



**ELECTRODEPOSITION OF ZINC OXIDE NANO PARTICLES ON MULTIWALLED  
CARBON NANOTUBES MODIFIED GLASSY CARBON ELECTRODE FOR  
SIMULTANEOUS DETECTION OF ACETAMINOPHEN AND CAFFEINE**

**By**

**TAKUNDA MUZVIDZIWA**

**(R113709Z)**

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**GWERU**

**ZIMBABWE**

**Supervisor: Dr.M. MOYO**

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## **DEDICATION**

I dedicate this work to my family and friends.

## **ACKNOWLEDGEMENTS**

I am thankful to God for making this project possible for me. I also thank my classmates for their unceasing support during my time of research. I thank my supervisor Dr. M. Moyofor his inspiration, guidance, suggestions, contributions and infinite patience in writing this research. I extend my gratitude to laboratory staff of Midlands State University Chemical Technology for its support. I want to thank my parents for their financial support.

## ABSTRACT

A new simple novel electrochemical sensing platform based on electrodeposition of nano zinc oxide on multiwalled carbon nanotubes modified glassy carbon electrode was constructed. The modified electrode was characterized by cyclic voltammetry and electrochemical impedance in a redox probe. The electrode surface area after modification was  $0.15 \text{ cm}^2$ , which was twice large that of a bare glassy carbon electrode. The new fabricated sensor was applied for individual and simultaneous determination of caffeine and acetaminophen. The limit of detection (LOD) for individual determination was  $3.69 \times 10^{-8} \text{ M}$  for acetaminophen and  $3.52 \times 10^{-8} \text{ M}$  for caffeine. The electrode showed good reproducibility, sensitivity and stability for detection of both acetaminophen and caffeine. A good recovery level obtained for real samples suggests practical utility of the ZnO/MWCNT/GCE as an effective sensor for simultaneous determination of acetaminophen and caffeine.

## DECLARATION

I, **TakundaMuzvidziwa**, hereby declare that I am the sole author of this dissertation. I authorize Midlands State University to lend this dissertation to other institutions or individuals for the purpose of scholarly research.

Signature.....

Date.....

## APPROVAL

This dissertation entitled “Electrodeposition of zinc oxidenano particles on multiwalled carbon nanotubes modifiedglassy carbon electrode for simultaneous detection of acetaminophen and caffeine” by TakundaMuzvidziwa meets the regulations governing the award of the degree of Chemical Technology of Midlands State University, and is approved for its contribution to knowledge and literal presentation.

Supervisor.....

Date.....

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## **ABBREVIATIONS**

**CV-** Cyclic voltammetry

**MWCNT-** Multiwalled carbon nanotubes

**DPV-** Differential pulse voltammetry

**EIS-** Electrochemical impedance spectroscopy

**GCE-** Glassy carbon electrode

**LOD-** Limit of detection

**LOQ-** Limit of quantification

**NMR-** Nuclear magnetic resonance

**WE-** Working electrode

**RE-** Reference electrode

**CE-** Counter electrode

**PBS-** Phosphate buffer solution

**SWV-** Square wave voltammetry

**UV-vis-** Ultraviolet visible spectroscopy

**HPLC-** High performance liquid chromatography

**GC-** Gas chromatography

**TLC-** Thin layer chromatography

**CVD-** Chemical vapour deposition

**DPV-** Differential pulse voltammetry

# CHAPTER ONE

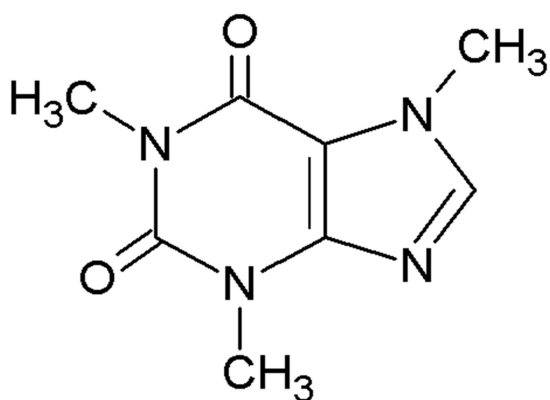
## Introduction

### 1.0 Introduction

The present chapter highlights the background of the research and the various methods that were used for the determination of caffeine and acetaminophen. The statement of problem and justification of study are discussed in the present chapter.

### 1.1 Background

Caffeine (3,7-dihydro-1, 3,7-trimethyl- H-purine-2, 6-dione) is an alkaloid mostly found in plants such as cocoa and cola nuts, coffee bean and tea and its structure is shown on Figure 1.1[1]. It has a molar mass of about 194.19 g/mol and is crystalline and white in its pure state. It always exists along with other N-methyl derivatives of xanthene such as theophylline and theobromine. It is found in various kinds of beverages for example soft and energy drinks [2].



**Fig 1.1:** Chemical structure of caffeine



It is commonly used for its psychoactive substance in daily human life. It has many important physiological effects such as stimulation of the central nervous system, gastric acid secretion and diuresis[3]. However if administered in high amounts it can cause nausea, trembling, nervousness, increased irritability, higher risk for bone loss in postmenopausal women, seizures and mutation effects such as inhibition of DNA[4]. A fatal dose of caffeine has been evaluated to be more than 10grams (about  $170 \text{ mg kg}^{-1}$  of body weight). Caffeine is a stimulant which perks up the mind and according to research it is also addictive[5]. Numerous methods for the analysis of caffeine have been developed and reported elsewhere, such as fluorescence spectrophotometry, microchip electrophoresis, thin layer chromatography, UV-Vis and gas chromatography [6].

Paracetamol is mainly used as an antipyretic and analgesic drug in most countries for the relief of mild to moderate pain associated with headache, arthritis, backache, toothaches and postoperative pain [7]. No harmful side effects caused by paracetamol have been reported when taken in normal therapeutic doses. Nevertheless, the abnormal level of paracetamol has been reported to cause the formation of some liver and nephrotoxic metabolites [8]. Furthermore, increased problems such as asthma, and eczema have been reported in children younger than one year after consuming paracetamol[9]. For quality control purposes and the wide spread use on a daily basis in different countries by different people, the monitoring of trace amounts of paracetamol is of great interest since little is known about potential chronic health effects associated with long term ingestion.

Current analytical techniques that have been reported for the quantification of paracetamol in different environmental matrices include capillary electrophoresis [8], high performance liquid

chromatography (HPLC) [10], liquid chromatography–mass spectrometry [6]. The aforementioned methods are sensitive and reliable.

Carbon nanotubes describe a family of nanomaterials made up entirely of carbon. In this family, multi-walled carbon nanotubes (MWCNTs) are of special interest for the industry[11].Structurally MWCNTs consist of multiple layers of graphite superimposed and rolled in on themselves to form a tubular shape.The cylindrical graphitic polymeric structures have novel or improved properties that make them potentially useful in a wide variety of applications in electronics, optics and other fields of materials science[12]. Carbon nanotubes are endowed with exceptionally high material properties, very close to their theoretical limits, such as electrical and thermal conductivity, high strength, stiffness, and toughness[13].MWCNTs are polymers of pure carbon and can be reacted and manipulated using the rich chemistry of carbon. This provides opportunity to modify the structure and to optimize solubility and dispersion, allowing innovative applications in materials, electronics, chemical processing and energy management[12].

Metal oxides have attracted considerable interest due to their superior properties which include large specific surface area, high uniformity, and excellent catalytic activity. Among the different metal oxide nanoparticles, zinc oxide nano-powder ( $n\text{ZnO}$ ) is an attractive semiconductor, with a wide band gap of 3.37 eV[14]. It is very effective as a photo catalyst, sometimes more effective than  $\text{TiO}_2$  in the visible region[15]. It has low cost synthesis, large excitonic energy, high mechanical energy and high-electron communication. The ZnO has been used previous for the fabrication of different sensors and biosensors[16].

## 1.2 Aim of study

- Development of an electrochemical sensor by electrodeposition of zinc oxide nano particles on multiwalled carbon nanotubesmodified glassy carbon electrode for simultaneous oxidation of acetaminophen and caffeine.

## 1.3 Objectives

- To modify the glassy carbon electrode with the multiwalled carbon nanotubes by the drop dry technique.
- To electrochemically deposit ZnO onmultiwalled carbon nanotubesmodified glassy carbon electrode using cyclic voltammetry.
- To characterize the modified electrode using cyclic voltammetry and electrochemical impedance in a redox probe.
- To study the effect of different supporting electrolytes on the electro catalytic oxidation of caffeine.
- To evaluate the effect of pH and scan rate on the peak currents and potentials.
- To study the effect of different modified electrodes on the electro catalytic oxidation of caffeine using cyclic voltammetry.
- To determine the electrode transfer kinetics, Tafel slopes, catalytic rate constants and relative catalytic effects of the modified sensor.
- To detect caffeine and acetaminophen on ZnO/MWCNT/GCE simultaneously.
- To calculate the detection limit of the ZnO/MWCNT/GCE on caffeine and acetaminophen oxidation.
- To study the stability of the developed electrode towards caffeine and acetaminophen oxidation.

- To determine caffeine in cola drink and acetaminophen in human urine by DPV.

#### **1.4 Problem statement**

High levels of caffeine in the blood stream can also be considered to be a risk factor for asthma, kidney malfunction and may affect behavior by causing depression and hyperactivity[17]. On the other hand acetaminophen if administered in high levels results in formation of some liver and nephrotoxic metabolites. As for children younger than one year asthma and eczema cases have been reported[16].

Several methods have been used for analysis of caffeine which includes HPLC, UV, NMR, TLC, GC and capillary electrophoresis in pharmaceutical formulations, biological samples and food stuffs[18]. Most of these methods have some major draw backs for instance they are expensive, time consuming procedures like pre-concentration or multi-solvent extraction and require highly skilled technicians. Therefore there is need to develop an easy, accurate, reliable and cheap technique for caffeine determination for present days. This fact has diverted the attention of most electrochemist towards electrochemical methods[19].

## 1.5 Justification

Numerous methods have been used for analysis of caffeine and acetaminophen which includes HPLC, UV, NMR, TLC, GC and capillary electrophoresis. Compared with other detection techniques, the electrochemical methods are proved to be popular tools ascribed to their advantages of stability, selectivity, simple and easy fabrication process[18]. The direct voltammetric analysis of substances at a bare electrode has led to electrode fouling due to unwanted precipitation and contamination thereby slowing electrochemical reaction rates of some species that require the use of high over potentials. This can be rectified by the use of electrodeposition of nano zinc oxide particles on multiwalled carbon nanotubes modified glassy carbon electrode[20]. Therefore due to the large band gap width (3.37 V), low synthesis cost, good electrochemical activity, high mechanical strength and biocompatibility properties exhibited by nano zinc oxide it was employed as one of the modifiers and multi-walled carbon nano tubes were also used due to their unique mechanical, chemical and electrical properties they were both combined to form a composite modifier[21].

## CHAPTER TWO

### Literature Review

#### 2.0 Introduction

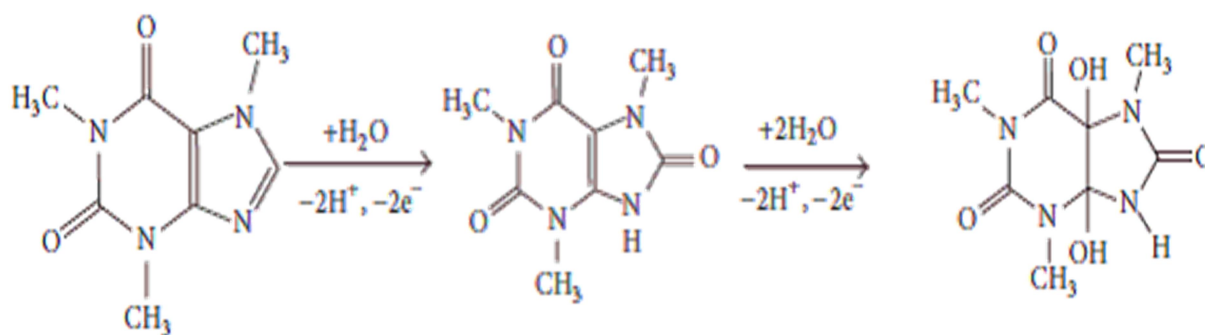
The present chapter gives an outline of caffeine, acetaminophen, carbon nano tubes, nano particles and all the theory of electrochemistry techniques.

#### 2.1 Caffeine

Caffeine is a stimulant that temporarily increases mental process and its chemical formula is  $C_8H_{10}N_4O_2$ . It has high popularity of coffee and caffeine containing products (coca, tea, energy and soft drinks), it is the most commonly used psychoactive substance in daily human life[22]. Caffeine is also distributed in plants where it serves as a natural insecticide since it may paralyze and kill some insects that feed on plant. The fatal dose of caffeine was evaluated to be more than 10 g that is about 170 mg per kg of body weight[6]. It is also considered to be a risk for cardiovascular diseases, kidney malfunction and may also cause hyperactivity. Caffeine is usually prescribed as analgesic adjuvant in drug formulations used for the treatment of migraine, in combination with other drugs for instance in aspirin or paracetamol[19]. The newest source of caffeine can be found in energy drinks where the caffeine typically comes from the guarana plant[9]. When consumed caffeine is absorbed by the body and takes less than five minutes to reach the brain and its half-life is on average four to six hours.

The electro-catalytic oxidation of caffeine occurs at very low pH and it is also affected by choice of supporting electrolyte mostly acid electrolytes are employed. The pH value of an electrolyte solution is an important factor that affects the redox behavior of electro active species[23]. It is

also believed that there is linear relationship between Epa and pH because there is equal of protons and electrons in the oxidation of caffeine at low pH. The process of oxidation of caffeine involves four protons and four electrons. The first step is a  $2\text{H}^+$ ,  $2\text{e}^-$  oxidation of the C-8 to N-9 bond to give the substituted uric acid. This is then followed by an immediate  $2\text{H}^+$ ,  $2\text{e}^-$  oxidation to the 4,5-diol analog of uric acid, which rapidly fragments[24]. This is shown in fig 2.1.



**Fig 2.1:** Caffeine electrochemical oxidation mechanism

Cyclic voltammetry was employed to study the properties of the modified electrode toward the oxidation of caffeine. Square wave voltammetry was used to plot the calibration curve and there was linear relationship between anodic peak current and caffeine concentration in the range  $2.0 \times 10^{-6} - 8.0 \times 10^{-4}$  M, with the correlation coefficient of 0.998 and a detection limit of  $1.43 \times 10^{-7}$  M [21].

## 2.2 Acetaminophen

Paracetamol is an antipyretic and analgesic drug which is widely used in the world. It is used mainly as an effective medicine for the relief pain and reduction of fever and a suitable alternative for patients who are sensitive to aspirin [25]. Paracetamol is likewise helpful in managing more serious pains, permitting lower doses of additional non-steroidal anti-inflammatory drugs (NSAIDs) or opioid analgesics to be utilized, thus minimizing general side effects. It is the main ingredient in various cold and influenza medications, and also numerous



solution analgesics. It is viewed as safe for human utilization in recommended doses, but since it's widely accessible, deliberate or coincidental overdoses are normal which leads to toxicity [20,26,27].

Paracetamol is basically metabolized in the liver, where the greater part of it (60–90% of a therapeutic dose) is transformed to inactive compounds by conjugation with sulfate and glucuronide, and afterward excreted by the kidneys. Just a small portion (5–10% of a therapeutic dose) is metabolized by means of the hepatic cytochrome P450 protein system, the toxicity of paracetamol is due to a minor alkylating metabolite (N-acetyl-p-benzo-quinone imine, NAPQI) that is created through this enzyme, not paracetamol itself or any of its major metabolites [1].

Paracetamol overdoses lead to hepatotoxicity with initial symptoms being nausea, abdominal pains, vomiting and diarrhea. Clinical indications of hepatic damage manifest within two days after ingestion of a toxic dose with the severity of the damage increasing with the dose ingested and also with the previous consumption of other drugs that induce liver cytochrome P450 enzyme [28].

