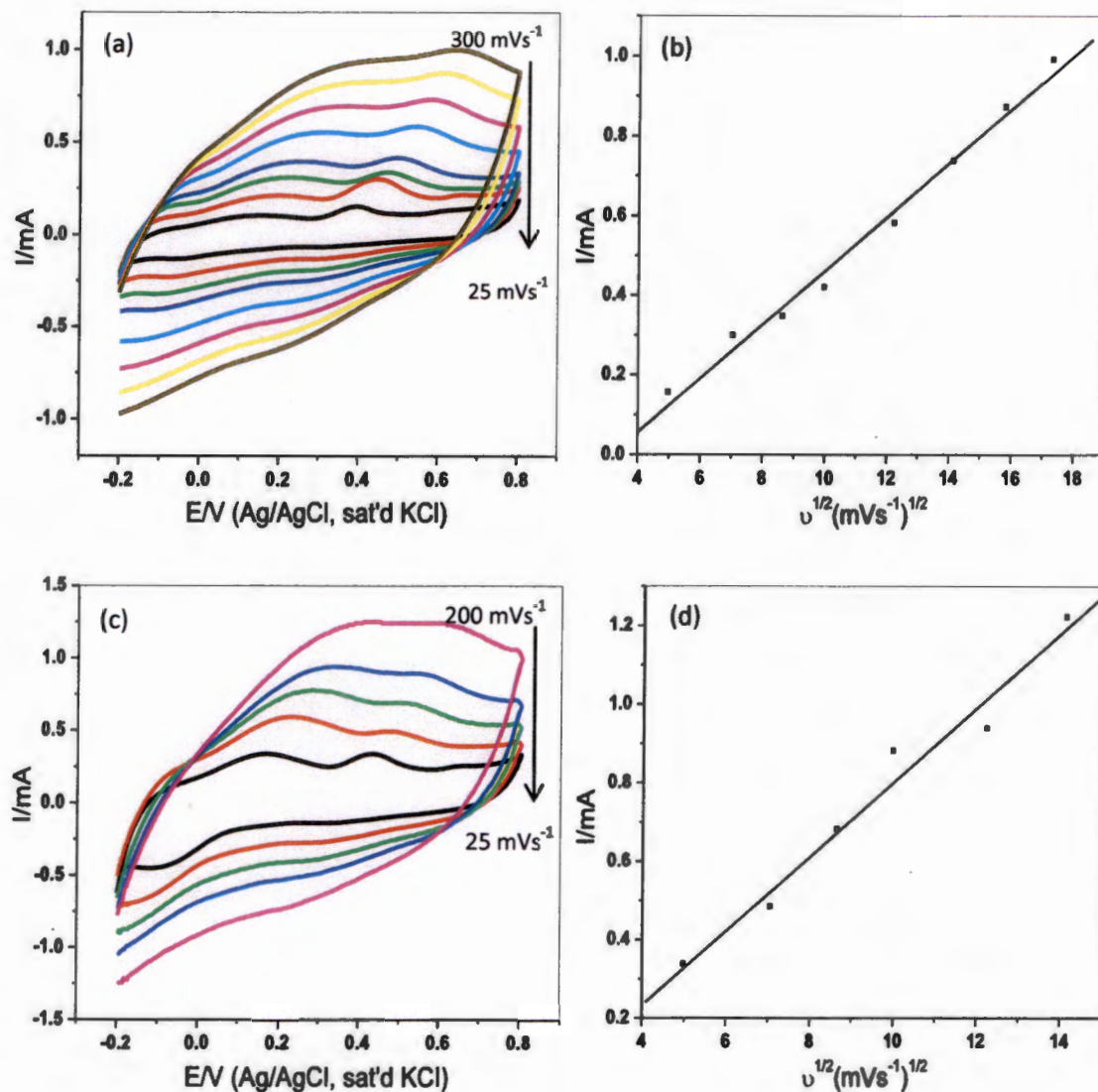


Figure 4.19: Cyclic voltammetric evolutions for (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ modified electrodes at scan rate (25 – 300 mVs⁻¹) and (25 – 200 mVs⁻¹) respectively in pH 7.0 PBS containing 3×10^{-4} M of ST. (b) and (d) are peak current vs. square root of scan rate plots of Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂.

