Boron-doped Diamond Sensors for the Determination of Organic Compounds in Aqueous Media

By

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ABSTRACT

In electrochemical oxidation treatment of wastewater, the electrode material is an important parameter in optimizing oxidative electrochemical processes, since the mechanism and products of several anodic reactions are known to depend on the anode material. The electrochemical oxidation of benzaldehyde, nitrobenzene and m-cresol on bare boron-doped diamond (BDD) electrode was investigated. Oxidation intermediates were identified for benzaldehyde (Epa, 319 mV), nitrobenzene (Epa, 910 mV) and m- cresol (Epa, 1247 mV). Their oxidation was observed to be irreversible and detection was possible as individual compounds as well as within a mixture of the three compounds, with detection limits ranging from 1.02×10^{-6} M for m-cresol to 2.78×10^{-4} M for benzaldehyde. In a separate electrochemical investigation quercitin, catechin and rutin were catalytically determined at a cytochrome c modified BDD electrode. Cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) have also been used to investigate the electrochemical properties of quercitin, catechin and rutin on modified cytochrome c BDD electrode. BDD electrode was functionalized with 4-nitrophenyl diazononium salt layer under UV-curing, for a few minutes. Cytochrome c was then electrochemically immobilsed onto the functionalized BDD electrode by cyclic voltammetry. Oxidation and reduction reaction mechanism of each flavonoid was studied. There was one oxidation and reduction peaks for quercitin and catechin respectively, and two oxidation and two reduction peaks for rutin. The cytochrome c modified BDD electrode showed good sensitivity for all three flavonoids and low detection limits i.e. 0.42 to 11.24 M as evaluated at oxidation and reduction peaks, respectively.

KEYWORDS

Boron-doped diamond (BDD) electrode

Benzaldehyde

Nitrobenzene

m-cresol

Cytochrome c/Diazonium salt/BDD electrode

Quercitin

Catechin

Rutin

Cyclic Voltammetry

Electrochemical Impedance Spectroscopy

UV/Vis spectrosopy

DECLARATION

I hereby declare that the work 'Boron-doped Diamond Sensors for the Determination of Organic Compounds in Aqueous Media' which I now submit for the assessment on the programme of study leading towards the award of a masters degree is entirely my work; quotes and phrases obtained from the work of others have been fully acknowledged and referenced.

Signed:

…….

EUÒDIA HESS **Date: DECEMBER 2010**

DEDICATIONS

This article is dedicated to:

The Glory of our Heavenly Father

For His love and grace.

Parents

My father John and mother Myrtle, whose love and support carries me and has lifted me above the clouds of doubt, fear and mediocrity

My sisters, brother and niece

Donell, Anastasia, John-Carl and Jemca for their trust and encouragement.

My special and true friend

Aldridge Wallenstein, for his consistent support, guidance and encouragement.

The Church

For their persistent prayers and support.

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PUBLICATIONS

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CONTENTS

LIST OF ABBREVIATIONS

CV	Cyclic voltammetry
EIS	Electrochemical Impedance Spectroscopy
SWV	Square Wave Voltammetry
Ipa	Peak anodic current
Ipc	Peak cathodic current
$E^{0\prime}$	Formal potential
Epa	Peak anodic potential
Epc	Peak cathodic potential
Rct	Charge transfer resistance
PBS	Phosphate Buffer Saline
ΔEp	Difference in potential peak potentials
De	Diffussion coefficient
BDD	Boron-doped diamond
CDEO	Conductive-diamond electrochemical oxidation
ICE	Instantaneous current efficiency
OCC	Oxygen-equivalent chemical oxidation capacity
AOP	Advanced oxidation process
BAT	Best available technology
TOC	Total organic carbon

PAE	Phthalic acid esters
ECD	Electrochemical degradation
CB	Chlorobenzene
NB	Nitrobenzene
CVD	Chemical vapour deposition
AA	Antioxidant activity
DMF	Dimethylformamide
DA	Dopamine
LOD	Limit of detection
LPO	Lipid peroxidation
LTC	Low temperature coal carbonization
HTC	High temperature coal carbonization
HPLC	High performance liquid chromatography
TLC	Thin-layer chromatography
DMP	Dimethyl phthalate
DEP	Diethyl phthalate
MWCNTs	Multiwalled carbon nanotubes

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CHAPTER ONE

Introduction

In recent years there has been increasing interest in environmental damage and human injury by industrial pollution, and the relevant legislation is always being made harsher. Electrochemistry offers new and interesting approaches to industrial wastewater treatment: in particular, electrochemical combustion is a very attractive process for solutions in which, although the pollutant concentration is low, its presence makes the waste toxic. Despite certain advantages, such as the versatility of the process and the simplicity of the reactors in terms of construction and management (which makes them particularly suitable for automation), the practical application of electrochemical techniques to wastewater treatment has been limited by the difficulty in finding anode materials with specific characteristics to make the process economically competitive. Several materials have been proposed as anode, such as Ti/PbO_2, Ti/ SnO_2, Ti/IrO_2 or glassy carbon but some of these have shown loss of activity due to surface fouling (glassy carbon) or limited service life (Ti/SnO_2). A new electrode material has recently attracted attention because of its very promising characteristics it consists of a silicon support coated by a layer of synthetic diamond, heavily doped with boron to acceptable electrical conductivity. The characteristics of this material, such as hardness, stability up to high anodic potentials and the wide potential range over which discharge of water does not occur, make it an excellent candidate as anode in the oxidation of organic compounds (Polcaro AM, et al., 2003). Thousands of tons of pharmaceutical drugs are consumed yearly worldwide in human and veterinary medicine and

agricultural products. Since their waste wasters are inefficiently destroyed in municipal sewage treatment plant, a fairly large number of these compounds have been recently detected in surface, ground and even drinking waters at low contents up to micrograms per litre. The possible interactions of these emerging pollutants with living beings in the environment are not well documented, although it is known that some drugs can affect endocrine system of fishes, can exert toxic effects on algae and invertebrates and can favour the development of multi-resistant strains of micro-organisms. On the other hand, dyes consumed in large amounts by textile industries are lost up fifteen percent during dyeing process and disposed out in effluents. The need of avoiding the possible adverse effects of accumulation of pharmaceuticals and dyes in aquatic environment on the health of living beings makes necessary to search powerful oxidation methods to decontaminate their industrial wastewaters, thus restoring the quality of drinking waters. The liberalization of common aromatic drugs and dyes such as clofibric acid, indigo carmine and cresols by means of different electrochemical advanced oxidation processes (EAOPs) using boron-doped diamond anode and different catalysts have been accomplished (Brillas E, et al., 2008).

Oxidation of organic compounds on electrocatalytic materials like PbO_2, SnO_2, Ti/SnO_2-Sb_2O_5 and more recently boron-doped diamond electrodes, where the over potential for oxygen evolution is particularly high. For this kind of electrodes, very high current efficiencies may be obtained, and complete mineralization of organic compounds can be achieved. Phenols are probably the most extensively studied compounds in the field of wastewater treatment, as they are persistent pollutants with high toxicity that can be released in the wastewaters of considerable number of industries. Degradation of organic compounds such as benzoic acid 2-naphol and 4-chlorophenoxyacetic acid are some of the organic

compounds studied on BDD electrodes. A model for the determination of current efficiencies in the electrochemical oxidation of organic compounds was presented by Comninellis and Platner. In this model, the instantaneous current efficiency (ŋ) is calculated from experimentally obtained oxygen flow rate. ŋ can also be calculated by determination of the rate of chemical oxygen demand (COD) removal, during an electrolytic process, using the following relationship:

$$ŋ = [(COD)t – (COD)t+\Delta t / 8I\ \Delta t] \times FV$$

where $(COD)_t$ and $(COD)_{t+\Delta t}$ are the CODs at times t and $t+\Delta t$ (in mol O_2 dm^{-3}), respectively, and I is the current (A), F is the Faraday constant (96, 487 C mol^{-1}), V is the volume of electrolyte (dm^3). This equation makes no assumptions about the nature or number of components in the system, since the observed COD differences along the time are exclusively related to the degree of oxidation of the organic matter as a whole (Marao A, et al., 2004). Many papers have demonstrated that BDD anodes allow the complete mineralization-up to near 100% current efficiency-of large number of organic pollutants, such as carboxylic acids, benzoic acid, cyanides, cresols, herbicides, drugs, napthol, phenolic compounds, polyhydroxybenzenes, poly acrylates, surfactants, and real wastewaters. Comninelis and co-workers observed that independently of organic pollutant nature, current efficiency and the amount of intermediates were affected by local concentration of ·OH relative to organics concentration on the anode surface. In particular, for high organic concentrations or low current densities, COD decreased linearly, forming a large amount of intermediates, while instantaneous current efficiency (ICE) remained about 100%, indicating a kinetically controlled process. Conversely for low organic concentrations or high current densities,

pollutants were directly mineralised to CO_2 but ICE was below 100%, due to mass transport limitation and side reactions of oxygen evolution.

Table 1: Some examples of organic compounds oxidised on diamond electrodes

pollutant	experimental conditions	remarks	ref
carboxylic acids	i = 30 mA cm^{-2}; T = 30 °C; 1 M H_2SO_4	average current efficiency: 70 − 90%	155−158
benzoic acid	i = 7−36 mA cm^{-2}; 0.5 M $HClO_4$	oxidation intermediates: salicylic acid, hydroquinone, hydroxybenzoic acid	161, 162
cyanides	i = 36 mA cm^{-2}; 1 M KCN + 1 M KOH	95% of CN^- elimination after passage of 220 Ah/l	163, 164
o- and *p*-cresol	27 129 ≤ Re ≤ 42 631, i = 15−60 mA cm^{-2}	*o*-cresol is more recalcitrant than *p*-cresol	165
herbicides	i = 30−150 mA cm^{-2}; pH = 2−12, T = 15−60 °C	herbicides decay follows a pseudo-first-order kinetics	29, 66, 166−170
naphthol	i = 15−75 mA cm^{-2}; T = 30−60 °C, 1 M H_2SO_4	efficiency increases with naphthol concentration	16, 56
phenol	flow-cell, batch one compartment cell, bipolar trickle tower reactor	microwave and ultrasound enhanced phenol oxidation	171−175
phenols, chlorophenols, and nitrophenols	i = 15−60 mA cm^{-2}; 5000 mg/L Na_2SO_4 or 1 M H_2SO_4	without diffusion limitation the current efficiency is 100%	19, 25, 26, 176−182
phenolic compounds and Triazines	i = 50 mA cm^{-2}; impinging cell	efficiency of 100% up to the near-complete mineralization	183−185
mixture of phenols	i = 30 mA cm^{-2}; 0.1 M Na_2CO_3	model proposed for the degradation of mixture of organics	186, 187
polyhydroxybenzenes	i = 15−60 mA cm^{-2}	influence of temperature, pH and, supporting media was studied	188
polyacrylates	i = 1−30 mA cm^{-2}; 1 M $HClO_4$	initial current efficiency 100%	189
surfactants	i = 4−30 mA cm^{-2}; SDBS = 25−300 mg dm^{-3}	complete removal of COD and TOC	138, 190−192
tridecane dicarboxylic acid wastewater	E = 1.6−5 V, pH = 3.4−8.26, COD about 10 000 mg dm^{-3}	COD removal rate 99%, specific energy consumption 6.4 kWh kg_{COD}^{-1}.	159
malic acid, EDTA, and triethanolamine	i = 7−36 mA cm^{-2}; initial COD 1500−8000 mg dm^{-3}	current efficiency of 85−100%	160
olive mill wastewater	T = 25 °C; i = 30 mA cm^{-2}	complete mineralization with high efficiency	193−195
effluent of a fine chemical plant	i = 150−600 A m^{-2}; pH = 2−12; T = 25−60 °C	efficiencies of the process depend on the pH and the temperature	196
Ink-manufacturing process wastewater	i = 15−60 mA cm^{-2}; T = 25−60 °C	Oxidation favored by the formation of electrogenerated oxidants.	197
wastewater from the automotive industry	initial COD > 2500 mg dm^{-3}	current efficiency > 90% for COD values higher 500 mg dm^{-3}.	198
industrial effluent	i = 20−60 mA cm^{-2}; Q = 60 −180 dm^3 h^{-1}.	total mineralization of the aromatic sulphonated acids	35