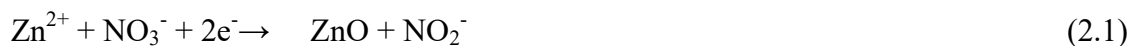
After having an overdose of paracetamol, biopsy of the liver reveal a range of histological abnormalities, which vary from focal hyperplasia of Kupffer cells and a few foci of hepatocytolytic necrosis to severe centrilobular necrosis, liver-cell loss, inflammation and some features of active regeneration. The consumption of alcohol heavily is related to more severe paracetamol hepatotoxicity by causing hepatocellular injury and renal tubular necrosis [4].

A simple, specific, precise and accurate reversed phase liquid chromatographic (RP-LC) method was developed for the determination of paracetamol in tablet dosage form. The chromatographic separation was achieved on a LiChrosorb C18, 250 mm × 4.6 mm, 5 μm column at a detector wavelength of 254 nm and a flow rate of 1.0 ml/min. The mobile phase was 1 mM sodium

pentane sulphonate in a mixture of 0.4 volume of formic acid, 25 volume of methanol and 75 volume of water. The retention time for paracetamol was found to be 6.09 minutes. The method was validated for parameters like specificity, linearity, precision, accuracy and robustness. The method was found to be specific as no other peaks of impurities and excipients were observed. The square of correlation coefficients for paracetamol was 0.9999 while percentage mean recovery 98.76%. The limit of quantitation and limit of detection were calculated from the standard deviation of responses and slopes using signal-to-noise ratio as per ICH guidelines. The LOQs for paracetamol was found to be 2 µg/ml while the LODs were 0.5 µg/ml [26].

### 2.3 Nano zinc oxide

Metal oxides have attracted considerable interest due to their superior properties which include large specific surface area, high uniformity and excellent catalytic activity[29,30]. Many methods have been employed to attach nZnO onto the glassy carbon electrode in electrochemistry and these include drop casting and electro-deposition. The procedure for drop casting is very simple, firstly, the nZnO sample is dispersed in a suitable solvent in the required proportions and then it will be drop casted onto the electrode and then dried[31]. As for electro-deposition of crystalline ZnO thin films from aqueous solution this is promoted by basegeneration upon electroreduction of nitrate ions and dissolved oxygen respectively. Firstly, there is dissociation of zinc nitrate and analogous formation of Zn<sup>2+</sup> ions. Then the dissociated nitrate ions will undergo further reduction forming both NO<sub>2</sub><sup>-</sup> ions and oxygen and then the released oxygen will be captured by the anchored Zn<sup>2+</sup> forming ZnO[10]. This is shown in equations 2.1 and 2.2.



The advantage of the electrodeposition method over the drop and dry method is that electrodeposition method promotes better surface coverage of the electrode therefore better surface area for electrocatalysis. Zinc oxide nano particles have wide technological applications in catalytic, photocatalytic, electrical and optoelectronic processes and systems. Due to their diverse applications, size and morphology of ZnO particles they are of great necessity[32]. The morphology of the ZnO nanoparticles can be modified to serve specific requirements by employing suitable synthetic processes[32]. Zinc oxide has been widely used in near-UV emission, gas sensors, transparent conductor and piezoelectric application[33]. Most of the ZnO crystals have been synthesized by traditional high temperature solid state method which is energy consuming and difficult to control the particle properties. ZnO nanoparticles can be prepared on a large scale at low cost by simple solution based methods, such as chemical precipitation, sol-gel synthesis and hydrothermal reactions. Zinc oxide nano particles can be used to impart UV blocking property on cotton and wool in textile industry[34].

Zinc oxide is photoactive and generates superoxide and hydroxyl radicals (Reactive Oxygen Species; ROS) when irradiated with UV in the presence of oxygen and water[32].

## **2.4 Carbon nanotubes**

Carbon nanotubes can be defined as hollow cylinders of carbon atoms and they play an important role in most of their applications[35]. Their appearance is the same as that of rolled tubes of graphite in such a way that their hexagonal carbon rings are walls and they are formed in large quantities. The ends of carbon nanotubes are domed structures of six membered rings capped by a five membered ring[36]. There are two forms in which carbon nanotubes can be found, which are single-walled carbon nanotube and multi-walled carbon nanotube which differ in arrangement of their grapheme cylinders. There are also three types of nanotubes namely

armchair, zigzag and chiral. They differ symmetrically and can vary in function due to the way they roll up[37].

This type of nano particles has exceptional mechanical and electrical properties. Various methods have been thoroughly investigated for growth of carbon nanotubes. Among all the methods that have been employed for nanotube synthesis, the best and commonly used method is chemical vapour deposition (CVD)[38]. There are also some different techniques that can be used which are the carbon arc-discharge, reaction chamber CVD, hot filament CVD, plasma enhanced CVD, microwave CVD and the laser ablation technique. The structural uniformity of carbon nanotubes produced by plasma enhanced chemical vapour deposition gives uniform height and diameter[39]. Carbon nanotubes are approximately a nanometer wide and a few microns long. The classifications of the different symmetries of nanotubes are dependent on the unit cell. The unit cell is a section of the carbon nanotube, which is broken down into vectors that describe the spiral symmetry of the nanotube[40]. Graphite, carbonaceous materials and carbon fiber electrodes are commonly used in fuel cells, batteries and other electrochemical applications[40]. The advantages of considering nanotubes for energy storage are their small dimensions, smooth surface topology and perfect surface specificity. The efficiency of fuel cells is determined by the electron transfer rate at the carbon electrodes, which is the fastest on nanotubes following ideal Nernstian behavior[12].

Carbon nanotubes have been employed in biosensors due to its high electrical conductivity, mechanical strength and chemical stability and also CNT based electrodes demonstrate high sensitivity with awesome detection limits. These properties prove that CNT have a great capacity to change electron transfer reactions when utilized as electrode modifying materials[20]. Multiwalled carbon nanotubes comprise of various concentric layers of graphene that form a

tube shape. Electrochemical sensors modified with carbon nanotubes are presently an area of interest for usage in creating electrodes for determination of different analytes[41]. The captivation of nanotubes depends on their exceptional properties, chemical, structural, electronic and mechanical properties. Considering these electronic properties, carbon nanotubes are known for their capacity of boosting electron transfer reactions and enhancing sensitivity in electro analysis[42].

According to research findings it has demonstrated that use of MWCNTs can be upgraded by adding other functional groups or different nanomaterials onto their surfaces. The mix of MWCNTs with different nanomaterials has been accounted to be very helpful for creating electrochemical sensors[1].MWCNTs consist of concentrically nested multiple graphene sheets with interlayer distances similar to those of graphite, generally its structure is more like many single-wall carbon nanotubes nested together or a single graphene layer rolled up like a scroll[11]. Two structural models of multi-walled nanotubes have been proposed. In the Russian Doll model, a carbon nanotube contains another nanotube inside it (the inner nanotube has a smaller diameter than the outer nanotube)[43]. In the Parchment model, a single graphene sheet is rolled around itself multiple times, similar to a rolled up scroll of paper[43]. Multi-walled carbon nanotubes have similar properties to single walled nanotubes, yet the outer walls on multi-walled nanotubes can protect the inner carbon nanotubes from chemical interactions with external materials. Multi-walled nanotubes also have a higher tensile strength than single-walled nanotubes[44]. Electrochemical characterization involving conjugates of MWCNTs and nanoparticles is expanding rapidly in electro-analytical researches because the conjugates have more appreciable electro-catalysis effect compared to the nanotube or nanoparticles alone. A number of studies have been carried out involving MWCNTs conjugated with nanoparticles and

other compounds for promoting electrocatalysis[45]. Based on the highlighted properties MWCNTs were used in the recent study.

## **2.5 Modification techniques**

### **2.5.1 Adsorption modification**

The method can be done or rather achieved by dropping the modifier on the surface of the electrode or by dipping the electrode in the modifier solution or suspension followed by evaporation of solvent at room temperature[46]. Therefore monolayer fabrication there is physisorption from solution by drop casting then the solvent is evaporated. Van der Waal forces and coulombic interactions are accountable for the physisorption. This technique makes it easy to change or adjust the thickness of the film by increasing the amount of deposition on the electrode of the modifying substance[28]. Dip dry coating is a method in which the electrode is dipped in a solution of the polymer, modifier or catalyst for a certain period of time which is adequate for impulsive film formation to take place by adsorption. Subsequently, the electrode is removed from the solution and the solvent is then allowed to dry. Drop dry coating is an adsorption process that includes immersion, start up, deposition, drainage and evaporation[28]. A few drops of sample, polymer, modifier or catalyst solution is smeared onto the electrode surface and permitted to stand to make the solvent dry. Spin coating is an adsorption method that involves evaporation of a drop of modifying sample solution from an electrode surface by high speed rotations utilizing centrifugal force[41]. Droplet evaporation involves dropping the modifying sample onto the surface of the electrode then as the solvent evaporates, a modifying film is formed on the surface of the electrode. The major merit of this method is that the sample coverage is instantaneously known from the original sample solution concentration and volume of the droplet[8].

### **2.5.2 Chemical modification**

The chemical modification is divided into two broad groups, those resulting in acidic and basic surface. The third type of chemical modification involves the surface impregnation with active metals and their oxides. Chemical modifying with a thin film is either bound or coated onto the electrode surface to supply desirable properties thereby changing the transport properties to the electrode surface and accelerating the transfer reactions[47]. In this method, the chemical film is strongly and ideally irreversibly adsorbed onto the electrode surface.

### **2.5.3 Covalent attachment**

This method involves employing a linking agent to covalently join one of numerous monolayers of the chemical modifier to the surface of the electrode. In this method stronger attachment of the electrode surface to the substrate surfaces can be achieved by covalently linking of the anticipated component to the surface groups existing[21].

### **2.5.4 Electropolymerisation**

The technique involves application of voltage to two electrodes immersed in a solution containing a monomer to be electropolymerised. The layer of electropolymerised monomer builds on the electrode surface. Electropolymerisation involves the use of polymer coating as modifying agents on the electrode[48]. A solution of monomer is oxidized or reduced to an activated form that polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be targeted at exposed sites at the electrode surface, unless the polymer film itself is redox active, electrode passivation takes place and furthermore film growth is prohibited[48].

### **2.5.5 Salinization**

In this method the salinizing reagents react with the surface hydroxyl groups of the substrate to form a modified surface. This leads to the formation of a surface hydroxyl or oxide groups on the electrode surface which are then reacted with trichlorosilanes or trialkoxy to form at most three covalent bonds to the original electrode surfaces[37].

### **2.5.6 Composite**

The composite electrode is prepared by a simple impregnation of the bulk electrode material with the chemical modifier. The chemical modifier is simply mixed with an electrode matrix material, as in the case of an electron-transfer mediator, the electrocatalyst combined with the carbon particles of a carbon paste electrode[34]. A binder can also be incorporated within the composite. Instead, intercalation matrices such as certain Langmuir-Blodgett films, zeolites, molecular sieves and clays can be used to enclose the modifier[49].

### **2.5.2 Electrodeposition**

Electrodeposition was originally used for the preparation of metallic mirrors and corrosion resistant surfaces among other things. In its simplest form electrodeposition consists of an electrolyte containing metal ions, an electrode or substrate on which the deposition is desired, and a counter electrode. When a current flows through the electrolyte, the cations and anions move towards the cathode and anode, respectively, and may deposit on the electrode after undergoing a charge transfer reaction [48,50].

This technique is irreversible in such a way that as the polymer is oxidized or reduced to a soluble state. It is used for to form conducting polymers. Electrochemical deposition has been used to restrain electrocatalysts onto the electrode. It is attained by repetitive



voltammetric scanning of appropriate catalyst solution, within a particular range of potential. The first and second scans are similar, succeeding scans decrease with the peak current. Electrochemical deposition also includes the adsorptive interaction between the adsorbate and electrode surfaces, in which the density is shared by the adsorbed molecule and the surface [20]. Based on the advantages of this technique such as better electrode surface coverage of electrode, possibility to grow films over large areas as well as irregularly shaped surfaces, wide range of industrial experience can be drawn upon and it is especially attracted in terms of cost, high throughput and scalability, it was selected in the present study over other aforementioned techniques.

## **2.6 Electrochemistry**

Electrochemistry is a branch of chemistry which examines phenomena that is brought about by the alliance of electricity and chemicals and effects to each other. It studies chemical reactions that involve the transfer of electrons between electrodes and reactant molecules in a solution producing electric power[43]. The electrochemical techniques analyze or rather are used for determination and detection of quite a versatile range of substances. In this current study a three electrode system is used for the electrochemical study. The cell is monitored by a combination of a computer and a potentiostat/galvanostat[51]. A chemical reaction is brought about by application of excess voltage from an external power and the resultant current flow is measured to obtain characteristics of the analyte of interest.

### **2.6.1 Voltammetry**

Voltammetric methods enable the sensitive and selective measurement of compounds based on their specific electrochemical behavior at the working electrode surface. It involves a three electrode system that is a working electrode, reference electrode and counter/auxilliary

electrode[52]. The potential at the working electrode (WE) is controlled against a reference electrode (RE), Ag/AgCl(s)/(satd. KCl) electrode. The controlling potential that is applied across the WE and the auxiliary electrodes is the excitation signal. Electrochemical methods are advantageous if the selectivity achieved eliminates the need for separation procedures, which simplifies procedures, enables short analysis times and lower cost of analysis by using a relatively cheaper instrument and less consumables[53].

### **2.6.2 Modes of mass transfer in voltammetry**

Electron transfer can be influenced by the mass transfer for ions to move towards or away from the electrode. The movement of electron in an electrochemical cell is controlled by mass transport processes and mechanisms involved in the mass transport. There are three types of mass transfer processes by which species may migrate to or from an electrode surface[54].

- **Migration**

Migration is the movement of charged particles under the influence of an electric field. It is undesirable but can be eliminated by addition of large excess of supporting electrolyte.

- **Convection**

Convection is a mass transport due to the movement of the solution as a whole which is assisted by means of stirring the solution, solution flow or rotation or vibration of the electrodes. Convection can also be due to some external mechanical energy acting on the solution of the electrode.

- **Diffusion**

Diffusion is a mass transport due to the spontaneous movement of analyte species from a region of high concentration to a region of lower concentration under the influence of a concentration gradient.

There are various techniques that can be used in voltammetry which include cyclic voltammetry, impedance spectroscopy, linear scan, square wave voltammetry and differential pulse voltammetry[54].

### **2.6.3 Cyclic voltammetry**

Cyclic voltammetry (CV) is a versatile electroanalytical technique for the study of electro-active species. Cyclic voltammetry monitors redox behavior of chemical species within a wide potential range. The current at the working electrode is monitored as a triangular excitation potential is applied to the electrode. The resulting voltammogram can be analyzed for fundamental information regarding the redox reaction[55]. Cyclic voltammograms are the electrochemical equivalent to the spectra in optical spectroscopy. A cyclic voltammogram is the plot of the response current at the working electrode to the applied excitation potential [55].

Cyclic voltammetry is the most effective analytical technique available for the study of redox systems that provides an excellent and convenient tool to determine whether an electrochemical reaction is diffusion or kinetically controlled[56]. Apart from its use in quantitative determinations, CV it is widely used for the study of redox process, understanding reaction intermediates and for obtaining stability of reaction products. It also provides an effective way of determining the amount of modifier deposited on the electrode surface. The surface coverage is determined by cycling the electrode with the appropriate potential window in a solution that contains a supporting electrolyte and determining the area under the oxidation or reduction wave[14]. Surface area for the modified electrode is determined by using the  $\text{Fe}(\text{CN})_6^{-3/-4}$  redox system and the RandlesSelvicik equation. It offers a rapid location of redox potentials of electroactive species and it has found a wide range of applications in industrial quality control, geology, material chemistry, biomedical analysis and environmental monitoring[57]. In this

research modified electrode is used to develop an analytical method for the electrochemical sensing and characterization of paracetamol. Cyclic voltammetry monitors redox behavior of paracetamol species within a wide potential range[11]. The working electrode is where the electrochemical reaction of interest takes place and it provides high signal to noise ratio. In cyclic voltammetry studies, the working electrode potential is ramped linearly versus time using a triangular potential waveform from the initial value to some predetermined final value followed by reversing the scan[58]. To obtain a cyclic voltammogram, the current at the working electrode in an unstirred solution is measured during the potential scan. The potential is applied between the reference electrode and the working electrode and the current is measured between the working electrode and the counter electrode. The controlling potential that is useful across the working electrode and the reference electrode is called the excitation signal[58]. These data are then plotted as current ( $i$ ) vs. potential ( $E$ ). The peak height ( $I_p$ ) and peak potential ( $E_p$ ) are dependent on the state of the GCE surface. The current will increase as the potential reaches the reduction potential of the analyte, but then falls off as the concentration of the analyte increases[41]. If the redox couple is reversible then when the applied potential is reversed, it will reach the potential that will re-oxidize the product formed in the first reduction reaction and produce a current of reverse polarity from the forward scan. Cyclic voltammetric processes are either reversible, quasi reversible or irreversible depending on the nature of the reaction[52].