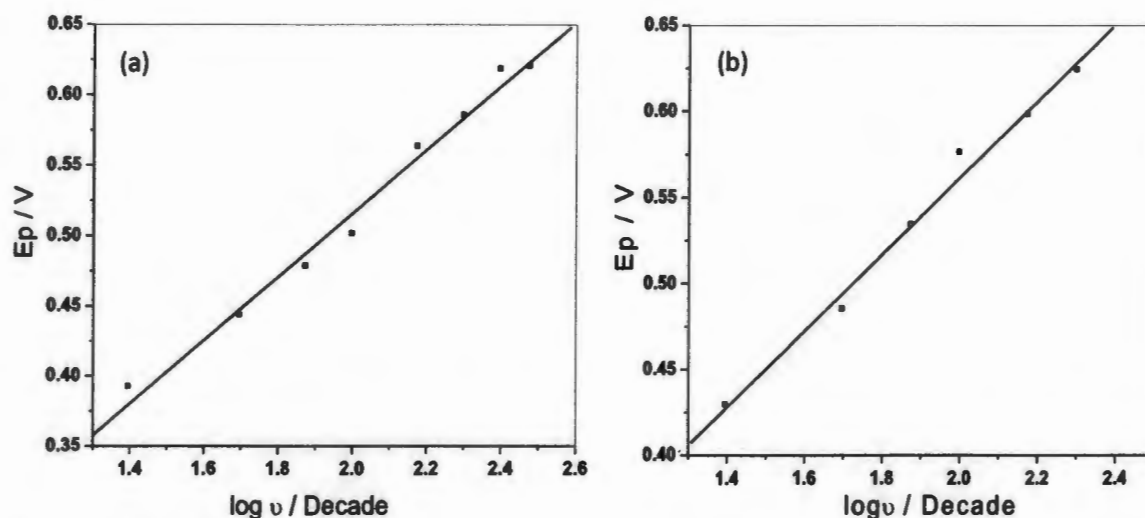


Figure 4.20: Plots of peak potential (E_p) versus $\log \nu$ of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ in pH 7.0 PBS containing 3×10^{-4} M of ST.

4.19 Concentration Study

Figure 4.21 shows that square wave voltammetry (SQW) has the effect of increasing the sensitivity and improving the characteristics for analytical applications. The plot of current response versus concentration showed a linear range of 0.14-1.5 μM with a calibration equation of $I_p (\mu\text{A}) = 56.524 [\text{ST}] (\mu\text{M}) + 198.3$ ($R^2 = 0.9928$) and $I_p (\mu\text{A}) = 9.627 [\text{ST}] (\mu\text{M}) + 323.8$ ($R^2 = 0.9906$) for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrode respectively. The limit of detection was calculated to be 0.26 and 0.32 μM for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ modified electrode respectively. The limits of detection of the electrodes were in line with values reported in literature toward serotonin detection [307-308]. The analyte concentration level in biological fluids is present at micromolar levels. Hence 0.26 and 0.32 μM limit of detection for Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ obtained in the study indicating that the fabricated sensor could be suitable for the modification of ultra-microelectrodes for in vivo detection of serotonin [309].