BDD application for wastewater treatment has also been widely studied by the group of Canizares, they obtained complete mineralization of organic wastes, independently of their characteristics (initial concentration, pH, and supporting media) and operating conditions

(temperature and current density). They also found that, depending on electrolyte composition, the organics were oxidized on the

electrode surfaces by reaction with hydroxyl radicals as well as in the bulk of the solution by inorganic oxidants electro generated on BDD anodes, such as peroxodisulfuric acid from sulfuric acid oxidation:

$$2H_2SO_4 \rightarrow H_2S_2O_8 + 2H^+ + 2e^-$$

This group has recently compared conductive-diamond electrochemical oxidation (CDEO) with two other advanced oxidation processes, Fenton oxidation and ozonation, for the treatment of synthetic and real wastewaters. To compare the performance of different advanced oxidation process (AOP), they introduced a new parameter, namely, oxygen-equivalent chemical oxidation capacity (OCC), which quantifies, in arbitrary units, the oxidants added to the waste and is defined as the kg of O_2, which is equivalent to the quantity of oxidant reagents used in each process to treat 1 m^3 of wastewater. This parameter is related to the various oxidants used in the three advanced oxidation processes and can be calculated according to the following equations:

$$OCC\ (kgO_2m^{-3}) = 0.298Q\ (kAhm^{-3}) \quad (35)$$

$$OCC\ (kgO_2m^{-3}) = 1.000[O_3](kgO_3m^{-3}) \quad (36)$$

$$OCC\ (kgO_2m^{-3}) = 0.471[H_2O_2]\ (kgH_2O_2m^{-3})$$

where OCC is the chemical oxidation capacity and Q refers to the charge. The group of Brillas, demonstrated that anodic oxidation with BDD is very effective for complete mineralization of herbicides and pharmaceutical drugs. Experiments carried out in batch

under steady conditions using a flow reactor reveal that the removal of pollutants is practically pH-independent, but it gets faster with increasing herbicide concentration, current density, temperature, and liquid flow rate. They reported that herbicide decay follows a pseudo-first-order kinetics, and that these persistent organic compounds are mainly destroyed by reaction with •OH produced at BDD surface, while generated weak oxidants, such as H_2O_2 and peroxodisulfate ions, have little influence on TOC decay (Panizza M, et al., 2009).

Problem statement and research motivation

Industrial effluents containing toxic and recalcitrant organic compounds lead to severe environmental problems. Traditional methods, including, biological, physical and chemical treatment are ineffective, and thus a number of alternatives have been researched, such as supercritical water oxidation, photochemical and peroxide/UV treatment, etc. electrochemical degradation (ECD) is one of the alternatives for the degradation of these compounds, and is suitable for low volume application and environmental compatibility (Liu H, et al., 2008). For the oxidative degradation of organics in wastewater treatment, the aim is the complete oxidation of organics to CO_2 or the conversion of toxic organics to biocompatible compounds. The electrode material is an important parameter when optimizing such processes since the mechanism and the products of several anodic reactions are known to depend on the anode material. Different types of electrodes have already been employed such as graphite, Pt, activated carbon, carbon fiber, PbO_2, Ti/IrO_2, and SnO_2. At most of these electrodes compounds are poorly degraded and not converted to CO_2 and H_2O. Many researchers compared BDD degradation ability with traditional electrodes such as SnO_2, PbO_2, RuO_2 and IrO_2 and demonstrated that under all experimental conditions, BDD enables

the highest oxidation rate and current efficiency. To explain it best Comninellis and co-workers speculated that on BDD-which has inert surface with weak adsorption properties-electro generated hydroxyl radicals are weakly adsorbed and consequently more reactive toward organic oxidation. Furthermore, they assumed that OH action is extended to a 'reaction cage' in the vicinity of the electrode surface, rather than limited to the surface itself (Panizza, M., et al, 2009).

The introduction of more stringent pollution regulations, coupled with financial and social pressures for sustainable development, has pressed toward 'zero-effluent' processes, as well as to researching and developing new or more efficient wastewater treatment technologies. A basic principle in environmental culture is to reduce waste pollution to 'As low as Reasonably Achievable' (ALARA) levels. To ensure acceptable levels of environmental impact, however, wastewater systems also have to be engineered in accordance with the principle of 'Best Available Techniques Not Entaling Excessive Cost' (BATNEEC) (Panizza, M. et al., 2009)

Aim

The project will investigate the electrochemical interactions between bare Boron-doped (BDD) electrode and volatile organic compounds (benzaldehyde, nitrobenzene and m-cresol). It will also investigate the electrochemical interactions between modified BDD electrode and flavonoids.

Objectives

The following are the research objectives outlined:

- Electrochemical characterisation and impedance modelling of the volatile organic compounds in solution.
- UV/Vis analysis of volatile organic compounds in solution.
- Surface characterisation of modified BDD electrode using scanning electron microscopy.
- Electrochemical characterisation and impedance modelling of modified BDD electrode and flavonoids.

Thesis statement

The properties of Boron-doped diamond electrode make it an essential electrode for the oxidation of organic compounds in wastewater treatment.

Brief overview of Chapters

This thesis is broken down into seven chapters.

- Chapter one presents a general introduction, motivation, thesis statement and research aim and objectives.

- Chapter two introduces literature review of volatile organic compounds and flavonoids

- Chapter three is on the methods. This chapter features the list of materials used, the research design and methods. A theoretical treatise of the analytical techniques used is also presented. The experimental consists of a general section that explains procedures common to the work in general.

- Chapter four comprises of the results obtained and discussion of volatile organic compounds, benzaldehyde, nitrobenzene, and m-cresol.

- Chapter five comprises of the results obtained and discussion of flavonoids, quercitin, catechin and rutin.

- Chapter six overall conclusion and future work

CHAPTER TWO

2.1 Literature review of volatile organic compounds (VOC's)

In principle, any successful chemical oxidation should have its electrochemical counterpart. In practice the two techniques are not parallel at present but appear to be almost entirely complimentary, a situation which will assuredly change as knowledge of factors influencing chemical and electrochemical oxidation increases. The advantages of electrochemical oxidation include the following; convenience on work up, low cost and yield. In addition, owing to its complimentary nature, unusual reaction products may be obtained from the electrochemical technique. It was recognized that oxidations in aqueous media occur by reaction of the substrate with anodically generated atomic oxygen, hydroxyl radicals, or peroxide species. Oxidation of the organic material proceeds by chemical reaction with the oxide species (or even through the oxide layer, the layer rather than metal surface acting as inert electrode). Electrooxidation of aromatics in aqueous media still remains an art than a science (Weinberg NL, Electrochemical oxidation of organic compounds). For the oxidative degradation of organics in wastewater treatment, the aim is the complete oxidation of organics to CO_2 or the conversion of toxic organics to biocompatible compounds. The electrode material is an important parameter when optimizing such processes since the mechanism and the products of several anodic reactions are known to depend on the anode material. In previous work a generalized mechanism for the oxidation of organic in the

potential region of O_2 evolution was proposed. This mechanism explains the complete oxidation of organics to CO_2 by electrogenerated hydroxyl radicals on 'non-active' electrodes and the selective oxidation on 'active' electrodes. According to this mechanism, BDD, a 'non-active' electrode, is an ideal anode for the complete oxidation of organics to CO_2 for wastewater treatment. Few electrochemical studies have been made on synthetic BDD films with the goal of developing application in the electrochemical oxidation of organics for wastewater treatment. Results have shown that only reactions involving simple electron transfer are active on diamond electrodes in the potential region of water stability. For the oxidation reactions with more complex mechanism, complex oxidation reactions can take place on diamond electrodes only in the potential region of water discharge. Diamond has been successfully used for the anodic oxidation of cyanide and the cathodic recovery of heavy metals (Gandini D, et al., 2000). High quality BDD electrodes possess several technologically important properties that distinguish them from conventional electrodes, such as an extremely wide potential window in aqueous and non-aqueous electrolytes: in the case of high quality diamond, hydrogen evolution commences at -1.5V vs SHE and oxygen evolution commences at +2.3 V vs SHE, therefore the potential window may exceed 3V, corrosion stability in very aggressive media: diamond electrode morphology is stable during long-term cycling from hydrogen to oxygen evolution, even in acidic fluoride media, inert surface with low adsorption properties and strong tendency to resist deactivation, very low double-layer capacitance and background current. BDD anodes promote the production of weakly adsorbed hydroxyl radicals, which unselectively and completely mineralize organic pollutants with high current efficiency:

$$BDD + H_2O \rightarrow BDD\ (\cdot OH) + H^+ + e^-$$

$$BDD(\cdot OH) + R \rightarrow BDD + CO_2 + H_2O$$

Many papers have demonstrated that BDD anodes allow complete mineralization up to 100% current efficiency of large organic pollutants such as carboxylic acids, benzoic acid, cyanides, cresols, herbicides, drugs, napthol, phenolic compounds, polyhydroxybenzenes, polyacrylates, surfactants, and real wastewaters. Depending on the electrolyte composition, organics are oxidized on the electrode surface by the reaction with hydroxyl radicals as well as in the bulk of the solution by inorganic oxidants electrogenerated on BDD anodes, such as peroxdisulfuric acid from sulphuric acid oxidation:

$$2H_2SO_4 \rightarrow H_2S_2O_8 + 2H^+ + 2e^-$$

Many researchers has compared BDD degradation ability with traditional electrodes, such as SnO_2, PbO_2, RuO_2 and IrO_2, and they demonstrated that under all experimental conditions, BDD enables the highest oxidation rate and current efficiency (Panizza M, et al., 2009). Boron-doped diamond (BDD) electrodes outperform conventional electrodes in terms of high response reproducibility and long term response stability, morphological and microstructural stability at extreme anodic and cathodic potentials and current densities, chemical inertness, wide potential window and low background current. Combining the superior properties of BDD electrodes with the merits of biosensors, such as specificity, sensitivity, and fast response makes BDD electrode an ideal substrate for biosensors (Zhou Y, et al., 2009). Oxidative electrochemical processes promising versatility, environmental compatibility and cost effectiveness have a continuously growing importance both in selective organic synthesis and in degradation of organic pollutants. For the oxidative degradation of organics in wastewater treatment, the aim is the complete oxidation of organics to CO_2 or the

conversion of the toxic organics to biocompatible compounds. The electrode material is clearly an important parameter when optimizing such processes since the mechanism and the products of several anodic reactions are known to depend on the anode material. For example, the anodic oxidation of phenol yields hydroquinone and benzoquinone at Ti/IrO_2 anodes and mainly carbon dioxide at Ti/SnO_2±Sb_2O_5 anodes. In previous work a generalized mechanism for the oxidation of organics in the potential region of O_2 evolution was proposed. This mechanism explains the complete oxidation of organics to CO_2 by electro-generated hydroxyl radicals on `non-active' electrodes and the selective oxidation on `active' electrodes. According to this mechanism, boron doped diamond, a `non-active' electrode, is an ideal anode for the complete oxidation of organics to CO_2 for wastewater treatment (Gandini D, et al., 2000). Recently, boron-doped diamond (BDD) thin film coating on a p-Silicon substrate becomes a new electrode material, which has attracted much attention. BDD electrode has the advantages of wide potential window, low background current, stable dimension and mechanical properties, exhibiting high chemical inertness and extended lifetime. Moreover, as it is known, •OH can be generated both on Pt and BDD electrodes, although the interaction of •OH with the latter was lower, causing a higher O_2 evolution potential and a higher efficiency for mineralization if enough high current (or potential) is applied, which leads to an excellent removal efficiency for organic pollutants. Thus, BDD may be suitable for the application as an anode in the treatment of wastewater with high current efficiency and favorable total organic carbon (TOC) removal (Liu L, et al., 2009).