#### **2.6.4 Reversible process**

A reversible process is the one in which the electron transfer process is rapid and the electroactive oxidized species in the forward scan is in equilibrium with the electroactive reduced species in the reverse scan. Reversibility is a direct and straight forward means of

probing the stability of an electroactive species[59]. At a temperature of 25 °C, the peak current in the reversible systems for the forward scan is given by Randles-Sevcik equation, (2.3).

$$I_{pc} = (2.69 \times 10^5) n^{3/2} A D^{1/4} \nu^{1/2} C \quad (2.3)$$

Where  $I_{pc}$  is peak current,  $A$  is the electrode area,  $\nu$  is scan rate,  $D$  is diffusion coefficient,  $C$  is concentration and  $n$  is number of electrons involved. A linear plot of  $I_{p\nu} \nu^{1/2}$  indicates that currents are controlled by planar diffusion to electrode surface. The potential where the current is half of its limiting value is known as half wave potential ( $E_{1/2}$ ) which is the average of the two peak potentials[59]. The formal reduction potential  $E^0$  for a reversible couple is the mean of  $E_{p,a}$  and  $E_{p,c}$  equation, (2.4).

$$E^0 = \frac{E_{pa} - E_{pc}}{2} \quad (2.4)$$

The number of the electrons involved in the redox reaction for reversible couple is related to the difference of peak potentials by equation (2.5).

$$E_{pc} - E_{pa} = \frac{57 \text{ mV}}{n} \quad (2.5)$$

### 2.6.5 Irreversible process

It is a process whereby the reaction goes one way, the most common is when only a single oxidation or reduction peak with a weak or no reverse peak. Irreversible processes are a result of slow electron transfer or chemical reactions at the surface of the working electrode. Totally irreversible systems are characterized by a shift of a peak potential  $E_{pa}$  and  $E_{pc}$ , with an increase in scan rate[60].

### 2.6.6 Electrochemical impedance spectroscopy

Impedance spectroscopy methods are based on the perturbation of the electrochemical cell with a signal of a small magnitude. It is regarded as a derivative of linear sweep voltammetry or

staircase voltammetry, with a series of regular voltage pulses overlaid on the potential linear sweep or stair steps. The current is measured instantaneously before each potential change, and the current difference is plotted as a function of potential[61]. The Nyquist plots obtained in electrochemical impedance spectroscopy comprise of a straight line and a semi-circle. The diameter of the semicircle corresponds to the charge transfer resistance and diffusion controlled process respectively. The straight line portion represents the Warburg impedance which takes into account the frequency dependence on diffusion transportation to the electrode surface [13].

### 2.6.7 Differential pulse voltammetry

The contrast which exists between differential pulse and normal pulse voltammetry shows that in differential pulse voltammetry potential pulse is fixed and superimposed on slowly changing base potential[62]. The current is measured at two points, firstly before the application of the pulse finally at the end of the pulse. The first current is subtracted from the second current, and the current difference is plotted versus the applied potential[63]. The highest produced peak current is proportional to the concentration of analyte and can be expressed by the equation 2.6.

Where  $\alpha = \exp \left[ \left( \frac{nF}{RT} \right) \left( \frac{\Delta E}{2} \right) \right]$  and  $\Delta E$  is pulse amplitude.

$$I_p = \frac{nFAD^{\frac{1}{2}}C}{\sqrt{\frac{\pi}{m}}} \left( \frac{1-\alpha}{1+\alpha} \right) \quad (2.6)$$

### 2.6.8 Square wave voltammetry

The technique was first introduced by Baker. It consists of regular square wave, superimposed on the base on of staircase potential, and is applied to working electrode[64]. The current is doubled during each square wave cycle, once at the end of the forward pulse and the other at the end of reverse pulse. The difference between the two measurements is plotted versus the base potential.

The advantage of this technique is its speed and when compared with differential pulse voltammetry for reversible and irreversible cases its peak currents are 4 and 3.3 times higher than the analogous differential pulse response[26].

## **2.7 Summary**

The analysis of drugs plays a very essential role in public healthy rendering it necessary to subjective checks of drugs in the human body. Various types of techniques have been utilized for determination of caffeine and acetaminophen, however electrochemical is becoming wide spread due to its advantages such as simplicity, fastness, high sensitivity and can obtain perfect results with minimum supervision. MWCNTs and metal nanoparticles are known to improve electrocatalysis by enhancing the surface area of the electrode and yielding excellent catalytic character unlike bare glassy carbon electrodes. Chemical and physical combination of these nano materials can produce very efficient electrochemical sensors. Based on this known attractive properties the research was being pursued following a series of sequential steps which are to follow in the next chapter.

## CHAPTER THREE

### Materials and methods

#### 3.0 Introduction

The present chapter highlights the reagents and the procedures which were applied in the study in order to attain the desired aims and objectives. The efficiency of the electrodeposited nano zinc oxide onto multi walled carbon nanotubes modified glassy carbon electrode was characterized using cyclic voltammetry and electrochemical impedance. Electro-oxidation of caffeine was studied using differential pulse voltammetry (DPV) and linear voltammetry. The simultaneous electro-oxidation of caffeine and acetaminophen was studied using DPV.

#### 3.1 Reagents and chemicals

All the chemicals which were used in this study were of pure analytical grade. Potassium ferrocyanide ( $K_3[Fe(CN)_6]$ ) was obtained from Sigma Aldrich (Johannesburg, South Africa), caffeine ( $C_8H_{10}N_4O_2$ ), potassium chloride (KCl), zinc nitrate ( $Zn(NO_3)_2$ ), sulphuric acid ( $H_2SO_4$ ) and potassium nitrate ( $KNO_3$ ) were from Associated Chemical Enterprises (Harare, Zimbabwe). Hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium di-hydrogen phosphate ( $KH_2PO_4$ ), perchloric acid ( $HClO_4$ ), di-potassium hydrogen phosphate ( $K_2HPO_4$ ), acetic acid ( $CH_3COOH$ ) and ethanol ( $C_2H_5OH$ ) were from Sky labs (Harare, Zimbabwe). Multiwalled carbon nanotubes (MWCNTs) were manufactured at Sigma Aldrich (Johannesburg, South Africa). Distilled water was from Midlands State University Chemical Technology laboratories. A stock solution of potassium ferricyanide was dissolved in 1M KCl in a 500 ml volumetric flask. Masses of about 13.06 grams of potassium di-hydrogen phosphate and 17.41 grams of di-



potassium hydrogen phosphate were weighed and each was dissolved and diluted in 100 ml volumetric flask to the mark. A volume of 30.75 ml  $K_2HPO_4$  and 19.25 ml  $KH_2PO_4$  were placed into a 500 ml volumetric flask then diluted to the mark to make a phosphate buffer solution at a pH of seven.

### **3.2 Equipment**

All the electrochemical experimental procedures were carried out using Autolab Potentiostat PGSTAT302F installed a 1.10 version NOVA software employing a convectional three electrode system. The three electrode system constituted of a bare glassy carbon electrode of 3 mm in diameter which was the working electrode, a platinum wire which was working as an auxiliary/counter electrode and Ag/AgCl (3.0 M) which was working as the reference electrode. All the experiments that were conducted in this study were carried out at room temperature conditions 25 °C (Zimbabwe). A digital analytical balance (G and G of model JJ224BC) was used for weighing. As for pH studies of the solutions, they were adjusted by a Thermoscientific Orion Star A211 pH meter.

### **3.3 Electrode modification**

Prior to modification the glassy carbon electrode was firstly polished using alumina slurry powder on a Buehler-felt pad. Then it was rinsed in distilled water followed by ultra-sonication in a solution of ethanol and distilled water(1:1)[65]. Multiwalled carbon nanotubes were dispersed in dimethylformamide (DMF) (1 mg:1 ml) and then ultra-sonication of the solution for about 30 minutes[66]. As for the MWCNTs the drop and dry method was employed where by 5 $\mu$ l of the solution was drop casted on the electrode surface and allowed to dry at room temperature [67]. A solution containing 0.05 M of  $Zn(NO_3)_2$  and  $KNO_3$  was prepared prior to

electrodeposition. The MWCNT/GCE was shifted to an electrochemical cell containing 0.05 M  $\text{Zn}(\text{NO}_3)_2$  and  $\text{KNO}_3$  solution. Then 10 cyclic voltammograms were performed in the potential range from 0 to -1.5 V at a scan rate of 0.05 V/s for the deposition of ZnO onto the electrode surface using cyclic voltammetry technique.

### **3.4 Reagents preparation**

A stock solution of 1 mM caffeine was prepared in a 1000 ml volumetric by dissolving about 0.19 grams of pure analytical grade caffeine in distilled water and it was used for the preceding experiments. A stock solution of 1 mM acetaminophen was prepared in a 1000 ml volumetric flask by dissolving 0.151 grams of pure acetaminophen.

### **3.5 Cyclic voltammetry**

#### **3.5.1 Electrochemical behavior of modifiers in 5mM $\text{K}_3[\text{Fe}(\text{CN})_6]$**

Cyclic voltammetry was used for the investigation of electrochemical behavior of the bare GCE, ZnO/GCE, MWCNT/GCE and ZnO/MWCNT/GCE electrodes in potassium ferrocyanide solution at a scan rate of 0.05 V/s from -0.3 to 0.6 V. A volume of about 80 ml of potassium ferricyanide was used for all analysis pertaining its use.

#### **3.5.2 Choice of supporting electrolyte**

The best electrode (ZnO/MWCNT/GCE) from ferrocyanide studies was used to determine its electrochemical behavior towards acetic acid (0.01 M), perchloric acid (0.01 M), sulphuric acid (0.01 M), phosphate buffer (0.01 M) and hydrochloric acid (0.01 M) in order to identify the best electrolyte for electrocatalytic oxidation of caffeine [51]. Cyclic voltammetry was used

for determination of the different electrolytes and they were scanned at 0.05 V/s from 0.6 to 1.6 V in 0.01 M concentrations of the afore mentioned electrolytes.

### **3.5.3 Effect of pH**

Eight different 100 ml volumetric flasks were filled with 100 mM caffeine solution. Then each solution was adjusted its pH using 0.1 M HCl solution for acidic pH and 0.1 M NaOH solution for basic pH from pH 1 to 8 at a scan rate of 0.1 V/s at a potential range of 0.6 to 1.6 V on ZnO/MWCNT/GCE.

### **3.5.4 Electrochemical oxidation of caffeine**

The electrochemical behavior of 100 mM of caffeine solution was studied using cyclic voltammetry on bare GCE, ZnO/GCE, MWCNT/GCE and ZnO/MWCNT/GCE electrodes in 0.01 M perchloric acid at pH 1 as the supporting electrolyte at 0.05 V/s from 0.6 to 1.6 V.

### **3.5.5 Effect of scan rate**

The effect of scan rate for ZnO/MWCNT/GCE in 100 mM of caffeine was carried out in pH 1 of perchloric acid solution. The scan rates which were applied various 0.05 V/s, 0.075 V/s, 0.1 V/s, 0.125 V/s, 0.15 V/s, 0.175 V/s, 0.2 V/s, 0.225 V/s, 0.250 V/s, 0.275 V/s and 0.3 V/s at a potential range of 0.6 to 1.6 V.

### **3.5.6 Order of reaction**

The order of reaction was of ZnO/MWCNT/GCE was studied using linear scan voltammetry in different concentrations of caffeine 200  $\mu$ M, 300  $\mu$ M, 400  $\mu$ M, 500  $\mu$ M, 600  $\mu$ M, 700  $\mu$ M, 800  $\mu$ M and 900  $\mu$ M at 0.1 V/s scan rate and potential of 0.6 V to 1.6 V.

### **3.5.7 Stability studies**

As for the stability studies of the produced sensor, cyclic voltammetry technique was employed. The assay reproducibility of the sensor was investigated through repetitive measurements of the 500  $\mu\text{M}$  caffeine sample in 0.01 M perchloric acid at pH 1 at a potential of 0.6 to 1.6 V.

### **3.6 Differential pulse voltammetry**

Differential pulse voltammograms of caffeine solution for individual determination at different concentrations were obtained from a range of 0 to 15  $\mu\text{M}$  at potential of 0.4 to 1.6V, scan rate of 0.01V/s and an amplitude pulse 0.05 V. Then for the individual oxidation of acetaminophen different concentrations ranging from 0 to 18  $\mu\text{M}$  at a potential of 0.4 to 1 V, scan rate 0.01 V/s, modulation time 0.5 s and amplitude of 0.05 V was used for analysis. As for simultaneous determination, 0.5 to 25  $\mu\text{M}$  of acetaminophen concentrations and caffeine at constant 100  $\mu\text{M}$  solution was prepared in pH 1 perchloric acid solution. Then 80 to 260  $\mu\text{M}$  concentrations of caffeine and acetaminophen solution at constant 10  $\mu\text{M}$  was prepared in pH 1 perchloric acid at a potential of 0.4 to 1.6 V scan rate 0.01 V/s, modulation time 0.5 s and an amplitude 0.05 V. Then 10 to 90  $\mu\text{M}$  of both caffeine and acetaminophen solution were prepared in pH 1 perchloric acid at a potential of 0.4 to 1.6 V and run at scan rate 0.01 V/s, modulation time 0.5 s and an amplitude 0.05 V.

### **3.7 Reproducibility studies**

The fabricated sensor was analyzed in a solution containing 100  $\mu\text{M}$  of caffeine and 10  $\mu\text{M}$  of acetaminophen employing the DPV technique. Before and after each analysis the electrode was

washed using distilled water in order to remove adhered particles at a scan rate of 0.01 V/s. potential of 0.4 to 1.6 V and amplitude of 0.05 V for a period of about seven times.

### **3.8 Effect of interference**

To the caffeine (150  $\mu\text{M}$ ) and acetaminophen (120  $\mu\text{M}$ ) solutions was added 5 ml volumes of 100  $\mu\text{M}$  ascorbic acid, 100  $\mu\text{M}$  citric acid and 10  $\mu\text{M}$  sodium nitrite one hundred fold of each were used from 0 to 1.6 V potential and amplitude of 0.05 V using DPV.

### **3.9 Real sample analysis**

A cola drink was bought from the Midlands State University tuck-shop and then poured into a beaker. An aliquot of cola drink was added to a 100 ml volumetric flask. Three standard caffeine solutions were spiked to flasks containing 5 ml aliquot of cola drink and then they were filled to the mark using 0.01 M perchloric acid at pH 1. Recovery tests were also carried for determination of acetaminophen in human urine samples. Human sample was collected after about 6 hours of administration of the paracetamol tablet in order to make sure there was complete adsorption and metabolism of the drug in the body. A volume of about 5 ml of urine sample was added to a 100 ml volumetric flask and three standard solutions of acetaminophen were spiked to each separate flask.