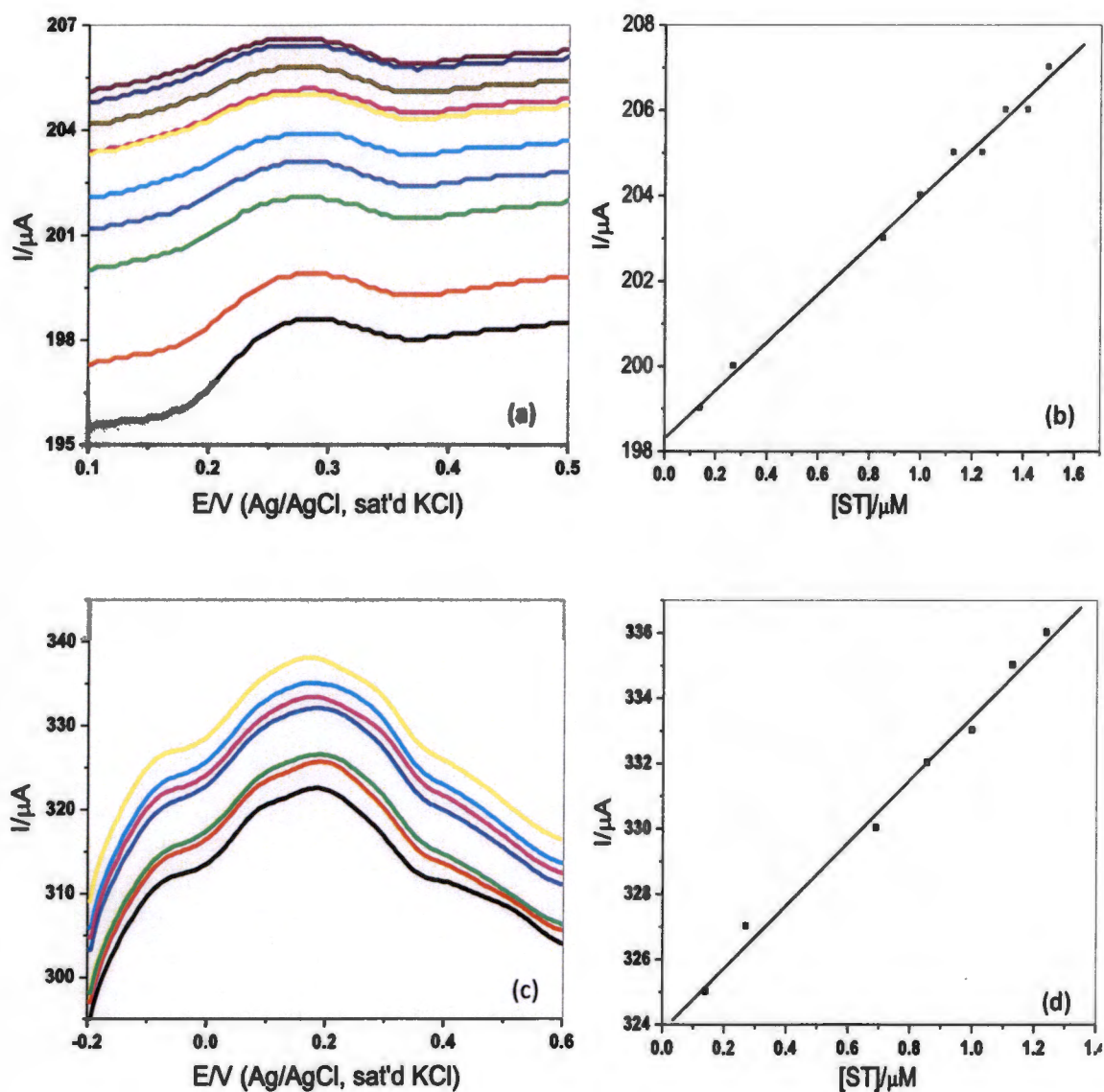


Figure 4.21: Square Wave Voltammogram of (a) Au-MWCNT-PANI-TiO₂ and (b) Au-MWCNT-PANI-RuO₂ electrode in (a) pH 7.0 PBS containing different concentrations of ST (0.14, 0.27, 0.69, 0.86, 1.00, 1.13 1.24 1.33 1.50 μM ; from inner to outer) and (b,d) are peak current vs. concentration of ST plots using MWCNT-PANI-TiO₂ and MWCNT-PANI-RuO₂ electrodes respectively.

CHAPTER FIVE

CONCLUSIONS

CONCLUSIONS

This work described the successful modification of Au electrode with PANI, MWCNT, TiO₂ and RuO₂ nanoparticles. Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ nanocomposite modified electrode were also developed and characterized using different spectroscopic techniques. Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrodes proved to have fast electron transport and better catalytic behaviour towards epinephrine and serotonin oxidation compared with other electrodes studied. Electrochemical impedance spectroscopy results showed a low charge transfer resistance of 3.27, 321 k Ω (EP, ST) and 5.20, 0.17 k Ω (EP, ST) for the analyte using Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ respectively, which confirms the reproducibility of the cyclic voltammetry on the electrode. The comparative study between the two electrodes has shown that Au-MWCNT-PANI-RuO₂ gave the best current response, because RuO₂ and MWCNT improved the electrochemical properties of the electrode. Electrocatalytic oxidation of epinephrine and serotonin on the Au-MWCNT-PANI-MO electrodes were diffusion controlled. This was through adsorption on the analytes on the surface of the electrode. A good stability was observed after cleaning and reusing the electrode. There was a current drop of less than 15 %. The electrode showed good resistance to electrode poisoning, and Au-MWCNT-PAINI-RuO₂ gave the best limit detection of 0.18 μ M and 0.26 μ M towards epinephrine and serotonin, and this is in line with values previously reported in literature. Au-MWCNT-PANI-TiO₂ and Au-MWCNT-PANI-RuO₂ electrode can easily detect epinephrine without interferences from ascorbic acid. The fabricated sensor shows excellent recovery towards epinephrine injection. The fabricated sensor is fast and easy to prepare.

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APPENDICES

Appendix 1

Formulae used

1. Tafel equation: $E_p = K + \frac{b}{2} \log v$

2. Laviron equation: $I_p = \frac{\pi^2 f^2 A \Gamma v}{4RT}$

3. limit of detection: $\text{LoD} = 3.3s/m$

Appendix 2

1. Synthesis, spectroscopic and electrochemical characterization of ternary Au-PANI-TiO₂ nanocomposite

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Conference: MAPET-15 3rd International Symposium on Electrochemistry
(26-28 May 2015, University of the Western Cape, SOUTH AFRICA)

Poster presentation by: Palesa Tsele

2. Electrochemical detection of Epinephrine using Polyaniline nanocomposite films doped with TiO₂ and RuO₂ Nanoparticles on Multi-walled Carbon Nanotube

Tebogo P. Tsele, Abolanle S. Adekunle, Omolola E. Fayemi, Eno E. Ebenso

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Electrochemical detection of Epinephrine using Polyaniline nanocomposite films doped with TiO₂ and RuO₂ Nanoparticles on Multi-walled Carbon Nanotube



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ABSTRACT