Aromatic compounds present in wastewater are highly hazardous pollutants produced in many industrial processes. The electrochemical oxidation of these pollutants is a promising technique for wastewater treatment. Several model aromatic compounds, polyaromatic

hydrocarbons, anilines and other benzene derivatives have been used to test the performance of different anode materials like platinum, porous carbon felts, Ti/PbO_2, Pb/PbO_2, Ti/SnO_2 and other DSA type electrodes. However the main problem in this process is the low current efficiency and low anode stability. Boron-doped diamond thin-films electrodes are emerging as excellent material for several applications, such as electrosynthesis, energy accumulation devices, electroanalysis, etc. These electrodes present some useful properties including high resistance to corrosion, high thermal stability, hardness, good electrical conductivity, etc. The electrochemical behavior of diamond thin films deposited on p-silicon substrates has been studied for its use on electrochemical oxidation of organic wastes (Montilla F, et al., 2002). In recent years many works have been published focusing on the application of boron-doped diamond electrodes to eliminate different kinds of pollutants, aiming at wastewater applications. Panizza and Cerisola review the subject in detail. Applications of BDD electrodes to eliminate persistent pollutants have focused on the degradation of dyes, phenols, and more recently textile auxillaries of low biodegradability. The use of BDD electrodes is very attractive for this kind of application, since chemical oxygen demand (COD) removal can be effected with very high current efficiencies using BDD as anodes, but especially due to the fact that complete mineralization of organic compounds, i.e., their complete oxidation to CO_2, can also be achieved. In order to obtain successful combustion of organic compounds, the applied potentials must be very high enough; otherwise polymerization reactions may take place, thus inactivating the electrode. At high potentials the combustion process involves indirect oxidation reactions, in the region very near the electrode, by $HO^{\cdot}$ Radicals, and also by strong oxidants generated by the oxidation of the supporting electrolyte, such as hydrogen peroxide, persulfate or peroxocarbonate (Pacheco MJ, et al., 2007). Phenolic compounds are common pollutants in many industrial wastewaters including those

coming from pesticides, dyes, pharmaceuticals and petrochemical industries. Most of these compounds are known by their high toxicity level and their persistence, and thus their recovery or their elimination is required prior to the discharge or reuse of the waste flow. Several technologies compete to treat industrial flow streams polluted with these compounds. When the waste that contains the phenolic compounds has a great purity and high concentration of the compound, recovery with the best available technology is recommended. If not, the destruction of aromatic compound is required. The best available technologies (BATs) to do this are the incineration for strongly polluted waste (high calorific power) and biological oxidation for biodegradable waste. Several technologies compete to treat other phenolic-polluted wastes, although the treatment costs increase strongly with them. Several works have been focused on the destruction of these synthetic wastes using different oxidation technologies such as chemical oxidation, advanced oxidation processes, catalytic wet air oxidation or electrochemical oxidation. Although they can be applied to a wide range of compounds, phenol and simple substituted phenols are frequently used as model pollutants in the study of the performance of different treatment technologies with synthetic wastewaters. It has been proven that all these technologies are able to reduce strongly the organic load and the toxicity of the waste. Within these technologies, electrochemical oxidation appears as one of the most promising technologies for the treatment of wastewater containing small amount of aromatic compounds. The use of new anodic materials like boron-doped diamond (BDD) has allowed achieving high efficiencies in the use of electric energy and, as a consequence to decrease strongly the operating cost of this technology (Canizares P, et al., 2005). The pollutants, especially organic compounds, released from industry to the environment are toxic to humans and other living organisms. The organic pollution is mainly due to the presence of polynuclear aromatic hydrocarbons, pesticides, etc.

Phenolic compounds in the wastewater stream mainly come from oil refineries, coal conversion plants, petrochemicals, polymeric resins, coal tar distillstion, pharmaceuticals etc. Phenols are the dominant organic contaminates in wastewater from coal conversion and coal cooking processes, and they generally comprise 40-80% of the chemical oxygen demand (COD). The phenolic compounds concentration is higher in coal carbonization wastewater which contributes about 8000mg/L^2. Phenolic compounds present in industrial wastewater are toxic and biorefractory in nature. Nowadays, various government agencies prescribe the legal limits and laws for toxic materials in the wastewater released from industries. Treatment technologies for phenolic waste are physical, chemical, biological and electrochemical processes (Rajkumar D, et al., 2003).

In recent years there has been increasing interest in environmental damage and human injury by industrial pollution, and the relevant legislation is always being made harsher. In this framework electrochemical methods offer a good opportunity to prevent and remedy pollution problems due to the discharge of industrial and sewage effluents. The electrochemical technologies have attracted a great deal of attention because of their versatility, which makes the treatment of liquids, gases and solids possible and environmental compatibility. In fact, the main reagent is the electron which is a ``clean reagent'' (Rajeshwar et al., 1994). As a consequence, the electrochemical methods and several applications, such as metal ion removal and recovery (Campbell et al., 1994; Armstrong et al., 1997; De Ponce and Pletcher, 1996; Widner et al., 1998), electrodialysis (Urano et al., 1984; Cherif et al., 1997), electrode ionisation (Auerswald, 1996; Ganzi et al., 1997) and, especially, destruction of toxic and non-biodegradable organics by direct or indirect anodic oxidation. Many studies

have been carried out on electro-chemical treatment of organic compounds and several anode materials have been tested. Smith De Sucre and Watkinson (1981) and Scharian and Kirk (1986) studied the oxidation of phenol at the lead dioxide anode, but complete Total Organic Carbon (TOC) removal was difficult. A reticulated glassy carbon anode was used for the oxidation of phenol, but they found a rapid decrease in the reaction rate due to the formation of insoluble and slow-to- oxidise products on the electrode surface. Recent studies demonstrated that the rate of phenol removal was much higher using high oxygen overvoltage anodes, such as Sb doped SnO_2, than using platinum ones. By using these anodes OH radicals are generated from the oxidation of water and organic compounds are completely degraded by reaction with adsorbed OH radicals. Besides direct oxidation, organic pollutants can also be treated by an indirect electrolysis generating chemical reactant to convert them to a less harmful product. Although a large number of electrogenerated oxidants can be used, such as Fenton's reagent or ozone, hypochlorite is the most traditional one and the most widely employed. The mechanism of its electrogeneration in basic solution containing chloride ions may be given by the following:

$$2Cl^- \rightarrow Cl_2 + 2e^-$$

$$Cl_2 + H_2O \rightarrow 4HOCl + H^+ + Cl^-$$

$$HOCl \rightarrow H^+ + OCl^-$$

Hypochlorite has been efficiently used in the treatment of landfill leachate and textile effluent, and in both cases the complete removal of ammonia and accompanying COD reduction was achieved. Other studies on synthetic wastewater containing formaldehyde showed that in the optimum condition formaldehyde can be removed by hypochlorite ions

electrogenerated on Sn, Pd, Ru, or TiO_2 anodes with a current efficiency near to 94%. (Panizza M, et al 2000).

In the recent years, there has developed great interest in the development of environmentally friendly electrochemical methods to degrade organic pollutants in wastewaters. Anodic oxidation and electro-Fenton are the most usual techniques, since they have high degradation effectiveness due to the electrogeneration of •OH as oxidant of contaminants. However, a limited number of papers have been published dealing with the destruction of several dyes by such electrochemical methods. The recent use of a boron-doped diamond (BDD) electrode in anodic oxidation has allowed the total decontamination of aqueous solutions of aromatics, whereas other conventional anodes (Pt, PbO_2, IrO_2, etc.) only lead to their partial degradation to carboxylic acid. Anodic oxidation with a BDD electrode then seems a promising technique for the treatment of wastewaters containing dyes. Large amounts of different dyes are used in the textile industries. However, about 15% of overall world production of dyes is lost during the dyeing process, being disposed out in the textile wastewaters. The release of such toxic, recalcitrant, and coloured industrial effluents in the environment is a dramatic source of esthetic pollution and perturbation of the aquatic life. To avoid the dangerous accumulation of dyes in the aquatic environment, research efforts are underway to develop powerful oxidation techniques for their removal in industrial wastewaters. Indigo is one of the oldest and most important dyes, mainly used in the dyeing of clothes (blue jeans) and other blue denim. Since its solubility in water is <2 mg l−1, indigo is transformed into more soluble products before industrial application. Its reaction with sulfuric acid yields indigo carmine, a common dye also used as food colouring, as indicator in analytical chemistry, and as a microscopic stain in biology. Recent studies have shown that indigo carmine reacts with

chemical oxidants as H_2O_2 catalyzed with transition metal ions, peroxodisulfate, and ozone. Direct ozonation of indigo carmine causes the breaking of its C=C double bond leading to the formation of two molecules of isatin 5-sulfonic acid. In contrast, Vautier et al. (2004) have reported that only indigo is formed when it is oxidized with hydroxyl radical (•OH) generated from an advanced oxidation process as TiO_2/UV. Under these conditions, it is almost completely mineralized to CO_2, with release of SO_4^{2-}, NH_4^+, and NO_3^- ions. Alizarin red, an anthraquinone dye, and Eriochrome black T, an azoic dye, have been used in textile dyeing for many years early antiquity, and they contain aromatic rings that make them difficult to treat with traditional processes, and although they are large molecules the functional groups are quite different. Electrochemical oxidation provides versatility, energy efficiency, amenability to automation, environmental compatibility and cost effectiveness, is a promising technique for the destruction of toxic or biorefractory organics in textile wastewaters. The overall performance of electrochemical processes is determined by the complex interplay of parameters that may be optimized to obtain effective and economical degradation of pollutants. The principal factors determining the electrolysis performance are electrode potential and current density, mass transport regime, cell design, electrolyte composition and temperature. Above all, electrode materials should be totally stable and exhibit high activity towards organic oxidation and low activity towards secondary reactions (e.g. oxygen evolution). Many studies have demonstrated that the complete mineralisation of organics can be obtained with high efficiency by direct electro-oxidation using only high oxygen overvoltage anodes such as SnO_2, PbO_2 and boron-doped diamond (BDD). With the use of these anodes, organics are oxidised to CO_2 by hydroxyl radical's electrogenerated from water discharge. Of these, SnO_2 and PbO_2 have the common drawbacks of a short service-life and the release of toxic ions, while BDD electrodes exhibit good chemical and electrochemical

stability, a long life and a wide potential window for water discharge, and are thus promising anodes for industrial-scale wastewater treatment. In fact, it has been demonstrated that many biorefractory compounds such as phenols, chlorophenols, nitrophenol, pesticides, synthetic dyes and industrial wastes can be completely mineralised with high current efficiency, even close to 100%, using BDD anodes (Saez C, et al., 2007). Electrochemical oxidation techniques have been applied to the treatment of effluents from industrial plants. These techniques have various advantages, for instance, easy control, mild operation conditions, amenability to automation, environmental compatibility, high efficiency, and low cost. As a high performance material with many excellent electrochemical features as wide potential, low background current, high oxygen evolution potential, and stable dimension and mechanical properties, boron-doped diamond (BDD) electrode has been arousing a lot of research interests, and is extensively used to electrochemical degradation of organic pollutants. Many of the biorefractory pollutants were decontaminated completely using BDD electrode. The electrochemical oxidations for naphthalenesulfonates, sodium dodecylbenzenesulfonate, and hexadecyltrimethyl ammonium chloride were studied, and these surfactants were mineralized completely. Alizarin red, eriochrome black T, indigo carmine, and other dye wastewaters were degraded by BDD electrode and the chemical oxygen demand (COD) removal efficiency was higher than 95%. Highly toxic herbicide or pesticide, such as 4-chloro-2-methylphenoxyacetic acid, 2-(4-chlorophenoxy) - 2-methylpropionic acid, 2-(4-chloro-2 methylphenoxy) propionic acid, diuron, and 3,4-dichloroanilin,were also mineralized completely by BDD electrode. Electrochemical methods for wastewater treatment mainly involve the direct and indirect electrochemical oxidation. The direct electrolysis is a process that organic pollutants are directly oxidized on the anode electrochemically with no intermediates. By indirect electrolysis, organic pollutants can be