## CHAPTER FOUR

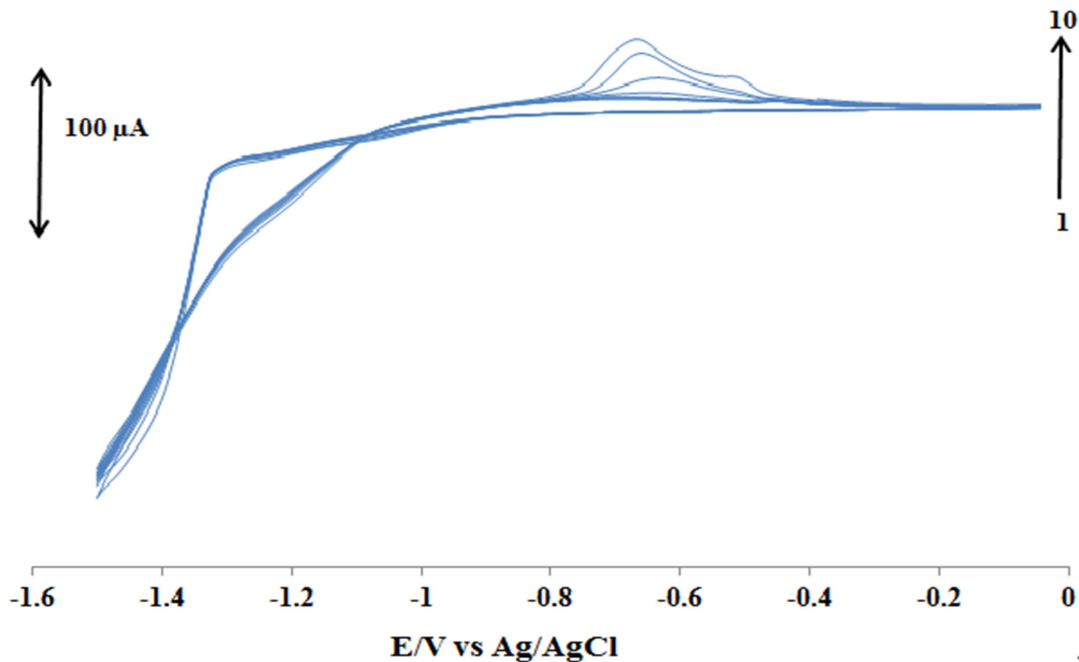
### RESULTS AND DISCUSSION

#### 4.0 Introduction

This chapter deals with the research findings and authentication of the procedures in the methodology section. Preliminary studies were carried out employing cyclic voltammetry with the GCE, ZnO/GCE, MWCNT/GCE and ZnO/MWCNT/GCE electrode in 5 mM potassium ferricyanide. Differential pulse voltammetry, linear sweep voltammetry and cyclic voltammetry were used in the electrochemical studies. Selectivity, sensitivity and reproducibility studies were conducted in authenticating the developed sensor.

#### 4.1 Electrochemical deposition of zinc oxide on glassy carbon electrode

Figure 4.1 shows 10 successive cyclic voltammograms for deposition of zinc oxide on multiwalled carbon nanotube modified electrode at scan rate of 0.05 V/s between -1.6 and 0 V in 0.05 M solution of  $\text{Zn}(\text{NO}_3)_2$  and  $\text{KNO}_3$  to form ZnO/MWCNT/GCE.



**Fig 4.1:** Cyclic voltammograms for deposition of zinc oxide on glassy carbon electrode in 0.05M  $Zn(NO_3)_2$  and  $KNO_3$  solution.

It can be observed that the peak current increased during deposition with each successive scan at scan rate of 0.05 V/s between 0 and -1.5V in 0.05M solution of  $Zn(NO_3)_2$  and  $KNO_3$  to form ZnO/MWCNT-GCE.

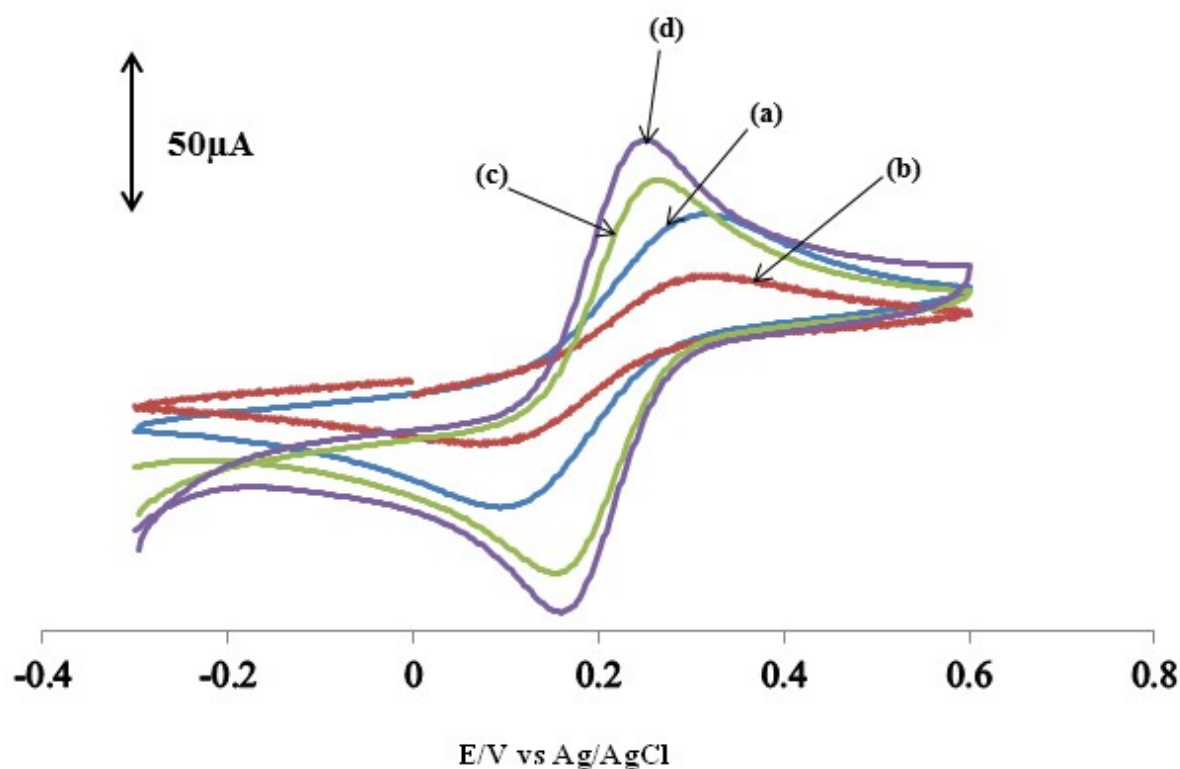
#### 4.2 Electrochemical behavior of modified electrode in redox probe

The electrochemical properties of bare GCE, ZnO/GCE, MWCNT/GCE and ZnO/MWCNT/GCE were characterized by CV in 5 mM  $K_3[Fe(CN)_6]$  solution containing 0.1 M KCl at 0.1 V/s. All electrodes were scanned in 5mM  $K_3[Fe(CN)_6]$  in KCl and the outcome voltammograms are illustrated on Fig 4.2. The different electrodes gave different signals of peak current when they were analyzed in the following descending order ZnO/MWCNT/GCE

>MWCNT/GCE > GCE >ZnO/GCE. It can be seen in Fig. 4.2 (curve a-d) that a pair of peaks corresponding to the redox reaction of ferricyanide were observed. The peak-to-peak potential separation is a good measure of the electron transfer ability of the electrode with lower values showing good electron transfer ability, following the order: ZnO/MWCNT/GCE (0.82 V), MWCNT/GCE (0.85 V), GCE (0.98 V) and ZnO (1.23 V). It can be seen that the modification of the GCE with ZnO results in a reduction in anodic peak current of the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  ( $I_{\text{pa}}$ : 77  $\mu\text{A}$ ) compared with the GCE ( $I_{\text{pa}}$ : 113.9  $\mu\text{A}$ ). Compared with ZnO/GCE, the anodic peak current of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  ( $I_{\text{pa}}$ : 228  $\mu\text{A}$ ) on MWCNT/GCE further increased. However, the ZnO/MWCNT/GCE significantly enhanced the redox peak currents, giving the highest anodic peak current  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  ( $I_{\text{pa}}$ : 266  $\mu\text{A}$ ) compared to other electrodes. The highest electrocatalytic activity was probably attributed to ZnO and MWCNT providing an increase in effective surface area on the electrode. The obtained results suggested that the ZnO/MWCNT was successfully adsorbed.

The best electrode was found to be ZnO/MWCNT/GCE which showed enhanced electron-transfer kinetics forming well defined redox peaks with high peak currents. The high peak current potentials exhibited by MWCNT/ZnO modified glassy carbon electrode might have been due to large surface area being provided by the MWCNT and the nano zinc oxide which are both in the nano range [69]. Therefore after the new sensor exhibited better electron transfer properties than the others it was applied for further studies.

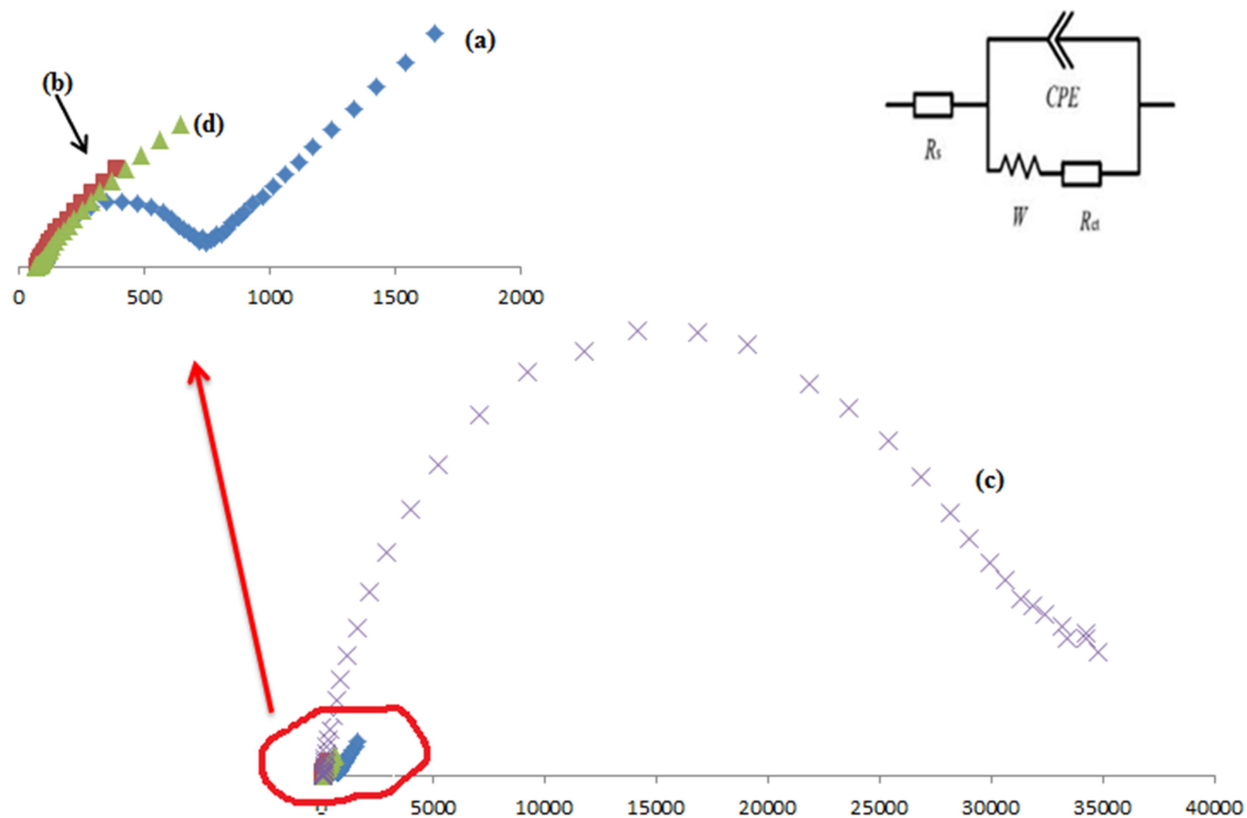




**Fig 4.2:** Voltammograms for (a) GCE, (b) ZnO/GCE, (c) MWCNT/GCE and (d) ZnO/MWCNT/GCE in 5 mM potassium ferricyanide solution at 0.05 V/s at a potential of -0.3 to 0.6 V

### 4.3 Electrochemical impedance spectroscopy

The electrochemical behavior of the four electrodes was further investigated using the electrochemical impedance spectroscopy (EIS) in 5.0 mM of potassium ferricyanide solution. The Nyquist plots in Figure 4.3 of the electrodes comprised of semicircles and straight line portions. The semicircle part at higher frequencies represents the electron transfer limited process and its diameter is equated to the electron transfer resistance showing the electron transfer kinetics of the redox probe on the interface of the electrode[61].



**Fig 4.3:** Electrochemical impedance graphs for (a) GCE, (b) MWCNT/GCE, (c) ZnO/GCE and (d) ZnO/MWCNT/GCE: *Inset* shows the equivalent circuit used for data fitting

The representative circuit for the Nyquist plots shown by insert in Figure 4.3 where,  $R_s$ ,  $CPE$ ,  $R_{ct}$  and  $W$  represent solution resistance, a constant phase element, the charge transfer resistance and the Warburg impedance respectively[13]. The plots show that ZnO/GCE had a large semi-circle with a large diameter suggesting that ZnO acted as an insulating layer and barrier. The GCE displays a small semi-circle indicating a low resistance in transfer of electrons. The MWCNT/GCE showed less resistance and the ZnO/MWCNT/GCE showed a plot which was almost a straight line indicating that the sensor had a very high electron transfer.

#### 4.4 Surface area determination

Scan rate studies for the bare GCE and all modified were done in 5 mM potassium ferricyanide solution by applying the Randles-Sevcik equation given on equation 4.1.

$$I_p = (2.69 \times 10^5)n^{3/2}AD^{1/2}V^{1/2}C \quad (4.1)$$

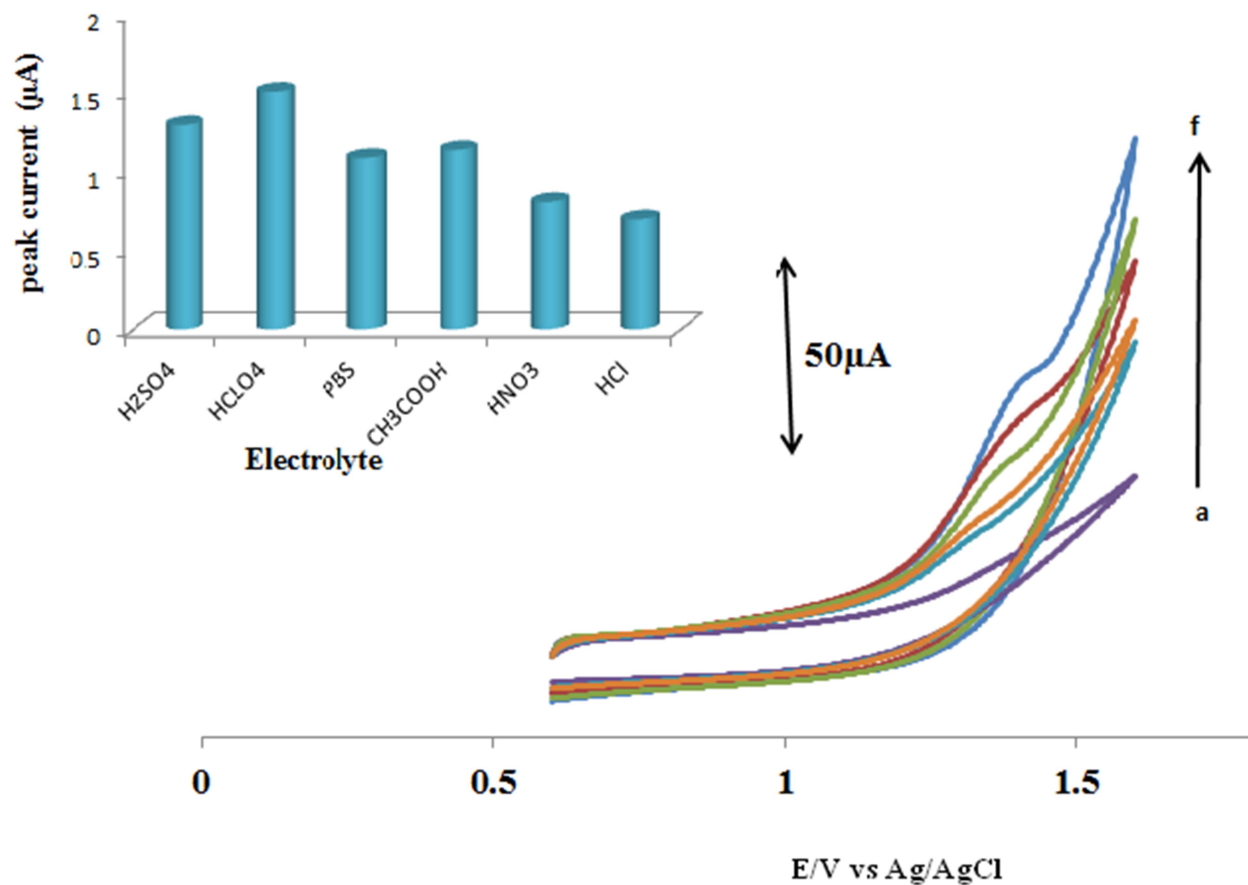
Where  $I_p$  is the peak current,  $n$  is equal to the number of electrons transferred to the surface of the electrode which is obtained from equation 4.2 below.