degraded by generating in situ strong oxidative intermediates that convert these pollutants into less hazardous products. The main oxidizing agent is active chlorine compounds, such as gaseous chlorine, hypochlorous acid, and hypochlorite ions, which are anodically produced from original chlorides present in the wastewater. The active chlorine compounds can electrochemically oxidize organic molecules quickly. This process is irreversible due to the intense oxidative activity of active chlorine compounds. It is obvious that a much faster COD removal will be obtained by indirect oxidation owing to strong oxidative chlorine compounds formed during electrolysis. However, gaseous chlorine, hypochlorous acid, and hypochlorite ions produced during the indirect electrolysis are highly toxic and caustic. Moreover, gaseous chlorine can be easily interacted with organic molecules and form carcinogenic, mutagenic, and teratogenic halogen compounds. The efficiency of electrochemical oxidation is pertinent to electrode materials and supporting medium, and some studies have been performed in wastewaters containing low concentration of organic pollutants (Wu M, et al., 2009). Phthalic acid esters (PAEs), also known as phthalates, are commonly used as plasticizers, and in the manufacture of insecticide carriers, propellants, and cosmetics. Over the past few decades, about 80 kinds of PAEs have been produced by various industries. PAEs exist in a free state in plastic and other products, and so readily enter the environment, being detectable even in the Atlantic and Arctic Oceans. PAEs are relatively stable in the natural environment. They have a high octanol–water partition coefficient and hence may tend to bioconcentrate in animal fat, promote chromosome injuries to human leucocytes, and interfere with the reproductive system. Following the 1999 UNEP Protocol on Long-Range Trans boundary Air Pollution, a considerable body of knowledge has been built up concerning about the characteristics of PAEs in terms of bioaccumulation, toxicity, environmental degradation, and adverse affects on human health. Much recent research effort has been directed towards

determining effective methods for the degradation of PAEs. Several investigations have considered the biodegradation processes of PAEs and their by-products in aqueous media, and found that Pseudomonas and white rot fungus are microorganisms that are particularly efficient at degradation of PAEs. Immobilized bacteria and other bio-augmented measures have also been processed in order to strengthen the PAEs removal effect. It has been suggested that monoesters and phthalic acid are the main intermediates in PAEs degradation. Biodegradation experiments involving different PAEs have indicated that those with shorter alkyl chains such as dimethyl phthalate (DMP) and diethyl phthalate (DEP) are very easily biodegraded, whereas PAEs with longer alkyl chains are poorly degraded with some considered resistant to biological treatment. Another study of PAEs biodegradation found that the degradation process could take from several days to a few months and so be only capable of handling trace to minor concentrations of these compounds with risk of generating secondary pollution. Advanced oxidation processes (AOPs) of PAEs involve several treatment procedures that are characterized by the in situ generation of the hydroxyl radical (OH). These include hydrothermal oxidation, photochemical degradation with UV or UV/H_2O_2, photocatalysis with Fe (III) or TiO_2 as the catalyst, sonication methods, and electrochemical oxidation. Among these methods, electrochemical oxidation is a promising environmentally clean process that is versatile, energy efficient and can be automated. As part of the electrochemical process, organic compounds are broken down by means of the hydroxyl radical formed through water oxidation at the surface of a high O_2-overpotential anode. The reaction equation is as follows:

$$M + H_2O \rightarrow M(^{\cdot}OH) + H^+ + e^-$$

where M refers to the anode. As a consequence, the most important factors in implementing electrochemical technology for the effective degradation of organic pollutants are usually the electrode material and its stability. Numerous experiments have been performed to degrade organic pollutants through anodic oxidation with different electrode materials. Classical anodes such as Pt, graphite and oxide films including PbO_2, SnO_2, and IrO_2, have all been used for anodic oxidation but produce a poor degradation effect due to weak generation of hydroxyl radicals. Pt tends to form an adsorbed film on the surface which deactivates the anode and reduces the useful lifespan of the electrodes. Compared to conventional anodes, boron-doped diamond (BDD) anodes have the following advantages: extremely wide potential window, corrosion stability, inert surface, high O_2-overpotential, and strong oxidation capacity. BDD anodes have been demonstrated to give a superior performance than conventional anodes applied to the treatment of many kinds of organic compounds (Li H, et al., 2010). Residual dyestuffs, although only present in small amounts, are sources of aesthetic pollution and eutrophication of water bodies and thereby have to be removed from wastewater before it is discharged. At the moment, several biological, physical and chemical processes are used to treat dye effluents. However, conventional sewage plant treatments are usually ineffective for dye removal because of their high biochemical stability, their relatively high molecular weight and the presence of aromatic rings. Physical methods such as precipitation, coagulation, filtration, adsorption, although effective in decolourisation have the disadvantage of sludge formation which requires the regular regeneration of the adsorbents. Chemical oxidation with ozone, Fenton's reagent and advanced oxidation processes are quite expensive and present operational problems. For these reasons there has been increasing interest in the use of new methods such as electrochemical oxidation which has been proven to be a promising and attractive technique for the effective oxidation of

wastewater containing organic compounds. Many studies have demonstrated that the complete mineralisation of organics can be obtained with high efficiency by direct electro-oxidation using only high oxygen overvoltage anodes such as SnO_2, PbO_2 and boron-doped diamond anodes. With the use of these anodes organics are incinerated to CO_2 by hydroxyl radicals electrogenerated from water discharge:

$$2H_2O \rightarrow 2OH\bullet + 2H^+ + 2e^-$$

Of these, the SnO_2 and PbO_2 have the common drawbacks of a short service-life and the release of toxic ions, while BDD electrodes exhibit good chemical and electrochemical stability, a long life and a wide potential window for water discharge and are thus promising anodes for industrial-scale wastewater treatment. In fact, it has been demonstrated that many biologically refractory compounds can be completely mineralised with high current efficiency, even close to 100%, using BDD anodes. Other than by direct electrolysis, organic pollutants can also be removed electrochemically by indirect electrolysis, generating in situ chemical reactants that convert them to less harmful products. In this field, the main oxidising agent is active chlorine, in the form of gaseous chlorine, hypochlorous acid or hypochlorite ions, which is anodically produced from chlorides naturally present in the solution:

$$2Cl^- \rightarrow Cl_2 + 2e-$$

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

$$HOCl \rightarrow H^+ + OCl^-$$

In fact, the $Ti/TiRuO_2$, that is the core of the DSA anodes used for the chlor-alkali process, has high electrocatalytic activity for chlorine evolution, which is due to the surface redox

reactions taking place at transition metal ions that act as active sites for the absorption of chlorine atoms. The most suitable electrode materials for the in situ generation of active chlorine are based on a mixture of metal oxides (e.g. RuO_2, TiO_2, and IrO_2). These electrodes are used as the core of the DSA anodes used for the chloro-alkali process, because of their high electrocatalytic activity for chlorine evolution. Several authors have reported that it is possible to obtain the almost complete mineralisation of solutions containing model compounds with chlorine-mediated electrolysis and real wastewater, including textile and tannery effluents, landfill leachate and car wash wastewater (Panizza M, et al., 2006).

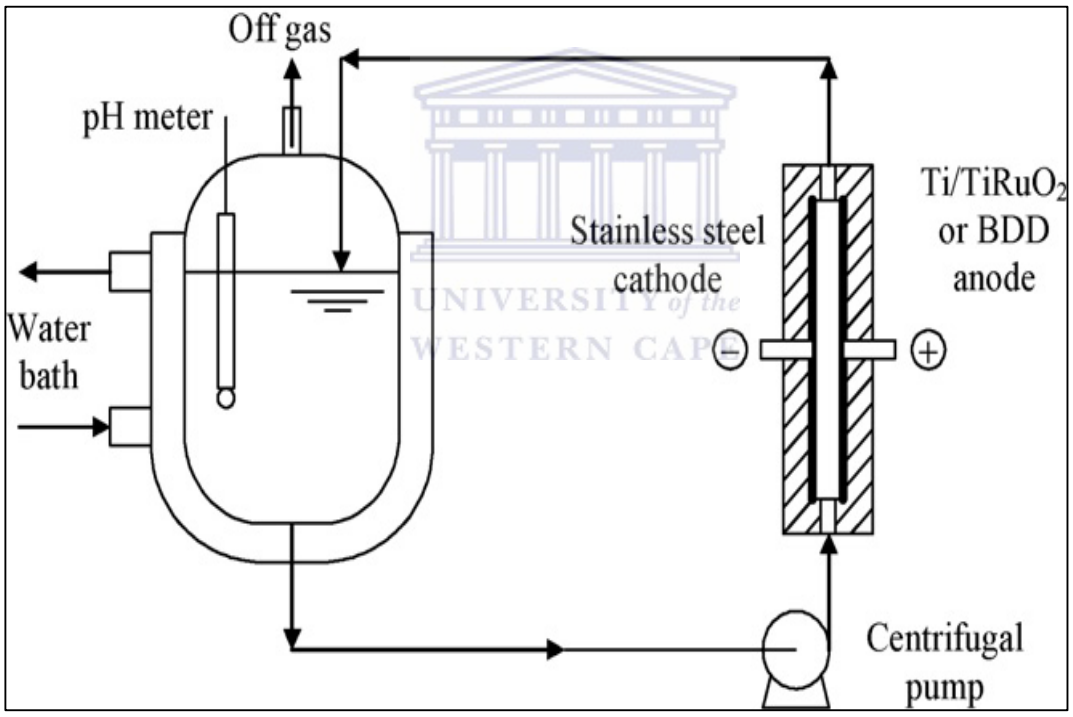

Figure 2. 1: Electrochemical cell for the oxidation of the methylene blue

In recent decades, halogenated aromatic hydrocarbons have been introduced to the environment from a variety of sources such as pharmacy, dyeing, chemosynthesis, petroleum refining and plastics. As a kind of typical harmful halogenated aromatic compound, chlorobenzene (CB) is widely found in many kinds of industrial wastewater. CB can accumulate in human body through food chain, which is obviously harmful. Cancer, teratogenesis, mutagenesis will be accordingly caused. Furthermore, it may also cause anesthetic effects and damage the central nervous system. Compared with aromatic compounds, halogenated aromatic compounds are more poisonous and more difficult to be treated by the ordinary biodegradation because of their high toxicity. Thus, it is of great importance and significance to find an efficient way to decontaminate them. Up to now, the degradation of CB was well investigated by several means, such as photocatalytic oxidation, Fenton oxidation, H_2O_2 oxidation, Mn catalytic oxidation and ultrasonic oxidation. Besides, electrochemical anodic oxidation should be taken into consideration as an effective and environmentally friendly process to clean up the halogenated aromatic hydrocarbons, owing to the advantages of strong oxidability, simplicity of operation and control and high efficiency. In aqueous system, electrochemical incineration of organic pollutants is assumed to follow two different pathways: direct oxidation at the electrode surface and indirect electrochemical oxidation mediated by appropriate formed aggressive oxidants (such as hydroxyl radicals, etc.). However, it has been reported that the hydroxyl radical (•OH) generation ability of different anodes varies a lot, resulting in the variation of degradation performances. Thus, it is of great importance to pursue a suitable anode for the efficient degradation. Pt electrode is widely used in electrochemistry and catalytic chemistry for its excellent electrochemical and catalytic properties. As is known, Pt has strong ability for dehalogenation, which is helpful to the degradation of halogen aromatic hydrocarbons.

However, the disadvantage of Pt is its relatively low oxygen evolution potential (ca. 1.2 V). Recently, boron-doped diamond (BDD) thin film coating on a p-Silicon substrate becomes a new electrode material, which has attracted much attention. Moreover, as it is known, •OH can be generated both on Pt and BDD electrodes, although the interaction of •OH with the latter was lower, causing a higher O_2 evolution potential and a higher efficiency for mineralization if enough high current (or potential) is applied, which leads to an excellent removal efficiency for organic pollutants. Thus, BDD may be suitable for the application as an anode in the treatment of wastewater with high current efficiency and favourable TOC removal. It has been reported that halogenated aromatic hydrocarbons could be easily dehalogenated by electrochemical reductive dehalogenation on Pt electrode and some other electrodes. Meanwhile, the electrochemical oxidative degradation of some halogenated aromatics has also been reported. Intensive and comparative investigations on the electrochemical oxidation and thoroughly mineralization of CB on BDD and Pt anode have not been reported yet. The differences of the kinetics behaviours and the reaction mechanisms between the two electrodes are still not very clear (Liu L, et al., 2009). Boron-doped diamond electrodes are passive in nature and do not interact or bind to organic pollutants nor do they catalyze the oxidation of pollutants presenting in the determined water samples. As (III) is known as a highly toxic and carcinogenic pollutant of the natural water even in ppb range of concentration. The oxidation of the As (III) turning it to less toxic As (V) by the oxygen dissolved in the water samples prior its laboratory determination yields analytical results not adequate to the real water toxicity. This fact defines the need of "in-situ" determination of the highly toxic As (III) and its distinction from As (V). The "in-situ" application of the spectral methods such as AAS and ICP is impossible because of the complicity of the employed equipment. On the other hand the AAS sensitivity to arsenic determination is not satisfying

and the two oxidation arsenic forms cannot be distinguished as well. Since, however only As (III) is electrochemically active the application of some of the voltammetric methods could solve the problem. A great variety of methods were developed for voltammetric As(III) determination most of them in acid media employing: direct cathodic reduction to As(0) ; direct oxidation to As(V); reduction-oxidation or reduction only after adsorptive accumulation realized as anodic or cathodic stripping voltammetry respectively allowing the achievement of very low LOD in the ppt range. A great variety of modified electrodes were reported as well applied for As (III) determination. Anodic Stripping Differential Alternative Pulses Voltammetry reported by the authors earlier providing high resolution was applied as an analytical method preventing the influence of interfering species having $E_{1/2}$ closed to that of As (III). The oxidation of the deposited arsenic on the electrode surface requires application of an electrode material providing excellent electrochemical resistance and wide anodic potential window. The mercury and silver are not suitable because the arsenic oxidation peak appears on the shoulder of their anodic oxidation and on the other hand the mercury is toxic. Au exhibit better characteristics than Pt in respect to the hydrogen over potential but some pretreatment procedure must be applied to keep the electrode surface free of oxide films greatly altering the kinetics of the electrode reaction. The BDD electrode characteristics however satisfy all the requirements for its application in As (III) determination. BDD electrode modified by gold was already successfully applied for anodic stripping determination of arsenic. In order to prevent the oxide films formation on the gold surface requiring complicated cleaning procedures application, the application of TiO_2 modified BDD for As (III) determination has been investigated. BDD electrode modified by TiO_2 usually employed as photocatalyst provides an additional advantage due to the adsorption properties of TiO_2 toward the As (III) allowing the sensitivity of the determination

increasing (Zlatev R, et al., Application of TiO_2 Modified Boron Doped Diamond (BDD) Electrode for As (III) Determination in Natural Waters, Engineering Institute of UABC). Semiconducting diamond materials have been investigated since 1952 following the discovery of naturally semiconducting diamonds by Custers et al. Ion implantation of natural, and later synthetic, diamond then became the focus of much research, as discussed in the 1975 review by Vavilov and observed in a number of Russian and Japanese publications in the subsequent decades. The significant step in the development and widespread use of boron doped diamond (BDD) was its fabrication via chemical vapour deposition (CVD) techniques to form a conducting polycrystalline thin film, introduced by Fujimori et al. in 1986. Investigations into the electrical and optical properties of these films soon followed, utilising cathodluminescence, electroluminescence, and Raman and IR spectroscopy. It was not until 1993 that Swain and Ramesham first noted the electrochemical potential BDD films held, with the first electroanalytical experiment performed by Tenne et al., also in 1993, involving the reduction of nitrate to ammonia. Since the addition of BDD electrodes to the electrochemist toolbox, the material has greatly advanced and become a significant asset. Now commercially available in a variety of forms and at varying levels of doping, the electrode is used in numerous areas of electrochemistry, ranging from analysis, to synthesis, and catalysis, as well as having significant application to waste water treatment, and uses in lithium batteries and as superconductors. Electroanalysis at BDD electrodes specifically has over 250 publications to date, including unmodified, pretreated, metal-modified, polymer modified, nanocrystalline and arrayed BDD electrode systems. A number of review papers regarding boron doped diamond have emerged over the last 15 years discussing their attributes analytically and electrolytically. A 2003 review by Marken et al. provides a general overview of the properties and types of diamond electrodes available to electroanalysis. More

recently Luong et al. have provided a concise and up-to-date review regarding the synthesis, electrochemical properties, analytical applications and brief overview of surface modification and functionalization of doped diamond electrodes, with Peckova more specifically reviewing the voltammetric determination of organic substances using BDD electrodes. Over the past decade, metal nanoparticle modification has developed to improve selectivity and sensitivity of BDD electrodes, increasing their diversity and application as electroanalytical sensors. A range of metal nanoparticle modifications and their analytical applications have been discussed. The modification of the essentially inert boron-doped diamond surface has been employed in order to introduce specific catalytic activity for example with platinum. The modification of boron-doped diamond with amine functionalities has been shown to dramatically change the reactivity towards oxygen. The deposition of stable deposits of iron and iron oxides is demonstrated to give a novel catalytic reactivity to boron-doped diamond electrode surfaces. Several previous studies have addressed the application of boron-doped diamond in stripping analysis and in particular in cathodic stripping voltammetry employing anodic deposition of metal oxides. Iron nanoparticles are known to be highly reactive and they have been employed in processes such as dehalogenation and remediation or nickel sequestration. Nanoscale zero valent colloidal iron has also been proposed for the removal of arsenic from drinking water. The use of iron nanoparticles deposited at electrode surfaces has been reported very rarely and the lack of this kind of approach has been pointed out in a recent review. In part this lack of study in this important field is due to the high reactivity of iron deposits and the rapid formation of inert oxide coatings. It is demonstrated that in the presence of fluoride both the high reactivity and the formation of oxide coatings can be utilized. Conventional anodic stripping voltammetry with trace amounts of iron is possible and iron deposits at boron-doped diamond electrodes are durable and catalytically very

active. Complexes of iron and fluoride are readily formed. The effect of fluoride anions on the surface chemistry of iron and iron oxides (hematite) has been studied (Saeza V, Catalytic Iron Metal Nanoparticles at Boron- Doped Diamond Electrodes: Sonoelectrochemical Deposition and Stripping, University of Alicante, Department Quimica) Electrochemical treatment, as one of the advanced oxidation processes (AOP), is able to convert organic materials into simple, relatively harmless, and inorganic molecules. In recent years, the electrochemical oxidation process has been shown to be promising for wastewater treatment, mainly due to its effectiveness and ease in operation, and for other applications like remediation of TBT contaminated sediments. The electrochemical methods find several applications, such as metal ion removal and recovery, electrodialysis, electrodeionisation, and especially, destruction of toxic and nonbiodegradable organics such as cyanide and EDTA. The main problem associated with such electrochemical treatment is its high cost. The use of new anodic materials like boron-doped diamond (BDD) has allowed achieving high efficiencies in the use of electric energy, and as consequence, to strongly decrease the operating cost of this technology. BDD anode has a high yield of hydroxyl radicals, which can facilitate degradation of organic compounds. It also has high chemical and mechanical stability. The over potential for water electrolysis of BDD is high so that the side reaction of oxygen evolution can be minimized, thus preventing loss of current efficiency. Different anode materials are studied to evaluate their suitability to eliminate organotins from shipyard process waters. The cyclic voltammetric response for dopamine (DA) has been studied at a variety of modified electrode surfaces. Well-characterized, DA is the most common excitable chemical neurotransmitter, and it plays an important role in the function of the central nervous system, renal, hormonal and cardio vascular systems. For example, the deficiency of DA can result in some grave diseases such as schizophrenia and Parkinson's disease. In

neurotransmission process, a selective binding of neurotransmitter with the specific receptor is very important. In in vivo binding strategy, hydrophobic aromatic interaction is an important factor that is responsible for selective binding in comparison with other factors, such as ionic interaction and hydrogen bonding. The development of micro/biosensors or modified electrodes is an attractive avenue for in vivo measurements of metabolites such as glucose, hormones, and neurotransmitters. Since metabolites can be stable for over a period of a few minutes, microbiosensors that can function in the tissues or bloodstream are powerful tools for clinical and neurochemical monitoring. In addition, they should provide the means to characterize the neurochemical microenvironment during nerve cell stimulation and pharmacological manipulations. Boron-doped diamond is a relatively new electrode material. Good quality, oxygen or hydrogen-terminated diamond thin-film electrodes exhibit properties well-suited for electrochemical studies. There are two types of diamond thin-film electrodes, micro- and nanocrystalline. Both types of films exhibit excellent electrochemical properties. We have exploited these properties and demonstrate that microcrystalline boron-doped diamond is a viable material for developing biosensors to study bioelectrochemical redox reactions in particular, the behaviour of DA. Ion exchange membranes of both anionic and cationic nature (e. g.: Nafion, poly(ester sulfonic acid), poly(4-vinylpyridine), etc have been developed to electrostatically accumulate/trap oppositely charged analyte molecules. In this context, the surface electrode modification with the cation-exchanger polymer Nafion has been largely used in electroanalysis it affords coatings that are chemically stable, present adequate antifouling ability towards surfactants and, at physiological pH, effectively incorporates DA, excluding anionic interfering species like ascorbate or urate anions. In addition, several investigations have studied the detection of DA at pH 7, mimicking physiological pH to determine if DA detection is feasible in biological fluids (Martínez-

Huitle CA, et al., Electrochemical behaviour of dopamine at Nafion®-modified boron doped diamond electrode: preliminary results University of Milan).

Benzene is a useful substance, widely used as a solvent, and also as precursor for many synthetic materials, such as: azo dyes, optical brightners, detergents, or concrete plasticizers. Unfortunately it is a highly toxic substance. At high levels of exposure, benzene acts on the central nervous system, causing drowsiness, dizziness, headaches, lightheadness, nausea, and decreased motor function coordination. With long-term low level exposure, its chronic effects include: impairment of formation of red and white blood cells and platelets, depression of bone marrow function, damage to central nervous system and induction of cancer. Many concerns have been expressed for the treatment of aromatic compounds, such as benzene, by traditional physical and chemical methods. Chemical remediation processes are expensive and can produce hazardous products. The treatment of benzene has encouraged the consideration of alternative or modified processes. One of these concerns is the anodic oxidation. The process of anodic oxidation of benzene is of environmental interest because it can be used as a model case for testing procedures for the removal of volatile carcinogenic compounds (benzene, toluene and xylene) from contaminated waters. The electrochemical oxidation of benzene could lead to the formation of useful organic compounds such as benzoquinone, which can be reduced to hydroquinone, thus providing an antioxidant for the fabrication of rubber and other products. Other major products are maleic acid and carbon dioxide. Metal and metal oxide electrodes can be selected for the study of benzene direct oxidation. Disadvantages of using metal and metal oxide surfaces as electrodes is that in some cases the products or by products formed between the combinations of benzene with

these materials are much more toxic than the original molecule itself (Oliveira R, et al., 2007). In relation to environment protection, the complete oxidation of organic molecules contained in pollutants becomes a crucial problem. The degradation of such organic pollutants, aldehydes, phenols and alcohols, has attracted considerable attention due to their high toxicity. The treatment at reasonable costs of hazardous wastewaters containing such pollutants is a difficult challenge. Among various techniques proposed, electrochemical oxidation is a possible alternative for eliminating organic compounds contained in water. It is a clean, attractive and one of the most environmentally friendly methods of treating industrial effluents. However, due to the fact that organic pollutants are generally present in low concentrations in industrial effluents, their complete oxidation to carbon dioxide is difficult. Among the molecules which need to be eliminated, aromatic compounds are the most difficult to oxidize due to their stability. Benzyl alcohol and benzaldehyde are amongst these stable molecules. Such compounds are harmful to aquatic organisms, and authorized emission levels are limited. Thus, it is crucial to reduce such emissions from industrial effluents. Among possible electrode materials able to oxidize benzyl alcohol only NiOOH electrocatalysts will be considered in this work. Nickel oxides are, among others, one of the most used electrocatalysts for the oxidation of alcohols and aldehydes. It is well known that the mechanism of oxidation of alcohols on Ni/ NiOOH depends on the position of the hydroxyl group in the molecule. The oxidation of primary alcohols at a Ni/NiOOH electrode in alkaline medium leads to the formation of organic acids. In the oxidation of secondary alcohols ketones are formed while tertiary alcohols are not oxidized. A general mechanism for the oxidation of primary alcohols was proposed by Fleischmann et al.:

$$Ni(OH)_2 + OH^- \rightarrow NiOOH + H_2O + e^-$$

$$NiOOH + RCH_2OH \rightarrow Ni(OH)_2 + RC^{\cdot} HOH$$

$$RC^{\cdot} HOH + 3OH^{-} \rightarrow RCOOH + 2H_2O + 3e^{-}$$

The electrochemical reactivity of benzyl alcohol was studied on platinum and palladium electrodes. Oxidation of the adsorbates formed from the alcohol adsorption led to CO_2 on both metals, but some reduction forming benzene was also observed at palladium (A.J. Motheo AJ, et al., 2006). The oxidation of aromatic alcohols to the corresponding aldehydes by sodium dichromate at elevated temperatures is a reaction that may be used for synthetic preparations that are difficult to accomplish using other oxidants. For example, Wakselman et al. described the preparation of 7-coumarincarbaldehyde in 84% yield by treatment of the corresponding alcohol with aqueous sodium dichromate at reflux temperature. The reaction is of particular interest because it is known that under acidic conditions primary alcohols are oxidized by chromium (VI) to give substantial amounts of carboxylic acids or esters unless the aldehyde can be distilled from the reaction mixture as it forms. Neutral chromium (VI) solutions do not oxidize aromatic aldehydes at an appreciable rate until considerably higher temperatures are attained (Donald G, et al., 1975). The reaction mechanism for the electroreduction of aromatic aldehydes, in particular, benzaldehyde, has been studied widely in aprotic solutions. It was found that the electron transfer to benzaldehyde is a fast process with an apparent rate constant of the order 0.1 cm s^{-1}. The reduction mechanism involves protonation of the resulting anion radical and dimerization. Nekrasov et al. suggested that the products of dimerization are pinacols and other substances with quinoid structure. The reduction of aromatic aldehydes in aprotic solvents has been studied in less detail. Anthoine et al. presented half-wave potentials of aldehydes in dimethylformamide (DMF) and found linear relationship between the energy of the lowest empty molecular orbital and the half

wave potential, $E_{1/2}$. They also found that the first reduction step is reversible in cyclic voltammetric experiments carried out at high sweep rates (20 Hz). Most papers are concerned with the reduction of benzaldehyde. Large scale electrolysis in DMF at potentials corresponding to the first polargraghic wave gave pinacol as main product. The kinetics of dimerization of benzaldehyde was investigated by Armstrong et al. in sulfolane. In the presence of 0.1 tetrabutylammonium perchlorate the second order rate constant was equal to the 2.4×10^3 M^{-1} s^{-1}.Kalinowski studied the reduction of benzaldehyde in DMF in the presence of alkali metal and found alkaline earth metals perchlorates and found a linear relationship between half-wave potential and the ionic potential. This result was attributed to ion pair formation between anion radicals and cations of supporting electrolyte with subsequent dimerization of the ion pair (Ronald Fawcett W, 1981). The use of ceric ion as oxidant in organic chemistry is well-known, in chemical literature with numerous applications, for the oxidation of side chain methyl groups to aldehyde groups in polycyclic aromatic hydrocarbons. The mediated electrochemical oxidation (MEO) process with cerium ions is also involved in the electrosynthesis process for the production of several carbonyl compounds commercially and for the destruction of various organic pollutants. Industrial applications of this oxidation process however are very limited, because of the high cost of the reagent and difficulties in disposing the effluent. Indirect electrochemical oxidation of p-methoxy-toluene to p-methoxy-benzaldehyde using ceric methanesulphonate was carried out under optimized conditions, and the yields achieved were 65-78% for the oxidation with continuous recycling and electrochemical regeneration of ceric was achieved with a current deficiency of 70-85% (Devadoss V, et al., Indirect Electrochemical Oxidation of p-Methoxy-Toluene to p-Methoxy-Benzaldehyde Using Ceric Methanesulphonate: A Scale-up Study, Central Electrochemical Research Institute, India).

Nitrobenzene is a priority pollutant and widely exists in water, soil, and sediments from a variety of industrial activities. Even at low concentrations, it presents a high risk to the environment and poses a great threat to human health. If the concentration of nitrobenzene in waste exceeds 2 mg/L, the waste can be declared hazardous. The degradation and detection of nitrobenzene are significant in public security and environmental protection, which also promote the development of detecting techniques for nitrobenzene. Several techniques have been developed for the determination of nitrobenzene, including high-performance liquid chromatography, gas chromatography, and UV-visible spectrophotometry. However expensive equipment, time consuming and complicated operations restrict their extensive application. Electrochemical methods are powerful techniques for organic and inorganic compound detection because they are highly sensitive, cheap, simple and convenient. Cathodic electrochemical detection of organic and inorganic compounds at different electrodes has been studied extensively (Luo L, et al., 2010). Nitrobenzene, is a major environmental pollutant because of its carcinogenesis and mutagenesis. The commercial uses of nitrobenzene are reduction to aniline, solvent, synthetic products of benzene, metal polishes, shoeblack, perfume, dye intermediates, plastics, explosives, pharmaceuticals, pesticides, and a combustible propellant. Therefore, the remediation of nitrobenzene in aqueous solution is of environmental concern because of its toxicity and quantity of its production. Many inexpensive and effective processes for water treatment are available because of the various chemical reduction treatments and advanced oxidation processes (AOPs) that have been studied for the degradation of nitrobenzene in aqueous solution such as FeO reduction, photocatalysis, photo-assisted Fenton oxidation, supercritical oxidation, and catalytic ozonation. Ultrasound is a very suitable method for the degradation of organic compounds in aqueous solution, specially, dual-field ultrasonic processors that are widely

used. It has been found that the degradation rate of pentachlorophenol by dual-frequency ultrasonic irradiation has the highest degradation when compared with those monofrequency ultrasonic systems, and the combined dual-frequency system has a synergistic effect that appears remarkably sensitive to frequency. Sonochemical degradation of *p*-nitrophenol in aqueous solution has been carried out to assess the ultrasound dual frequency effects (Zhao L, et al., 2009). Nitroaromatic explosives, which are derived from nitration of toluene, constitute a big problem at sites of military explosive production, ammunition testing and storage. Many nitroaromatic compounds accumulated in the environment are biodegraded by bacteria. Bioremediation can be defined as any process that uses microorganisms to return a polluted environment to its original conditions. Adding specific substrate is possible to induce or accelerate a determinate biodegradation process. This process is called stimulation. Since mechanical cleanup of nitroaromatic polluted areas is an economic issue, bioremediation is an important alternative. However, compounds like nitrobenzene have more than one biodegradation pathway. Comamonas sp.strain JS765 is able to oxidize nitrobenzene; on the other hand Pseudomonas pseudoalcaligenes strain JS45 is able to reduce nitrobenzene. Both degradation pathways lead to mineralization of the compound, some products of the reductive pathway are transient more toxic products (especially aniline). Many of this products are cytotoxic and/or mutagenic (Caravatti I, et al., 2007).

Cresols can be found in wastewaters from coal-conversion processes and from the production of phenolic resins and pesticides. Cresols are also well known disinfectants and sterilizers as raw materials for synthetic surfactants. They may cause chemical burns and dermatitis upon contact with skin. The oxidative degradation of cresols smoothly proceeded toward inorganic end products when a gaseous plasma generated by means of dc glow discharge was sustained

in contact with the surface of aqueous solution containing organic compounds (Tomizawa S, et al., 2006).Thermal decomposition of cresols in supercritical water in the absence of oxygen showed the rate of reactivity in order of o-cresol, p-cresol and m-cresol. Photocatalytic oxidation of cresols with ultraviolet light/titanium dioxide (UV/TiO_2) system showed complete removal after 2.5 hours of reaction time at pH 7. Microbial degradation of cresols by Pseudomonas sp. was reported by Ahamad and Kunhi. The rate of degradation of the three isomers was in order of o-cresol, p-cresol and m-cresol. Broholm and Arvin reported that cresols and other phenolic compounds in coal carbonization effluent were degraded under aerobic conditions and mixed nitrate and iron-reducing conditions. Electrochemical oxidation of cresols on glassy carbon electrodes modified with cobalt (II) phtalocyanine and cobalt (II) octabutoxyphalocyanine were reported for the electrochemical analyses of o-,m- and p-cresols (Rajkumar D, et al., 2003). A DNA biosensor has been constructed by immobilizing DNA on a glassy carbon electrode modified with multiwall carbon nanotubes (MWCNTs). Phenol, m-cresol and catechol showed noticeable inhibition towards the response of the electrode due to their interactions with the DNA (Zheng Y,et al., 2009). The electrochemical oxidation of cresols (particularly p-cresol) to hydroxybenzaldehydes is of industrial importance. Because the oxidation of cresols occurs at easily accessible potentials, voltammetry may be used in their quantification. However, as with other phenolic compounds, radicals produced following the oxidation of cresols at solid electrodes couple to form polymeric species which adsorb onto the electrode and deactivate it. There have been some reports on the modification of the electrodes using electroactive conducting polymers which have been shown to prevent such deactivation of the electrodes by oxidation products of cresols. Relatively stable response was obtained on glassy carbon electrodes (GCE) modified with poly(3-methylthiophene) for the detection of cresols and other phenolic

compounds. Horseradish peroxidase-modified graphite and carbon paste electrodes have been used as biosensors for the determination of cresols and other phenolic compounds. Metallophthalocyanine (MPc) complexes are known to be good electrocatalysts for many reactions. Cobalt (II) phthalocyanine (CoPc) complexes, in particular, have been used to improve the stability of GCE for the detection of cresols and chlorophenols. The oxidations of the three cresols are too close to allow the selective oxidation of one in the presence of the others. Further, the fact that m- and p-cresols have similar boiling points makes direct separation of the two by fractional distillation difficult. However, electrochemical separation of m- and p-cresol by selective oxidation could be possible if the oxidation of these cresols occurred at significantly different potentials (Grootboom N, et al., 2001). Phenolic compounds in the wastewater stream mainly come from oil refineries, coal conversion plants, petrochemicals, polymeric resins, coal tar distillation, pharmaceuticals, etc. Phenols are the dominant organic contaminants in wastewater from coal conversion and coal coking process and they generally comprise 40-80% of the chemical oxygen demand (COD). Shivaraman and Pandey have reported that phenolic concentration is higher in low temperature coal carbonization (LTC) wastewater as compared to that of high temperature coal carbonization (HTC) waste. The LTC waste contains about 2500 mg/L of phenol, 250 mg/L resorcinol, 5300 mg/L catechol, 480 mg/L o-cresol, 200 mg/L m-cresol, 470 mg/L p-cresol, 2000 mg/L pyrogallol, and 400 mg/L of xylenol. Wastewater from the oil shale treatment contains high concentration of phenol, p-cresol, and resorcinol. The ash dump wastewater from oil shale contains phenols in the concentration of 400-670 mg/L. Phenolic compounds are toxic to plants, fish, and many other organisms. Phenolic compounds inhibit the normal function of microbial population, thereby affecting the biological treatment process. Hence for the treatment of bio-refractory organic compounds, chemical methods of degradation might

provide better alternatives (Rajkumar D, et al., 2005). Different analytical methods were used to determine o-. p-, and m-cresols simultaneously present in solution, in particular, spectrophotometry at different wavelengths. This method does not necessarily give sufficiently accurate results and is time-consuming. HPLC on the reversed-phase adsorbents can separate the mixture into two peaks: one for o-cresol and another for the sum of m- and p-cresols. To solve analytical problem, HPLC with scanning UV-detection at different wavelengths, this approach is difficult as the absorbance spectra of cresols in the ultraviolet region are close to one another. A method using microscale HPLC using two detectors based on different physical principles and connected in series was used to determine different cresols (Ruban VF, et al., 2007).

2.2 Literature review of flavonoids

Basic introduction of the modifier cytochrome c

The classica1 viewpoint maintains that electron transfer and energy transduction by cytochrome c and cytochrome c oxidases are achieved by at least two different transition metals (copper and iron) organized in three distinct centres. The widely accepted ratio of Cu/Fe ratio is almost 1.5, which would suggest the presence of three copper ions per mole of functional unit in both eukaryotic and prokaryotic cytochrome c and cytochrome c oxidase. The Fe of cytochrome a_3 and CuB, are very close (< 5 Å) to one another, and form a magnetically coupled heterobinuclear centre. This implies that electronic changes at either metal centre during inhibition events may have an effect on the electronic environment of the

other metal centre (Malatesta 1995). Most biosensor systems in which cytochrome c protein or enzyme is used as biorecognition element relies on the inhibition of the heme site for the inhibition signal. Cyanide, a well known inhibitor of cytochrome c, is thought to bind to the CuB active site of the cytochrome oxidase, thus inhibiting electron transfer from the biomolecule to the metal electrode (Su, 2005). In an investigation of the reduction of ferricytochrome c at boron doped diamond, the structural unit responsible for the electron exchange was attributed to the haem c with a Fe(III/II) redox centre (Marken, 2002). Most recently Zn^{2+} has been shown to inhibit enzymatic activity of the mitochondrial and bacterial cytochrome c oxidase and the inhibitory effect was measured as enzyme turnover studies based on titrimetric and spectroscopic methods. The inhibition was associated with blocking of proton transfer pathways in the biomolecule and the requirement of a reducing environment was confirmed, since the Zn^{2+} binding group at the positively charged side of the enzyme is not accessible at the oxidised enzyme (Vygodina, 2008). The reduction of CuB is proposed to be responsible for facilitating Zn^{2+} binding. The reduction of CuB is associated with a rearrangement of its co-ordination sphere, from tetrahedral to planar (Tsukihara, 1996).