Therefore  $n$  is equivalent to 1,  $D$  gives the diffusion coefficient of the analyte in solution  $7.6 \times 10^{-6} \text{ cm}^2/\text{s}$  and  $C$  is the concentration of the solution in  $\text{mol}/\text{cm}^3$ .  $A$  is the surface area and  $v$  scan rate in volts per second. The surface area for the electrode modifiers were obtained as follows ZnO/GCE was  $0.034 \text{ cm}^2$ , GCE was  $0.0712 \text{ cm}^2$ , MWCNT/GCE was  $0.088 \text{ cm}^2$  and  $0.15 \text{ cm}^2$  for ZnO/MWCNT/GCE. The surface of the newly modified electrode ZnO/MWCNT/GCE was as twice as that of the bare electrode meaning that the electron catalytic activity of the electrode was enhanced.

#### 4.5 Effect of supporting electrolyte

The nature of the electrolyte solution has been reported to affect the electrochemical behavior of caffeine. Since caffeine is oxidized at very high peak potentials of about 1.4 V a strongly acidic media is required to avoid simultaneous oxidation of water [1]. The determination of a supporting electrolyte for the caffeine solution was studied in different acids. All the acids were suitable for oxidation of caffeine, the highest signal to noise ratio as well as the best reproducibility was obtained in perchloric acid. The Figure 4.4 shows the relationship of the peak current to the different electrolytes which were used in the study for determining the supporting electrolyte.

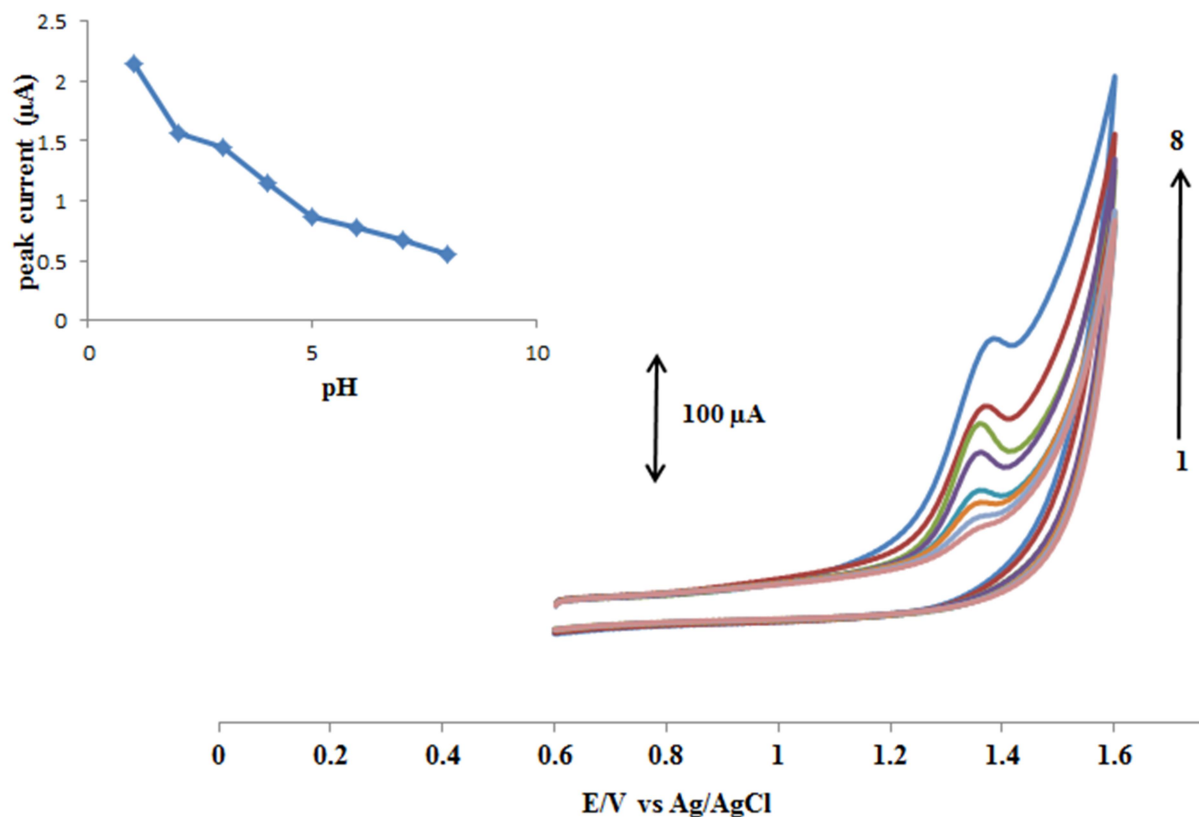


**Fig 4.4:** Cyclic voltammograms for peak current response of (a) HCl, (b) HNO<sub>3</sub>, (c) CH<sub>3</sub>COOH, (d) PBS, (e) HClO<sub>4</sub> and (f) H<sub>2</sub>SO<sub>4</sub>: *Inset* shows bar graph of peak current relative to electrolyte used

#### 4.6 pH studies

The redox behavior of most biomolecules and drugs is affected by the pH of supporting electrolyte [66]. The dissociation or reactivity of biological molecules vary from molecule to molecule some react easily at low and some at high pH which is due the effect of different

functional groups within the molecule structure. The presence of electron donating groups tend to cause an increase acidity as they decrease polarity of a drug molecule [70].

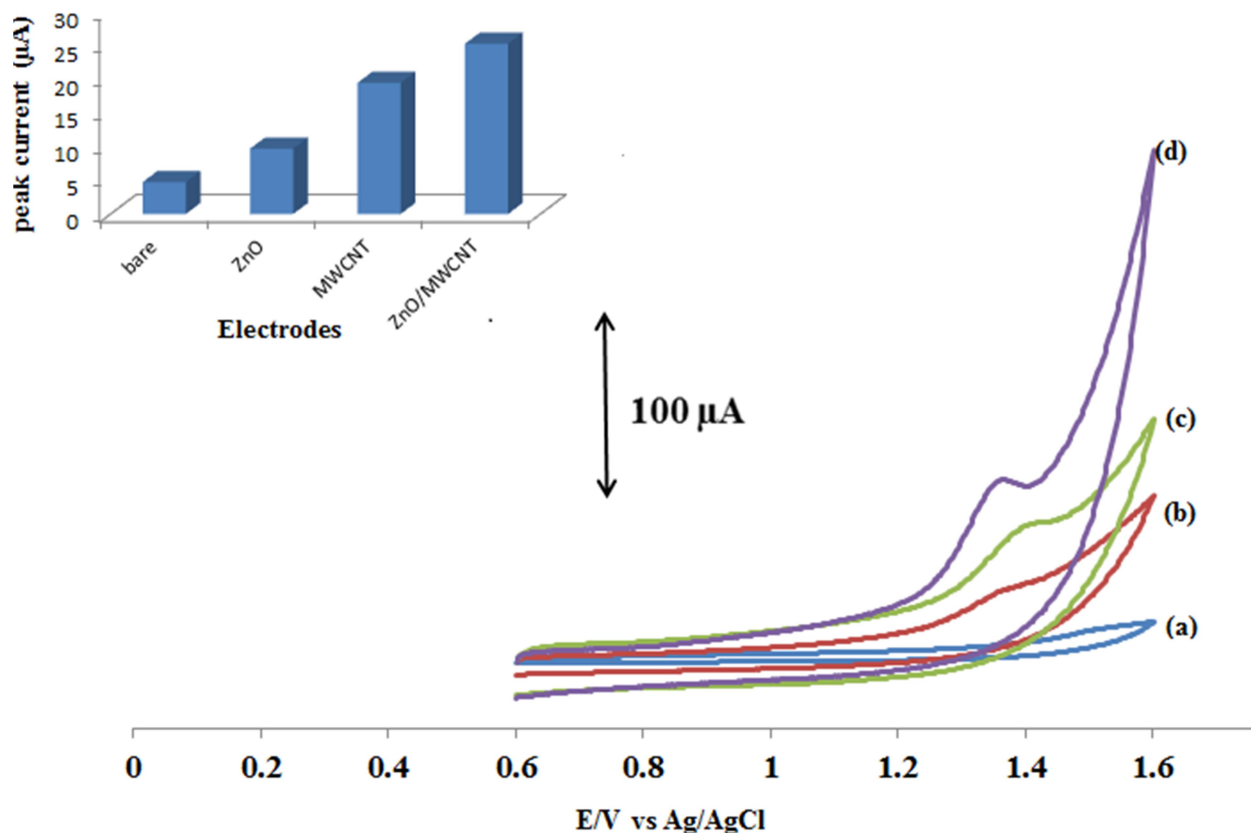


**Fig 4.5:** Cyclic voltammograms of 100 mM caffeine solution at different pH values of 0.01 M perchloric acid: *Inset* shows plot of current against pH

The effect of pH on ZnO/MWCNT/GCE was examined and the resulting voltammograms were obtained as shown on Fig 4.5. As the pH decreased from pH 8 to pH 1 the peak current increased as well. The maximum peak response was obtained at pH 1. The plot of peak current against pH showed that at high pH values there is little oxidation of caffeine as compared to low pH values.

#### 4.7 Electrochemical oxidation of caffeine

The different cyclic voltammograms of caffeine on GCE, ZnO/GCE, MWCNT/GCE and ZnO/MWCNT/GCE in a 0.01 M HClO<sub>4</sub> (pH 1.0) at a scan rate of 0.1 V/s are shown in Fig. 4.6.



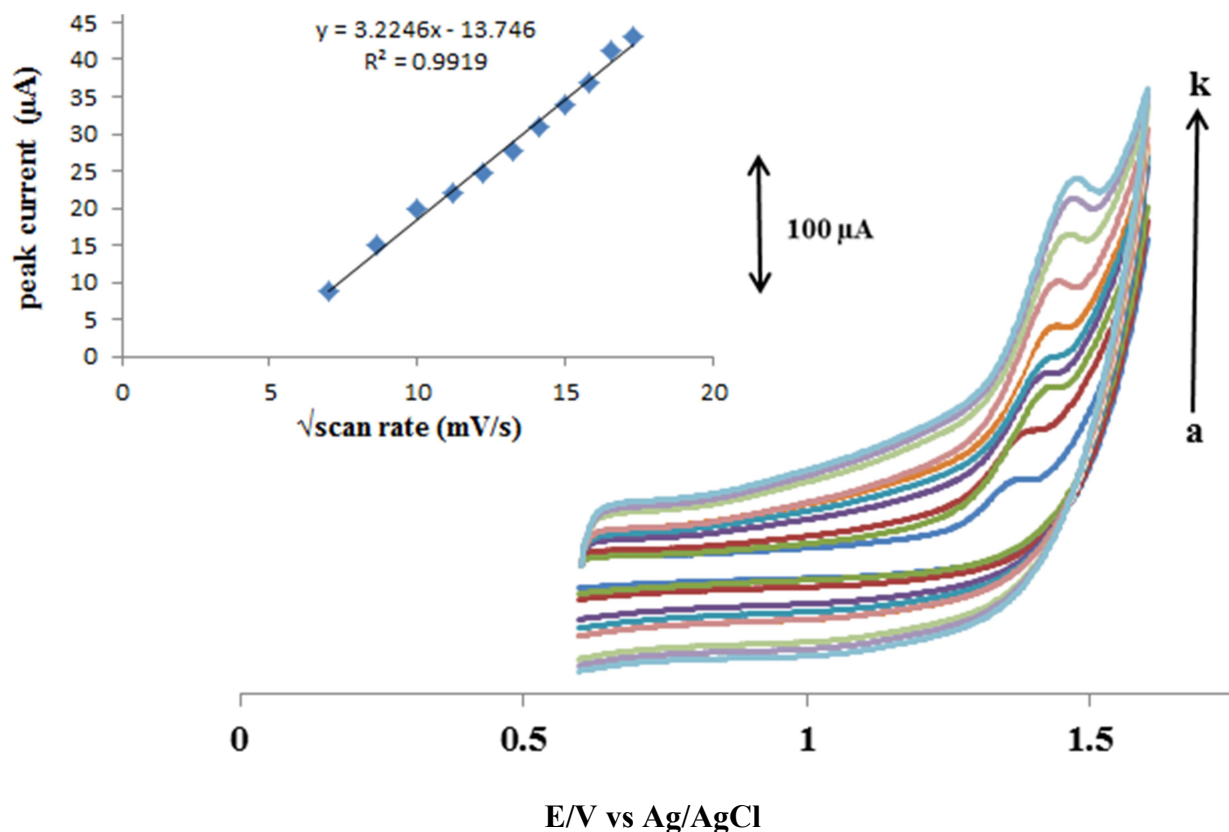
**Fig 4.6:** CVs caffeine on GCE, ZnO/GCE, MWCNT/GCE and ZnO/MWCNT/GCE in a 0.01 M HClO<sub>4</sub> (pH 1.0) at a scan rate of 100 mV s<sup>-1</sup>

As shown in Fig 4.6, peak current increased according to the following sequence of electrode modification on the electrode GCE < ZnO/GCE < MWCNT/GCE < ZnO/MWCNT/GCE. In the presence of the GCE the oxidation of the caffeine exhibited high peak potential value while the ZnO/MWCNT/GCE showed to have the lowest potential due to synergistic effect of the combination of the nano structures. The inset Fig 4.6 shows the relationship between peak

currents and all the probes used in the study. According to the results it shows that there was a decrease in peak potential from GCE which had the highest to ZnO/MWCNT/GCE which had the lowest peak potential. The new probe reduced the over potentials which were being exhibited by the bare electrode proving that it was an improved sensor and that it had better electrocatalytic properties.

#### **4.8 Kinetics**

The effect of varying scan rate of caffeine was determined at ZnO/MWCNT/GCE and Figure 4.7 illustrates an inset plot of current against square root of scan rate and it showed the existence linearity between current and scan rate. The relationship had a correlation value of 0.9919 showing that the electrocatalytic oxidation of caffeine was diffusion controlled.