Flavonoids are naturally occurring phenolic antioxidants that are present in the human diet. They contribute to the antioxidant properties of green vegetables, fruits, olive and soybean oils, red wine, chocolate, and teas. They may be responsible for the protective effects of some diets against coronary heart disease. Some flavonoid derivatives are used as drugs mainly in vascular and hepatic diseases. Clark and Mackay's stated that flavonoids are poorly transferred from digestive lumen into the blood stream. Evidence now shows that some

flavonoids can cross the intestinal barrier into the bloodstream. Conjugates of a range of dietary phenolic compounds including catechin, quercitin and ferulic acid have been measured in the plasma with and without oral supplementation. Detected levels are extremely low in relation to the ingested amounts. The radical scavenging and the transition metal ion reducing activities of these phenolic compounds depend on their redox potentials. In addition, the antioxidant action is related to their ability to chelate transition metal ions. The flavonoid antioxidant activity efficacy in biological systems also depends on the partition coefficient between the liphophilic and aqueous phases, the binding to macromolecules, and the interaction with other antioxidants (Filipe P, et al., 2001). Many enzymes involved in intracellular signaling may be affected by flavonoids. The effects of flavonoids on protein kinesis are of importance since they directly influence immune functions in the host (Middleton and Kandaswami, 1992). Apart from the purely academic study of their natural occurrence, distribution, biosynthesis, metabolism, and function in plants, flavonoids are becoming of increasing importance in applied science. Flavonoids are commonly referred to as bioflavonoids. The term refers to a class of plant secondary metabolites. According to literature the IUPAC nomenclature, they can be classified into:

- Flavonoids, derived from 2-phenylchromen-4-one structure
- Isoflavonoids, derived from 3-phenylchromen-4-one structure
- Neoflavonoids, derived from 4-phenylcoumarine structure

Quercitin, one kind of flavonoids, is widely distributed in the plant kingdom. Many studies have revealed various beneficial effects on human health, including cardiovascular

protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti-inflammatory effects due to their polyphenolic nature and radical scavenging activity and metal-chelating properties, of which the former may dominate. Owing to their electrochemical activity, over the past few years, alot of studies were focused on this fields, for example, the electroanalysis of quercitin, the interaction of quercitin and DNA, the properties of quercitin and copper-chelating, the determination by capillary electrophoresis and HPLC using electrochemical detection, especially the literature reported that the electrochemistry of quercitin relates with the five hydroxyl groups, among these, the two catechol hydroxyl groups are electron-donating groups. But, it has been challenge to electroanalyze quercitin fast, convenient and sensitive methods till date. The electrochemical behavior of quercitin has been studied on multi-wall carbon nanotubes-modified paraffin-impregnated graphite disk electrode (Jin G, 2006). Quercitin is the aglycone (i.e., minus the sugar group) of a number of other flavonoids, including rutin, quercetrin, isoquercitin, and hyperoside. Activity comparison studies have identified other flavonoids as often having similar effects as quercitin, but quercitin usually has greatest activity. Its chemical structure contains the resorcinol group in the ring A (with the m-hydroxyls in the positions C5 and C7), the catechol group in the ring B (with the o-hydroxyls in the positions C3 and C4) and the hydroxyl group in the position C3 of the ring C, the carbonyl group in the position C4 in the ring C and C=C bond between the carbons C2 and C3 in the ring C. Quercitin concentration in onions ranges from trace amount in white to 2.5- 3 mmol kg^{-1} in red varieties in which it occurs as various O-β-glycosides with D –glucose as the main sugar residue. Analytical methods suitable for measurement of quercitin and its glucosides have mainly been based on HPLC with UV-detection and mass spectral characteristics, HPLC with diode-array detection, UV-spectra for identification, and HPLC with fluorometric or

electrochemical detection. The literature on the electrochemistry of flavonoids is limited. While most work has concentrated on the detection of flavonoids in biological samples by HPLC with electrochemical detection, little information can be found on their basic electrochemical properties. Flavonoids with 3.4,5 – trihydroxy (pyrogallol-type) and 3,4 – dihydroxy (catechol type) substitution patterns were the most easily oxidized, while flavonoids with 1,3,5 – hydroxyl (phloroglucinol-type) and phenol type substitution patterns were much harder to oxidize. The study of electrochemical behavior of flavonoids and flavonoid-rich extract by cyclic voltammetry (CV) is one of the most used techniques. Other voltammetric techniques, such as differential pulse voltammetry (DPV) and adsorptive stripping voltammetry (ASV), can also be used (Zeilinska D, et al., 2008). Various epidemiological studies have shown that quercitin and related isoflavonoids suppress cancerous tumor growth in vivo and in vitro. The results of electrochemical oxidation are relevant to interpret the anti-oxidant or estrogenic behavior of quercitin and other flavonoids. It is important to understand the chemical properties of quercitin under redox environment. Any structural and chemical similarities between the quercitin and synthetic estrogens can also provide insights into their mode of action. The electrochemical behavior of quercitin has been widely studied in different media including organic, aqueous, hydro-alcoholic or physiological media. The stability of the intermediate species resulting from oxidation reactions is substantially different depending on the surroundings, offering a very rich chemistry. The electrochemical behavior in biological-like conditions is relevant to get insight into the quercitin action as antioxidant. The overall cyclic voltammetric profile of quercitin, the effect of solution pH as well as the reversibility of each peak associated with oxidation of the five electroactive functional OH groups of quercitin has been investigated. Jorgensen et al isolated one characteristic product after electrolysis of quercitin in

acetronitrile, and this intermediate has been recognized as very stable and can serve as biomarker for anti-oxidant action of the compounds in vivo. Timbola et al. reported two other possible fractions by electrochemical oxidation quercitin in hydro-alcoholic solution but the absence of structural characterization coupled with low yield prevented further spectroscopic identification. The difficulties in isolating the oxidation products formed at each particular oxidation peak and the complex chemical reactivity associated with some of intermediates have not allowed a straightforward establishment of the reaction scheme or the correct identification of the intermediates. This often demands a knowledge and final judicious choice of combination of different separation and detection technique. The table below shows electrochemical oxidation products of quercitin (Zhou A, et al., 2007):

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Identified oxidative products following the electrochemical oxidation of quercetin

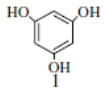
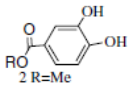
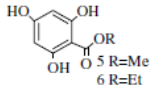
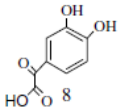
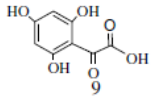
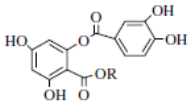
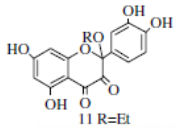
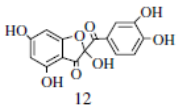
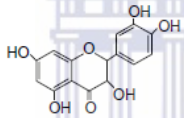
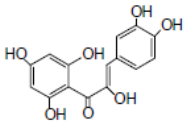
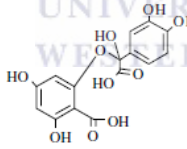
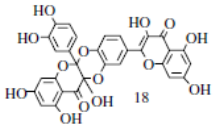
Name	Structural formula	Name	Structural formula
2,4,6-Benzenetriol	 1	3,4-Dihydroxybenzoic acid or ester (protocatechuic acid)	 2 R=Me 3 R=Et 4 R= H
2,4,6-Trihydroxybenzoic acid or ester	 5 R=Me 6 R=Et 7 R= H	3,4-Dihydroxy-phenylglyoxylic acid	 8
2,4,6-Ttrihydroxy-phenylglyoxylic acid	 9	2-(3,4-Dihydroxybenzoyloxy)-4,6-dihydroxybenzoic acid or Ethyl ester	 10 R= Et 13 R=H
2-(3,4-Dihydroxyphenyl)-2-hydroxy(ethoxy)-5,7-dihydroxy-chroman-3,4-dione	 11 R=Et 14 R= H	2-(3,4-Dihydroxybenzoyl)-2,4,6-trihydroxy-benzofuran-3-one	 12
Dehydroquercetin (Taxifolin)	 15	3-(3,4-Dihydroxy-phenyl)-2-hydroxy-1-(2,4,6-trihydroxy-phenyl)-propenone	 16
2-[Carboxy-(3,4-dihydroxyphenyl)-hydroxy-methoxy]-4,6-dihydroxybenzoic acid	 17	Dimer	 18

Figure 2. 2 : Electrochemical oxidation products of quercitin

Catechin is a representative of a large family of compounds that have the structure of flavones (2-phenyl-chromone) constituted by the two benzene rings (A and B) joined together by a c-pyrane ring (C-ring). The basic skeleton suffers a great number of substitutions in the natural compounds and over 4000 different flavonoids are known today. Many of these compounds exhibit antioxidant properties in a number of biochemical systems. Flavonoids

are present in body cells and fluids as a result of ingestion of fruits, vegetables, and plant-derived food and beverages, such as wine, tea and chocolate. Application of (+)-catechin as a phenolic standard in quantitation of reducing equivalents in food and biological fluids and its oxidation mechanism are therefore of particular interest. One of the most recent methods used to measure antioxidant activity of phenolic compounds is selective electro-oxidation by cyclic voltammetry. The applicability of electrochemistry only seems logical since the antioxidant power of a polyphenol in a reaction with a free radical is directly correlated to its ability to act as a reducing agent at an electrode. In antioxidant activity measurements, a wide span of pHs is of interest, including the stomach acid pH 2, wine pH 3.6 and physiological pH 7.4. The influence of pH and deprotonation reactions on the oxidation pathway and the antioxidant activity of phenolic compounds, determined by chemical or electrochemical methods has been recognized only in a limited number of papers dealing with the subject. Catechin is a weak polyprotic acid which, depending on the pH of the solution, may exist undissociated or in any of its anionic forms. The hydrogen or electron abstraction reactions of catechin may lead to the formation of various radical

$R(OH)_2 \rightarrow R(OH)^{\cdot+}_2 + e^-$ (electron abstraction)

$R(OH)_2 \rightarrow ROHO\cdot + H^+ + e^-$ (hydrogen abstraction)

$ROHO^- \rightarrow ROHO\cdot + e^-$ (electron abstraction)

$ROHO^- \rightarrow RO_2\cdot^- + H^+ + e^-$ (hydrogen abstraction)

Where, up to the physiological pH of the media, the parent molecules are catechin and/or phenolate monanions. The phenoxyl radical (ROHO·) formed in the first oxidation step is most likely to undergo a second oxidation step and form a more stable catechin o-quinone (RO_2). However, at physiological pH, the chemical and electrochemical oxidation pathway of catechin is complicated by the subsequent dimerization reactions. Both, enzymatic and non-enzymatic chemical oxidation of catechin produce o-quinone species that are prone to a nucleophilic attack by a catechin unit on the B-ring in a Michael-type addition whereby a dimeric product is formed. It has also been found that radicals formed by the one-electron abstraction from phenolate anions at an electrode, at physiological pH, may enter subsequent polymerization reactions rather than be further oxidized by a second-electron abstraction to more stable quinonic forms. Dimerization reaction is thought to proceed as an irreversible coupling of the two radicals or as an irreversible coupling of the phenoxyl radicals with the excess phenolate anions that yield a dimer radical anion. Dimer radicals can be immediately oxidized at the electrode surface and/or by electron exchange with a radical phenoxyl. In acidic solutions at low concentrations, the coupling reactions are significantly suppressed and o-quinone is the most abundant oxidation product. The motivation behind the present research was a desire to understand more fully the mechanisms of catechin electro-oxidation at various pHs with an emphasis on the molecular properties that dictate the oxidation pathway. It is hoped that such an improved understanding of phenolic oxidation would widen the areas of cyclic voltammetry application in determination of antioxidant activity.