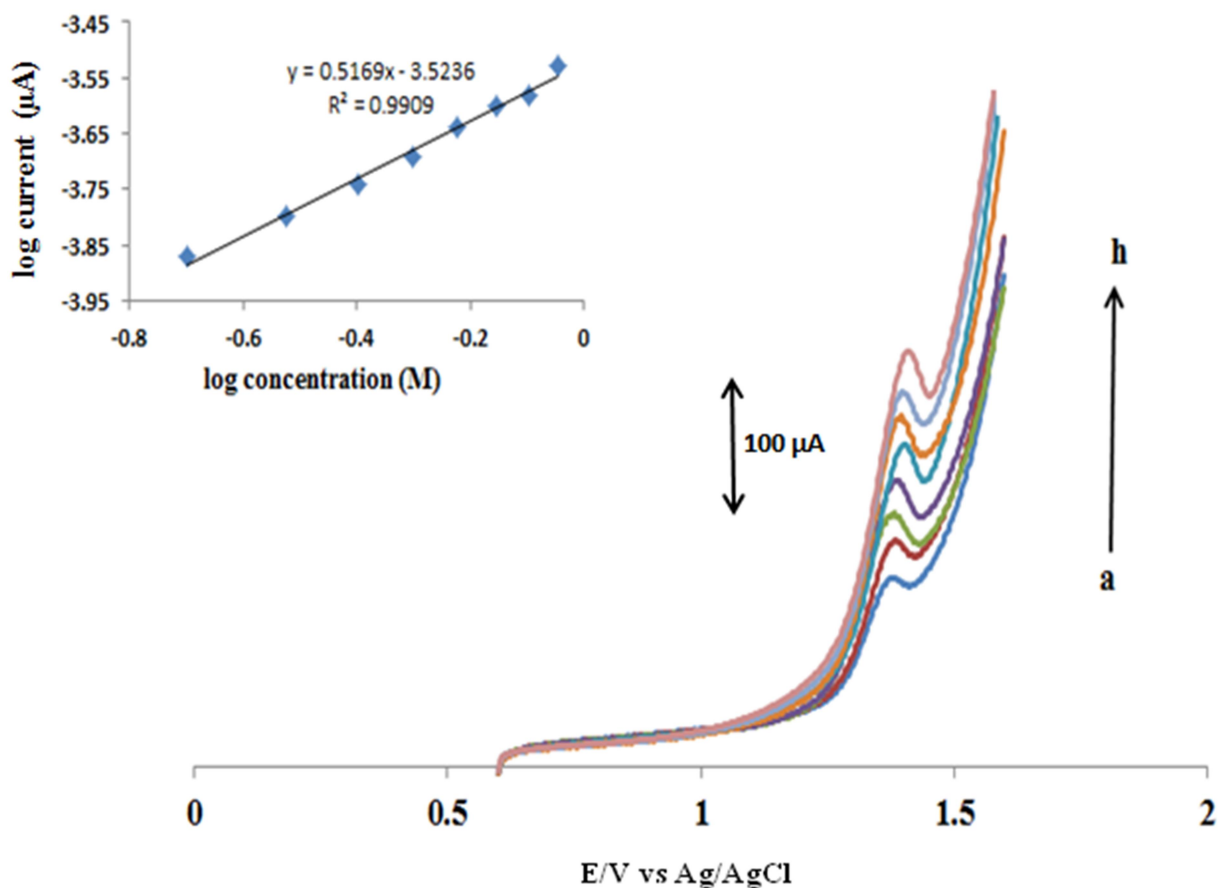


**Fig 4.7:** Cyclic voltammograms for the study of effect of scan rate from 0.05 to 0.3 V/s denoted by (a) 0.05 V/s, (b) 0.075 V/s, (c) 0.1 V/s, (d) 0.125 V/s, (e) 0.15 V/s, (f) 0.175 V/s, (g) 0.2 V/s, (h) 0.225 V/s, (i) 0.25 V/s, (j) 0.275 V/s and (k) 0.3 V/s: *Inset* plot of current against square root of scan rate

#### 4.9 Order of reaction

The plot shows a linear relationship and from this plot, the order of reaction can also be deduced or rather be estimated. Therefore according to Figure 4.8 the correlation value was 0.9909 giving evidence that there was linear relationship between log concentration and log of current.





**Fig 4.8:** Linear voltammograms for (a) 200 $\mu$ M, (b) 300 $\mu$ M, (c) 400 $\mu$ M, (d) 500 $\mu$ M, (e) 600 $\mu$ M, (f) 700 $\mu$ M, (g) 800 $\mu$ M and (h) 900 $\mu$ M of caffeine concentrations. *Inset* plot of log current against log concentration of caffeine

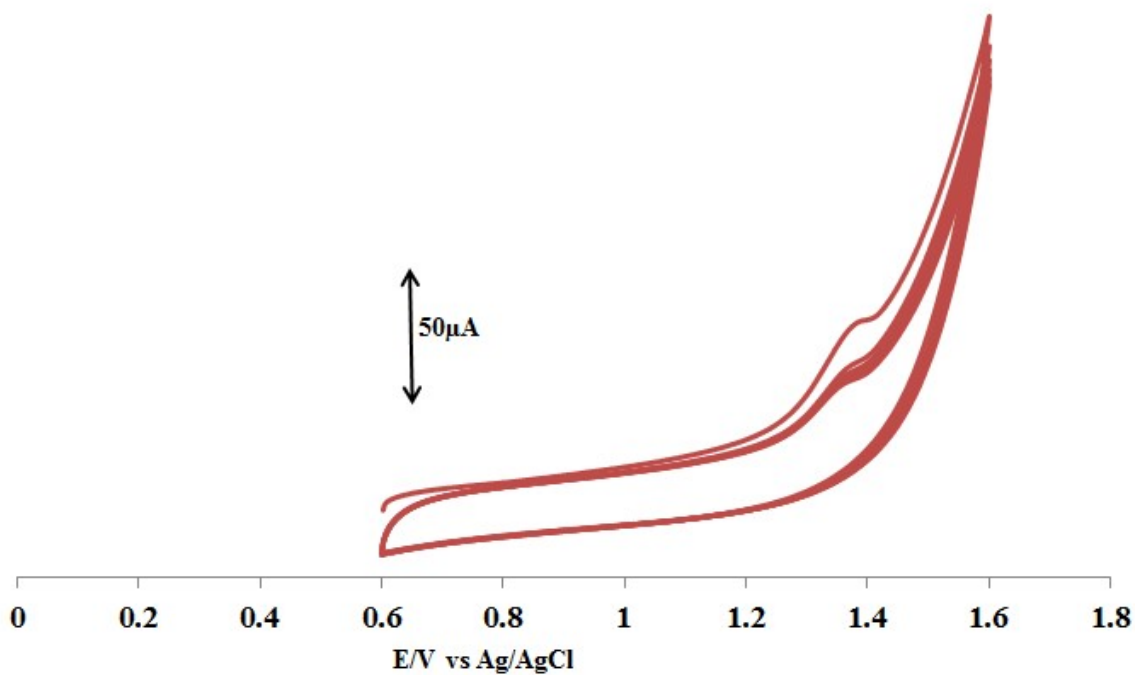
The electrocatalysis is approximately first order implying that one analyte molecule interacts with one molecule of ZnO/MWCNT. The Tafel was found to be 103.4 mV/decade meaning that the process involved 1 electron in rate determining step[71]. This was calculated applying the equation 4.3.

$$E_p = \frac{b}{2 \log + \text{constant}} \quad (4.3)$$

where  $v$  is the scan rate and  $b$  is the Tafel slop.

#### 4.10 Stability

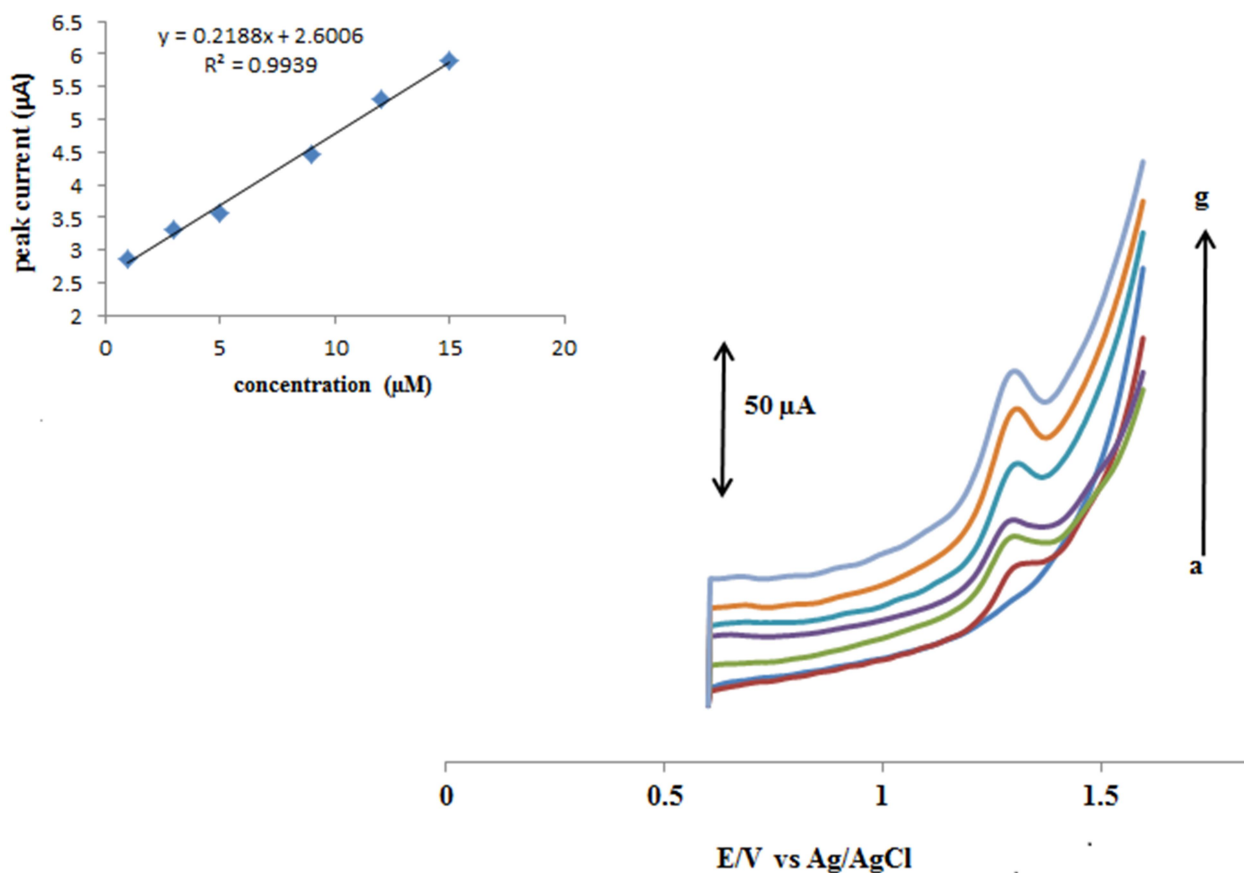
The stability of the ZnO/MWCNT/GCE sensor was studied using cyclic voltammetry by running 30 cycles in 500  $\mu\text{M}$  caffeine solution. The results show that the oxidation peak current slightly decreased with an increase in number of cycles. After a period of about one week a small decrease in peak current and after one month the electrode being stored at room temperature 94.8% response of anodic peak current was retained. These results show that the fabricated sensor possessed an acceptable stability and can be used continuously.



**Fig 4.9:** Cyclic voltammograms for successive runs in 500  $\mu\text{M}$  caffeine solution in perchloric acid at 0.05V/s at a potential of 0.6 to 1.6 V

#### 4.11 Limit of detection caffeine

The DPV technique was selected due to its high sensitivity as compared to cyclic voltammetry. The plot of peak current against concentration produced a linear relationship. Under optimized conditions and method parameters, the DPVs of different concentration of caffeine are shown in fig 4.10.



**Fig 4.10:**Differential pulse voltammograms of caffeine at (a) 0  $\mu\text{M}$ , (b) 1 $\mu\text{M}$ , (c) 3  $\mu\text{M}$ , (d) 5  $\mu\text{M}$ , (e)9  $\mu\text{M}$ , (f) 12  $\mu\text{M}$ , (g) 15  $\mu\text{M}$  concentrations:*Inset* plot of  $i_{pa}$  vs concentration of caffeine

The linear regression equation was acquired as  $i_p = 0.02188[\text{caffeine}] + 3.507$  and  $r^2 = 0.9939$ .

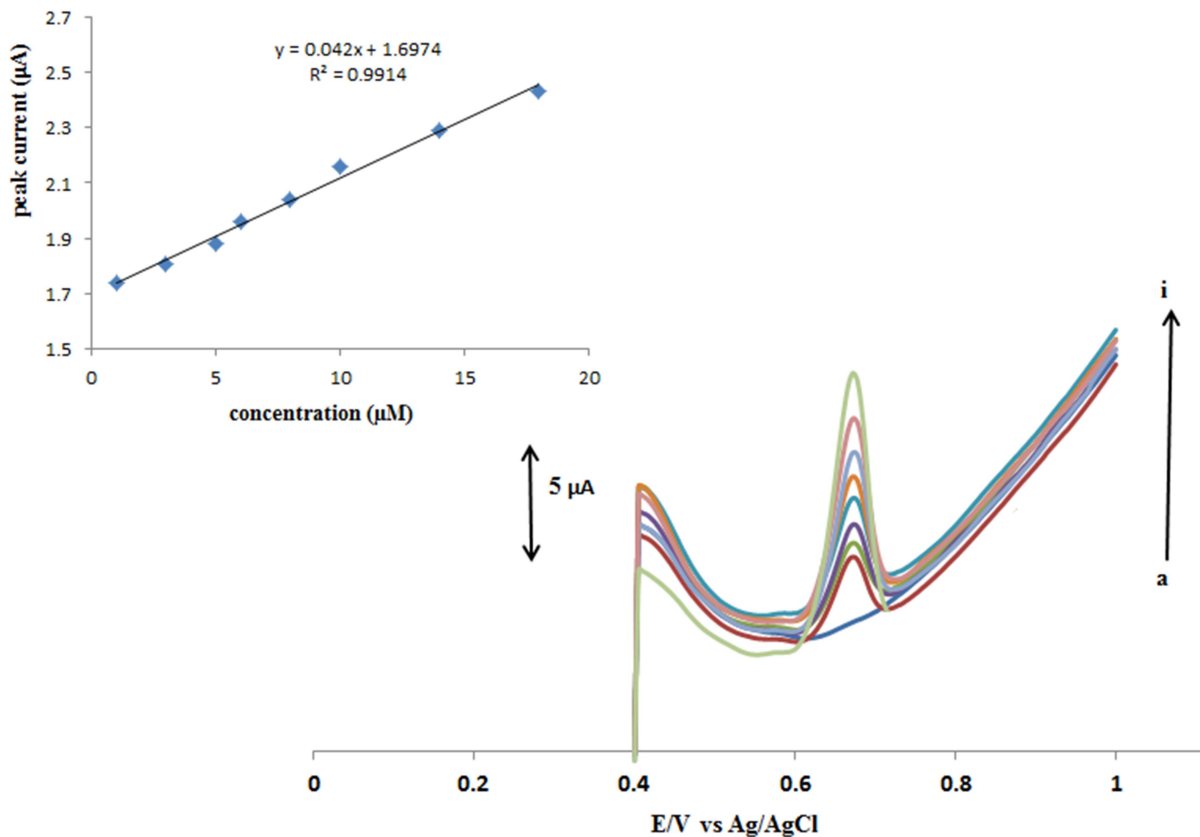
The limit of detection was calculated using the following equation 4.6 and nine blank measurements were carried out.

$$\text{LOD} = \frac{3S}{m} \quad (4.6)$$

Where  $S$  is the standard deviation of the blank and  $m$  is slope of the calibration curve. The limit of detection was to be  $3.52 \times 10^{-8}$  M. The limit of quantification was determined using the equation 4.7.

#### 4.12 Limit of detection acetaminophen

The linear regression equation was found to be  $i_p = 0.042[\text{acetaminophen}] + 1.6974$  and  $r^2 = 0.9914$ . The current is measured instantaneously before each potential change, and the current difference is plotted as a function of potential.



**Figure 4.11:** Differential pulse voltammograms of caffeine at (a) 0 µM, (b) 1 µM, (c) 3 µM, (d) 5

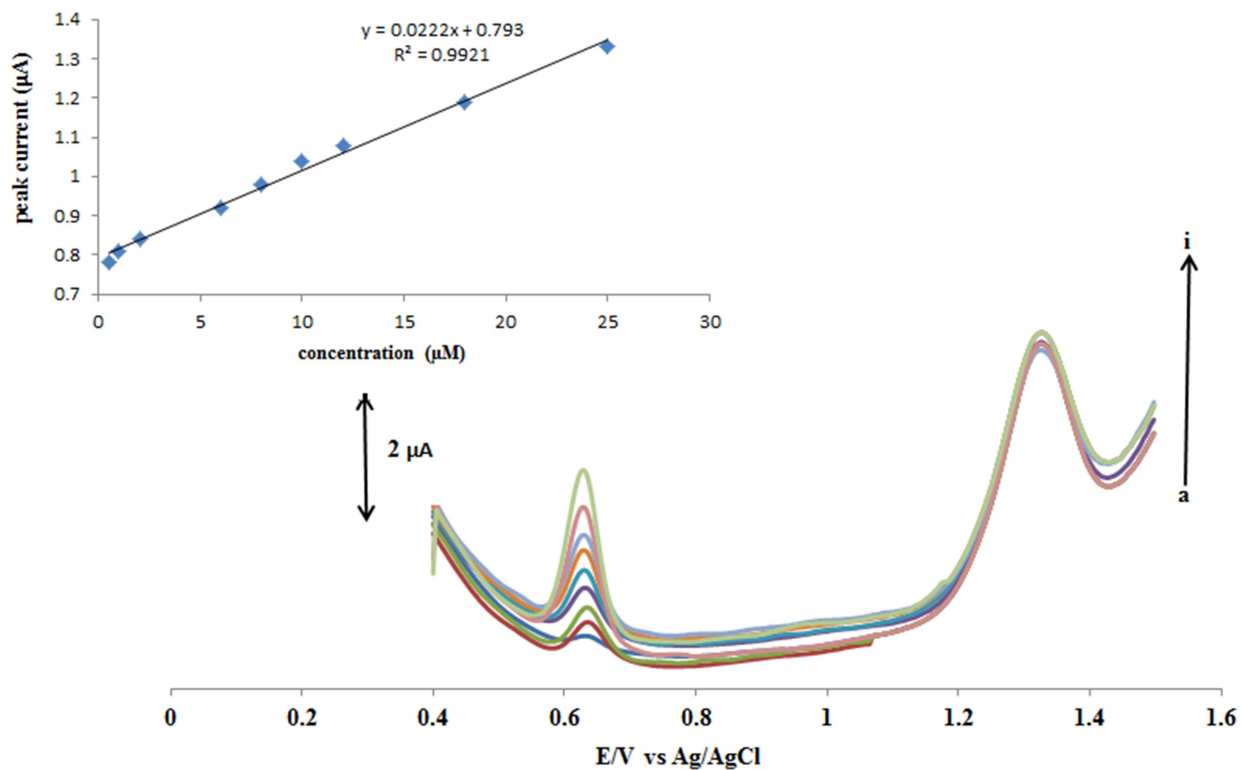
$\mu\text{M}$ , (e)  $6 \mu\text{M}$ , (f)  $8 \mu\text{M}$ , (g)  $10 \mu\text{M}$ , (h)  $14 \mu\text{M}$  (i)  $18 \mu\text{M}$  concentrations: *Inset* plot of  $i_{pa}$  vs concentration of acetaminophen.

The limit of detection of acetaminophen was found to be  $3.69 \times 10^{-8}$  M. The following equations

was used respectively  $\text{LOD} = \frac{3S}{m}$ .

#### **4.13 Simultaneous determination of caffeine and acetaminophen**

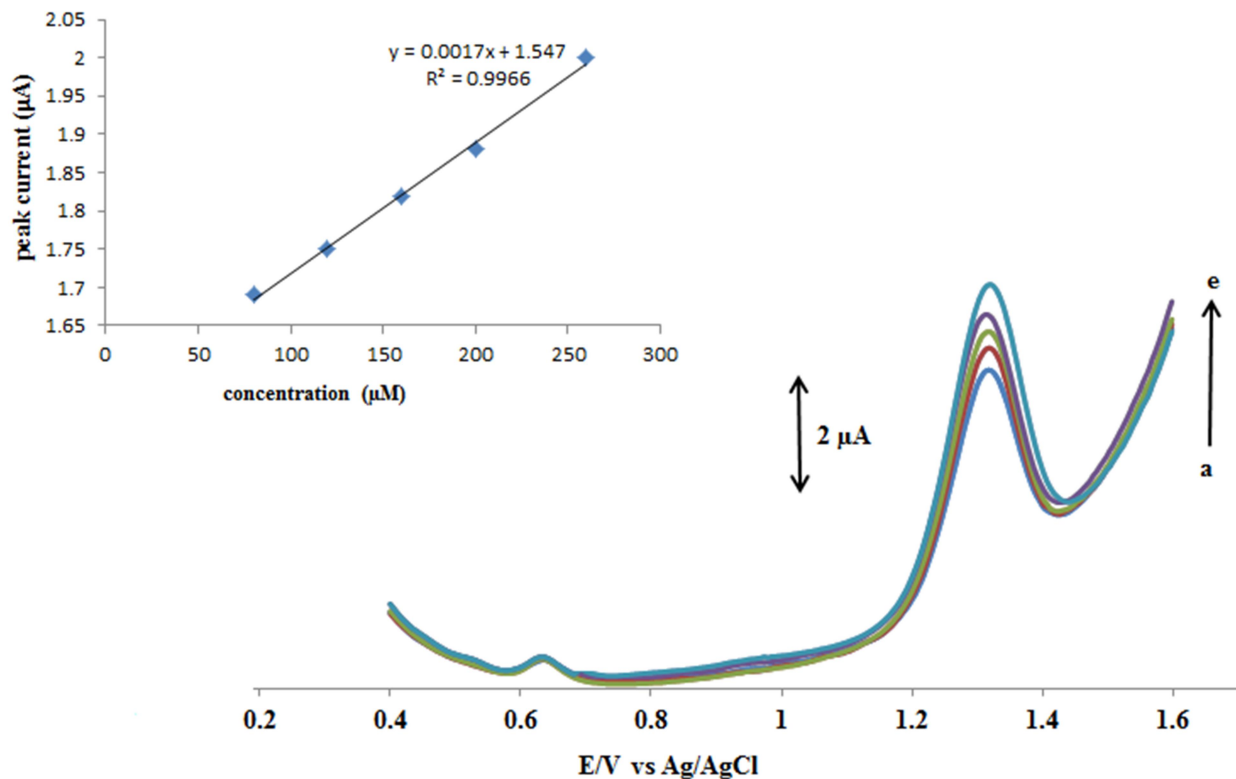
The proposed method was applied for simultaneous determination of caffeine and acetaminophen. In this esteem three cases were studied. In the first scenario the concentration of acetaminophen was being increased while that of caffeine was maintained constant. In the second scenario the concentration acetaminophen was held constant while that of caffeine was being varied. The third scenario both concentrations of acetaminophen and caffeine were increased simultaneously. According to the results all the above cases matched well with the results obtained when the two molecules were analyzed individually.



**Fig 4.12** Differential pulse voltammograms for (a) 0.5 μM, (b) 1 μM, (c) 2 μM, (d) 6 μM, (e) 8 μM, (f) 10 μM, (g) 12 μM, (h) 18 μM and (i) 25 μM of acetaminophen at constant caffeine concentration: : *Inset* plot of  $i_{pa}$  vs concentration.

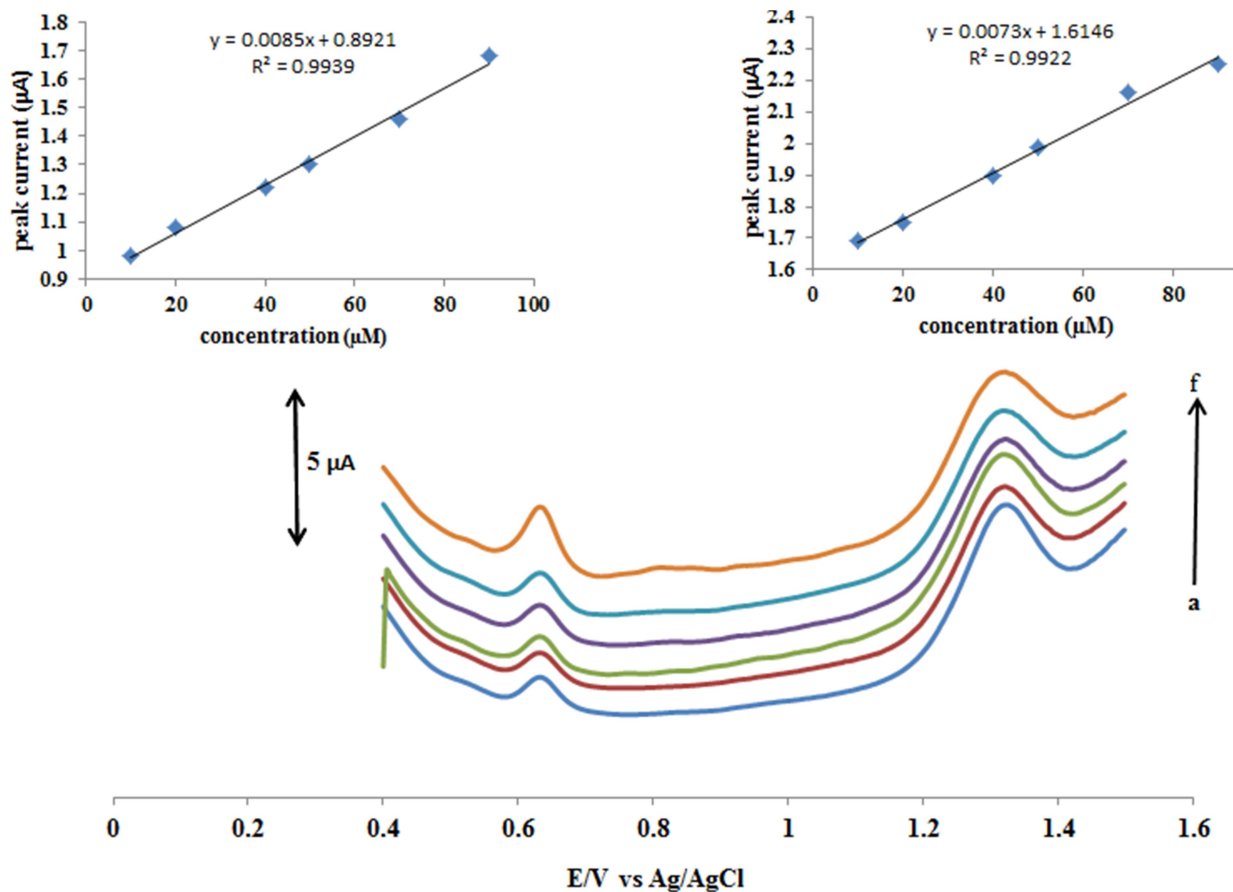
The limit of detection of acetaminophen was found to be  $4.04 \times 10^{-8}$  M. The following equation

was used respectively  $LOD = \frac{3S}{m}$ .



**Fig 4.13** Differential pulse voltammograms for caffeine (a) 80  $\mu\text{M}$ , (b) 120  $\mu\text{M}$ , (c) 160  $\mu\text{M}$ , (d) 200  $\mu\text{M}$ , (e) 260  $\mu\text{M}$  at constant acetaminophen concentration insert is a plot of current against concentration of caffeine: : *Inset* plot of  $i_{pa}$  vs concentration.

The plot of peak current against concentration produced a linear relationship. The statistical analysis was applied in the calculation of limit of detection by the least square regression method using excel linest formulae. The limit of detection was  $5.88 \times 10^{-8} \text{ M}$ .



**Fig 4.14** Differential pulse voltammograms for (a) 10  $\mu\text{M}$ , (b) 20  $\mu\text{M}$ , (c) 40  $\mu\text{M}$ , (d) 50  $\mu\text{M}$ , (e) 70  $\mu\text{M}$ , (f) 90  $\mu\text{M}$  of both acetaminophen and caffeine concentration: : *Inset* plot of  $i_{pa}$  vs concentration for acetaminophen left and for caffeine to the right

The limit of detection (LOD) was  $6.37 \times 10^{-8}$  M for acetaminophen and  $6.89 \times 10^{-8}$  M for caffeine. The plot of peak current against concentration produced a linear relationship. The statistical analysis was applied in the calculation of limit of detection by the least square regression method using excel linest formulae.

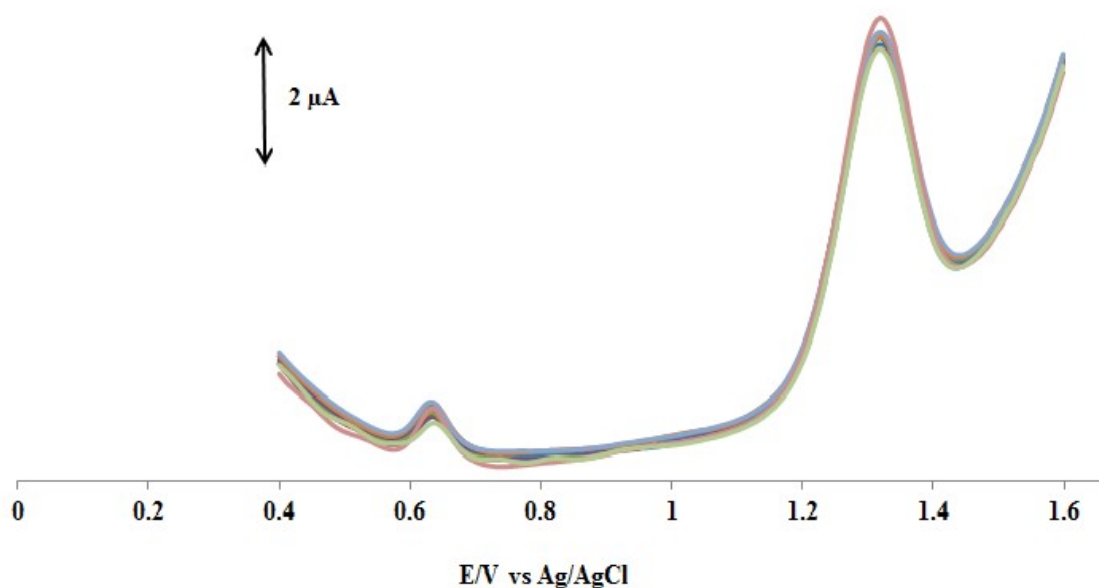
**Table 4.1:** Analytical parameters for electrochemical determination of caffeine and acetaminophen in 0.01 M  $\text{HClO}_4$  solution at pH1.



Number	Compound	LWR	LRE	R <sup>2</sup>	LOD	%RSD
Statistical data for individual molecules						
1	Caffeine	1 – 15 μM	I <sub>p</sub> (μA) = 0.2188(μM) + 2.6006	0.9939	3.52 x 10 <sup>-8</sup> M	0.77
2	Acetaminophen	1 – 18 μM	I <sub>p</sub> (μA) = 0.0042(μM) + 1.6974	0.9914	3.69 x 10 <sup>-8</sup> M	1.55
Statistical data for one ion when the concentration of the other molecule is constant						
3	Caffeine	80 - 260 μM	I <sub>p</sub> (μA) = 0.0017(μM) + 1.547	0.9966	5.88 x 10 <sup>-8</sup> M	1.01
4	Acetaminophen	0.5 – 25 μM	I <sub>p</sub> (μA) = 0.0222(μM) + 0.793	0.9921	4.04 x 10 <sup>-8</sup> M	0.9
Statistical data for all the two ions simultaneously						
5	Acetaminophen	10 – 90 μM	I <sub>p</sub> (μA) = 0.0085(μM) + 0.8921	0.9939	6.37 x 10 <sup>-8</sup> M	1.75
6	Caffeine	10 – 90 μM	I <sub>p</sub> (μA) = 0.0073(μM) + 1.6146	0.9922	6.89 x 10 <sup>-8</sup> M	1.8

### 4.13 Reproducibility

The newly developed sensor showed a slight decrease in the peak current after washing the electrode three different times with ethanol and water solution ratio one as to one. The relative standard deviation of 2.11 % and 4.1 % this indicated good repeatability of the which is below 5%[38].

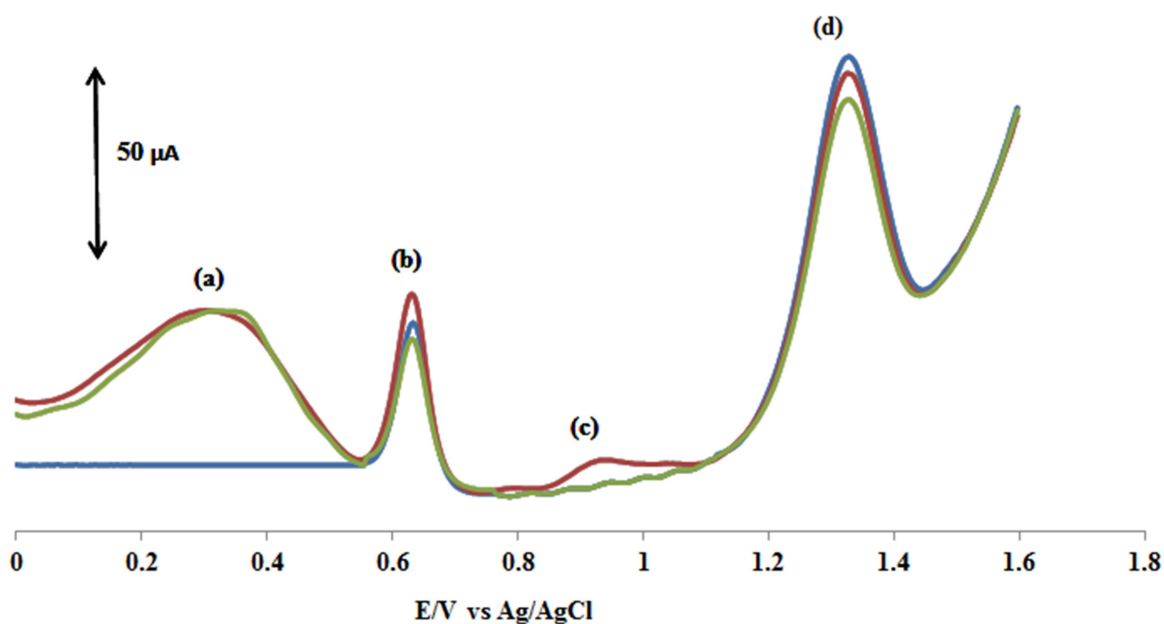


**Fig 4.15:** Differential pulse voltammograms for nine repetitions in caffeine-acetaminophen solution at ZnO/MWCNT/GCE electrode.

### 4.14 Interference

The selectivity of the ZnO/MWCNT/GCE modified electrode towards the detection of caffeine and acetaminophen was determined in the presence of three other different compounds in perchloric acid pH 1. The three species were ascorbic acid, sodium nitrite and citric acid. The

effect of these species was determined using DPV and it was observed that there no great significant change in the peak currents and peak potentials of the interfered species. The relative percentage response of the sensor calculated using the formulae  $(R\%) = [1 - (\frac{I^{analyte} + I^{inter}}{I^{inter}})] \times 100$ . All the interferences showed to have no effect as they had interference effect of less than 10%.



**Fig 4.16** DPV for (a) ascorbic acid, (b) paracetamol, (c) sodium nitrite and (d) caffeine.

The RSD % above 10 % shows that the compound interferes with the analyte [33]. The RSD (%) were 1.7 % for sodium nitrite and 2.2% for ascorbic acid.

## 4.15 Applications

### 4.15.1 Analysis of caffeine in cola drink

The DPV technique was employed for the standard addition method. In order to estimate the accuracy of the proposed analytical technique coca cola beverage drink was spiked with aliquots amount of caffeine standard. The average results for five replicate measurements with standard deviation are summarized in table 4.2.

**Table 4.2:** Results for determination of caffeine in cola drink (n = 5)

Samples	Added ( $\mu\text{M}$ )	Expected ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery (%)
1	0	-	2.8	96.2 $\pm$ 1.7
	5	7.8	7.3	
2	0	-	3.6	95.3 $\pm$ 1.3
	7	10.6	10.1	
3	0	-	5.2	98.6 $\pm$ 1.9
	9	14.2	14.0	

### 4.15.2 Analysis of acetaminophen in urine

Different concentrations of the acetaminophen standard solutions were added to the volumetric flasks, which were filled up to volume with 0.01M HClO<sub>4</sub> (pH 1.0). A volume of 5 mL of the solution was placed in a cell for determination and the results are summarized in Table4.3. The recoveries were found in the range of 96.2- 98.7% showing good efficiency of sensor in real sample analysis.

**Table 4.3:** Results for determination of acetaminophen in urine (n = 5)

Samples	Added ( $\mu\text{M}$ )	Expected ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery (%)
1	0	-	3.3	98.7 $\pm$ 1.2
	4.5	7.8	7.7	
2	0	-	1.9	96.2 $\pm$ 2.1
	8.5	10.4	10	
3	0	-	3.7	98.6 $\pm$ 1.6
	10.5	14.2	14.0	

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATION

#### 5.0 Conclusion

A new electrochemical sensor based on the electrodeposition of ZnO on modified multiwalled carbon nanotube glassy carbon electrode was successfully prepared, characterized and utilized for the electrochemical determination of caffeine and acetaminophen. The nano particles showed enhanced sensitivity of the new modified electrode this was achieved due to their ability capability to increase conductivity and an increase in electroactive surface area of the electrode. The new ZnO/MWCNT/GCE revealed good reproducibility, sensitivity and stability towards acetaminophen and caffeine determination without being affected from interference of some presence species like ascorbic acid, citric acid and sodium nitrite. The method showed to be simple and less time consuming for determination of both caffeine and acetaminophen with high accuracy and precision. The detection limit of caffeine was  $3.52 \times 10^{-8}$  M and  $3.69 \times 10^{-8}$  M for acetaminophen.

#### 5.1 Recommendations

Further studies can still be done in order to enhance the performance of this newly developed sensor. This can be achieved by employing new methods which effectively bind ZnO onto MWCNT surface and also better methods can be identified for the attachment of MWCNT onto electrode surface. As for determination more analytes can be analyzed simultaneous.

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## APPENDIX

### APPENDIX A: MATERIALS

#### List A1: Apparatus used for the study

Beakers, volumetric flasks, burette, spatula, sample bottles, funnels, measuring cylinders and syringes.

**Table A.1: Reagents and chemicals**

Name	Chemical formulae	Manufacturer	Concentration/mass
Caffeine	$C_8H_{10}N_4O_2$	ACE	0.001M
Distilled water	$H_2O$	MSU	-
Perchloric acid	$HClO_4$	Skylabs	0.1M
Hydrochloric acid	$HCl$	ACE	0.1M
Acetic acid	$CH_3COOH$	Cosmo chemicals	0.1M
Potassium ferricyanide	$K_3[Fe(CN)]_6$	ACE	5mM
Sodium hydroxide	$NaOH$	Skylabs	0.1M
Multiwalled carbon nanotubes	MWCNT	Sigma Aldrich	1mg/L
Ethanol	$C_2H_5OH$	Skylabs	99%
Di-potassium hydrogen phosphate	$K_2HPO_4$	Skylabs	17.41g
Zinc nitrate	$Zn(NO_3)_2$	ACE	0.05M
Potassium chloride	$KCl$	ACE	1M
Sulphuric acid	$H_2SO_4$	ACE	0.1M



**Table A.2: Instrumentation**

Name	Model	Manufacturer	Use
Analytical Balance	GA-110	OHAUS	Weighing
pH meter	Az-8601	OHAUS	pH measurement
PGSTAT	PGSTAT302F	Autolab	Electrocatalysis
Ultra-Sonicator	KQ-250B	China Corp	Ultra agitation

### Treatment of Glassware

Laboratory liquid soap was used for washing all glassware and they were rinsed using distilled water to remove contaminants and impurities.

## APPENDIX B

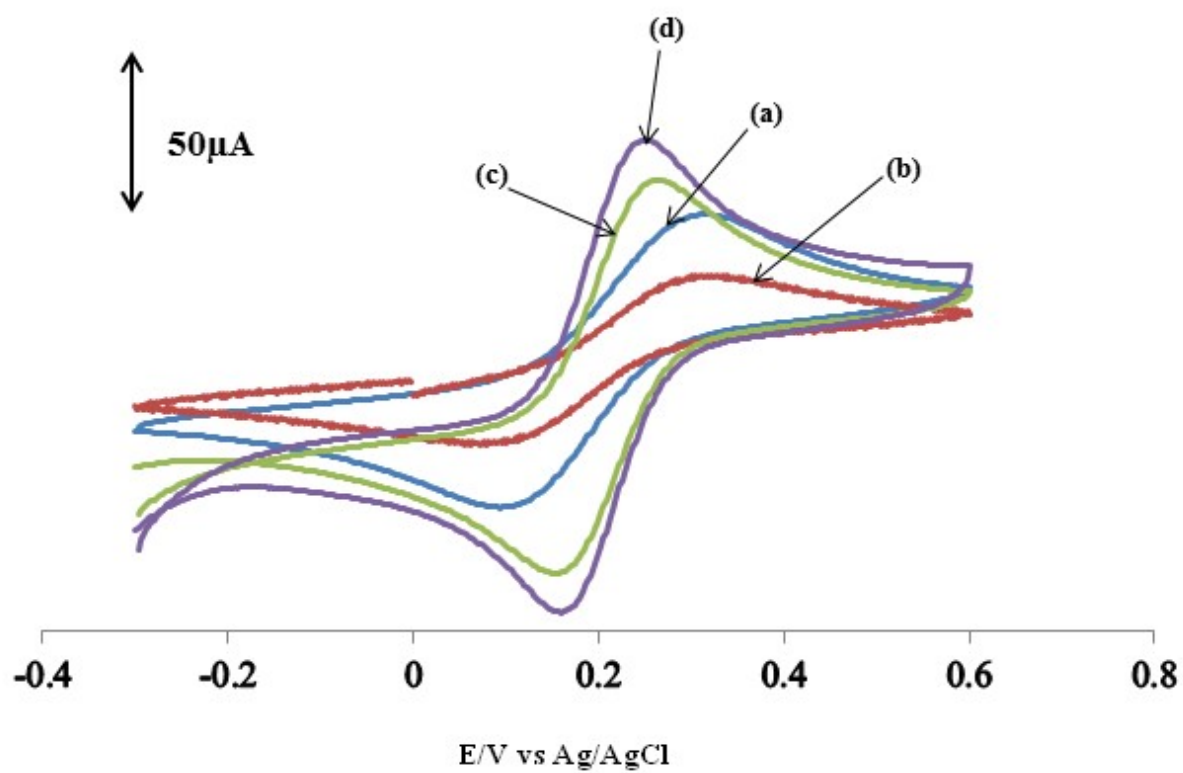
### B1: Surface area

Constant  $R = 8.314$ ,  $T = 273$  K

Randle-sevick equation

$$I_p = (2.69 \times 10^5)n^{3/2}AD^{1/2}V^{1/2}C$$

$$D = 7.6 \times 10^{-6} \text{ cm}^2/\text{s}, C = 1 \times 10^{-6} \text{ cm}^2, m = 1 \times 10^{-5} \text{ and } A = 0.15 \text{ cm}^2$$



Voltammograms for (a) GCE, (b) ZnO/GCE, (c) MWCNT/GCE and (d) ZnO/MWCNT/GCE in 5 mM potassium ferricyanide solution at 0.05 V/s at a potential of -0.3 to 0.6 V.

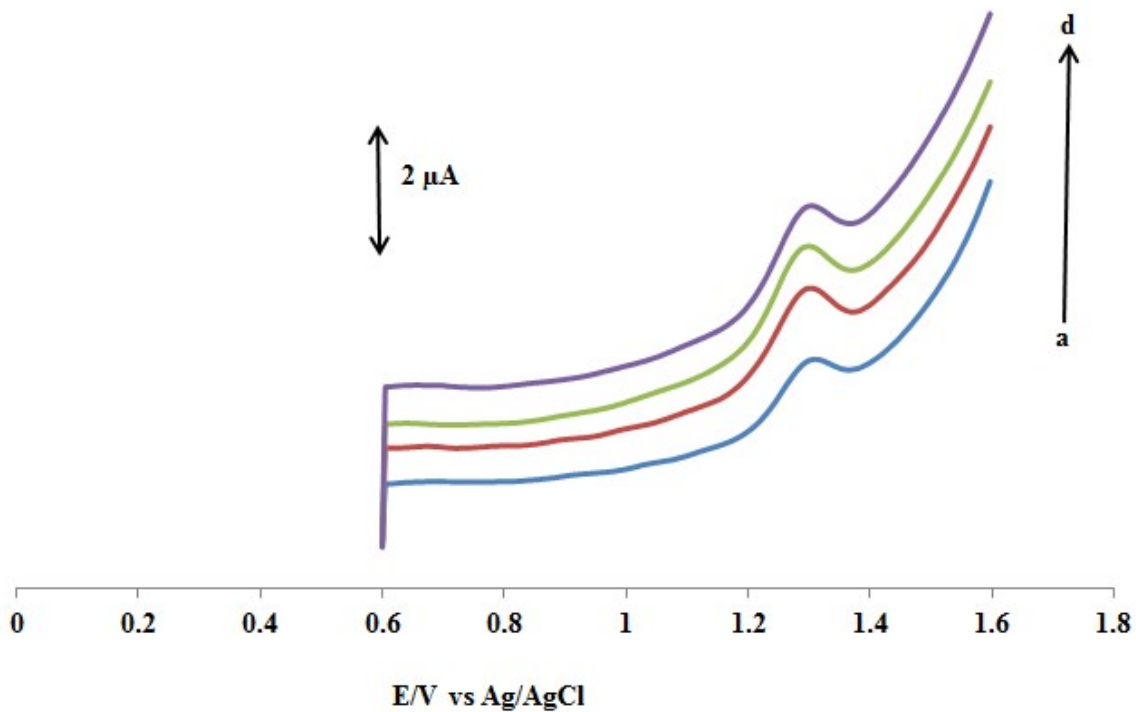
### B3: Limit of Detection and Limit of Quantification

#### Excel Linest function

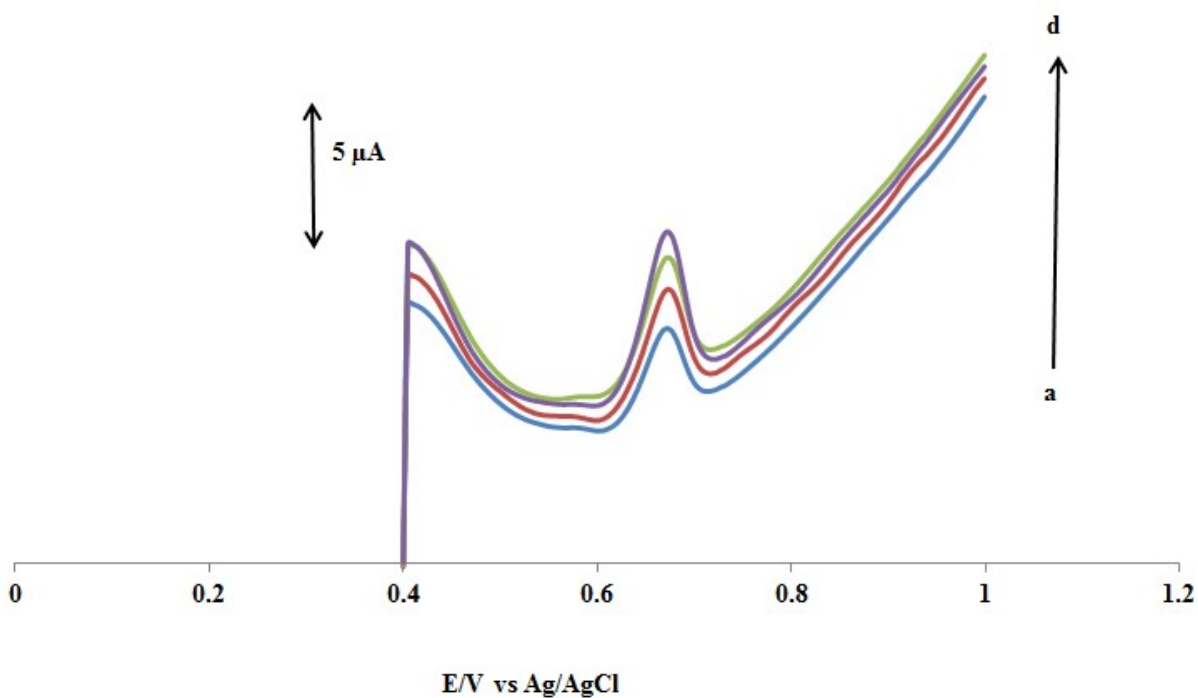
Slope	Intercept
Standard error of the slope	Standard error of the intercept
R <sup>2</sup>	Standard error in y

Limit of detection  $3.69 \times 10^{-8}$  M for acetaminophen and  $3.52 \times 10^{-8}$  M for caffeine.

### B4: Real sample analysis



DPV voltammograms for real sample analysis for caffeine (a) real caffeine sample in cola drink, (b) 5  $\mu\text{M}$ , (c) 7  $\mu\text{M}$  and (d) 9  $\mu\text{M}$  standard solutions for caffeine



Differential pulse voltammograms for real sample analysis for acetaminophen (a) real acetaminophen sample in human urine, (b) 4.5  $\mu\text{M}$ , (c) 8.5  $\mu\text{M}$  and (d) 10.5  $\mu\text{M}$  standard solutions for acetaminophen