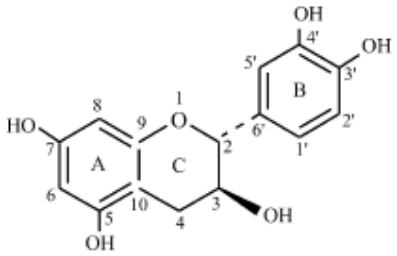


Figure 2. 3: Structure of Catechin.

(Martinez S, et al., 2005). The flavonoid (+)-catechin has two different pharmacophores, the catechol group in ring B and the resorcinol group in ring A and it has also the hydroxyl group at position 3 in ring C. Most of the problems encountered when describing catechin antioxidative activity are due to the lack of information on the intrinsic reactivity of each ring (A, B) and the lack of reliable thermodynamic constants. The A and B rings of (+)-catechin are not conjugated and ionisation of the OH groups of one ring system should not appreciably affect ionisation of the OH groups of the other ring. Hence, ionisations of OH groups of ring A are independent and distinguishable from those of ring B. It was concluded by spectroscopic methods that the influence of the A ring on the spectral properties of the radicals from the B ring is negligible in flavonoids where the C ring is completely saturated. Cyclic voltammetry has been used for the evaluation of antioxidant capacity of several polyphenols and their mixtures. The redox properties of polyphenols have been utilised as a measure of the antioxidant properties of wines on the basis of the measurement of the oxidation current at a constant potential by HPLC with electrochemical detection. So far, few attempts have been made to pursue a possible correlation between the oxidation potentials of antioxidants and their antioxidant activities. The phenolic groups of flavonoids can be electrochemically oxidised, and most flavonoids show an oxidation peak. Several antioxidants with two oxidizable moieties were studied by cyclic voltammetry and their free

radical scavenging activity against the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also determined. The mechanism of oxidation of catechins using electron spin resonance (ESR) data has been proposed, for (+)-catechin. Many published reports described the determination and quantification of catechins, using HPLC with ultraviolet, fluorescence, mass spectroscopy and electrochemical detection. The reduction potentials of flavonoids depend strongly on the electron-donating properties of the substituents in the B-ring (Janeiro P, et al., 2004). Catechins, the main constituents in green tea, have attracted lasting interest because of their wide biological effects. Recently, the antioxidant activity (AA) of catechins has been widely reported. Catechins effectively suppressed lipid peroxidation (LPO) in biological tissues and subcellular fractions such as mitochondria, microsomes, liposomes, and low density lipoproteins (LDL). On the other hand, they exhibited scavenging activity on free radicals, such as 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical, superoxide anion, hydroxyl radicals, and singlet oxygen. However, absolute as well as relative efficiency of catechins seems to vary from one assay to another, thus obscuring the structure–activity relationship. The specific mode of inhibition of LPO by catechins is not clear; they may act by chelating transition metals to inhibit the decomposition of lipid hydroperoxide (LOOH), or by scavenging chain-propagating peroxyl radicals, because the reduction potential of catechin radicals was reported to be lower than those of alkylperoxyl and hydroperoxyl radicals (Yang B, et al., 2001). Flavonoids are benzo-γ-pyrone derivatives containing several hydroxyl groups attached to the C6–C3–C6 ring and are found extensively in nature, in seeds, fruits and vegetables. The antioxidant activities of flavonoids are much higher than that of vitamin C and E. Catechin is the most important members of flavonoids. It has been acknowledged to be an antioxidant and radical scavenger. Therefore, catechin has been linked with many health benefits including prevention of DNA damage due to oxidation and improvement in

blood flow and liver function. On the other hand, catechin is susceptible to autoxidation to generate free radical intermediates, and active oxygen species, such as O_2 •− and hydrogen peroxide (H_2O_2) to induce fatty acid peroxidation, DNA damage and diseases. Catechin has been separated by high-performance liquid chromatography (HPLC) from Oolong tea, green tea, black tea, redwine vinegar and the seeds of red grape. It is constituted by benzene ring A and B. There is much evidence that the catechol group in B ring is the antioxidant active moiety. Traditional catechin analysis is mainly carried out by instrumental analysis, such as thin-layer chromatography (TLC), capillary electrophoresis (CE), HPLC-UV. However, such analysis is generally performed at centralized laboratories, requiring extensive labour and analytical resources, and often results in a lengthy turnaround time. Thus it is very important to establish a simple, fast, sensitive and low cost method for monitoring catechin. The electrochemical analysis has many advantages over conventional methods. And the sensitivity and selectivity of electrochemical analysis can be enhanced using chemically modified electrodes. Hence, various electrochemical modified electrodes have been developed for the determination of catechin. Wu et al. successfully investigated the characteristics of electrochemical reduction of the intermediate produced in the process of catechin autoxidation using Ru(bpy)3 3+-modified oxidized BDD electrode. Jarosz-Wilkołazka and co-workers immobilized laccase on the surface of graphite electrode for determination of catechin. El-Hady fabricated HP-β-CD incorporated carbon paste modified electrode for simple monitoring of catechin in some commercial drinks and biological fluids. Moreover, in the previous works, the oxidation mechanisms of catechin have been explored via electrochemical methods. All these methods could not reach a low enough detection limit. And the way to fabricate the modified electrodes was comparably complicated. Therefore, a low and simpler analytical method is urgently required. The subtle electronic properties

suggest that carbon nanotubes have the ability to promote electron-transfer reactions when used as an electrode material in electrochemical reactions (Yang L, et al., 2009).

The naturally occurring compound rutin (quercetin-3-O-rutinose,) is one of a range of bioactive flavonoid compounds, which are present in substantial amounts (0.5–1.5%) in plants. Fruits and vegetables contain so much flavonoid that human daily intake of these compounds was estimated to vary from 50 mg to 1 g. The literature on the electrochemistry of flavonoids is limited. However, Hendrickson et al. investigated the electrochemical properties of four structurally related flavonoids, quercetin, quercitrin, rutin and luteolin by using cyclic voltammetry and rotating ring-disk voltammetry. Hemoglobin consists of four polypeptide chains, each with one heme group. It is an important respiratory protein in red cells, being a carrier of oxygen. In addition, it is involved in many clinical diseases such as leukemia, anemia, heart disease, excessive loss of blood, etc. There have also been some reports about its direct electrochemistry at solid electrodes or modified ones. The nature and dynamics of binding small molecules to biomacromolecules represent an active area of investigation. Studies directed towards the design of site- and conformation-specific reagents provide routes towards rational drug design. As a kind of biochemical antioxidant, rutin can protect against hemoglobin oxidation inside human red blood cells in the presence of primaquine. The major focus of this article is on the electrochemical behavior of the interaction of rutin with hemoglobin. Some interesting results have been obtained, and the proposed method may be useful for analysis of clinical samples (Bao X, et al., 2001). Rutin, called as vitamin P, is a kind of flavonoid glycoside which widely presents in plants such as Flos Sophorae buds and the leaves of some species of plants. Rutin is also an electroactive compound, and its molecular. Some related investigations showed that rutin has a wide range

of physiological activities such as anti-inflammatory, antitumor and antibacteria. Therefore, it can be used in the treatment of diseases characterized by capillary bleeding with increased capillary fragility. Hence, it is necessary to develop some simple, economical and efficient methods for the determination of rutin in pharmaceutical preparations, crude drugs and so on. Some analytical methods, such as capillary electrophoresis, chemiluminescence, HPLC, sequential injection analysis and spectrophotometry have been applied to the determination of rutin. However, those methods are somewhat high-cost and complicated. Compared with these methods, electrochemical method has the advantages of fast, economic and simple. Therefore, the electrochemical determination of rutin attracts great interests. In addition, electrochemical sensors can be fabricated to extremely small dimensions and is an ideal placement directly into biological samples with minimal damage to tissues. Rutin was has been determined using MWNTs-IL gel modified electrode with the characteristics of sensitive, rapid and simple.

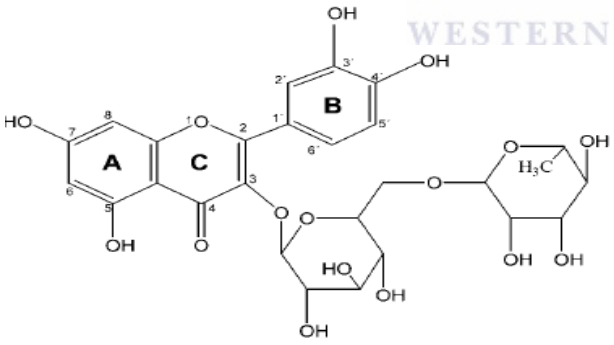


Figure 2. 4: Structure of Rutin.

(Liu X, et al., 2010). The study of the interaction of DNA with small molecules such as drugs, organic dyes and metals has been an intensive topic for decades because it provides insight into the screening design of new and more efficient drugs targeting to DNA, which can speed up the drug discovery and development processes. The recognition of DNA binders involves a complex interplay of difference interactive forces. It includes hydrophobic interaction along the minor groove of DNA, strong electrostatic interaction arising from the exterior sugar-phosphate backbone and intercalative interaction between the stacked bases pairs of native DNA from the major groove. A variety of analytical techniques have been developed for the characterization and identification of the interaction between DNA and small molecules with relative advantages and disadvantages. However, most of these methods suffer from high cost, low sensitivity and procedural complication. Up to now, electrochemical methodologies have attracted appreciable attention due to the inherent specificity and high sensitivity. Direct monitoring, simplicity and low cost facilitate to investigate the drug-targeting compound interactions and obtain the quantitative analysis information in pharmaceutical formulations and biological fluids. On the other hand, the electrochemical system serves as a versatile and illuminating model of biological system in an approach to the real action occurring in the living cells in vivo. The interaction mechanism can be elucidated in three different ways, involving the use of drug- or DNA-modified electrodes and interaction in solution. Owing to the antioxidant ability of flavonoids, they play an important role in inhibiting DNA and cells from oxidative damage, mutagenesis and carcinogenesis. Thereby they possess a broad range of pharmacological activities, such as anticancer, antibacterial, enzyme inhibitory, and pro-or antimutagenic properties. As flavonoids are electro-active, the electrochemical characters of most active components in flavonoids were investigated and reported in literatures. Brett and her co-workers investigated

the electro-oxidation of quercetin, catechin and rutin using various voltammetries and revealed that flavonoids can be oxidized via a complex and pH dependent electron transfer process. Volikakis and Efstathiou determinate 12 flavonoids using adsorptive stripping voltammetry in a flow injection system using nujol-graphite and diphenylethergraphite paste electrodes. Rutin, is a kind of the most abundant natural flavonoids, also the mainly active component of many natural Chinese traditional medicines. Ghica and Brett previously reported the electrochemistry of rutin, proposing that the oxidation of rutin is pH dependent adsorption process at glassy carbon electrode (Tian X, et al., 2008). Xu et al. used a 2-aminoethanethiol self-assembled monolayer modified gold electrode for the direct determination of rutin without the interference from coexisting ascorbic acid. Wei et al. Fabricated a CeO_2 nanoparticle modified electrode for the rutin determination and demonstrated a strong catalytic effect of nanoparticle towards electrochemical oxidation of rutin. Mousty et al. investigated the feasibility of amperometric detection of rutin at a biosensor using polyphenol oxidase (PPO) and obtained a better sensitivity for rutin. Room temperature ionic liquids (RTILs) are entirely composed of ions and exist as liquids at room temperature with the characteristics of negligible vapor pressure, good solubility and chemical stability. As a new "green" media, RTILs have many unique electrochemical properties such as high ionic conductivity and wide electrochemical windows. RTILs modified carbon paste electrode (IL-CPE) was used for the investigation of electrochemical behavior of rutin. It was found that IL-CPE showed good electrocatalytic activity for the oxidation of rutin with the increase of the redox peak current in the phosphate buffer solution (Sun W, et al., 2008). It was found that CeO_2 nanoparticles modified electrodes display good electrochemical catalytic activity for the oxidation of rutin in a phosphate buffer (pH 6.5). Cerium oxide (CeO_2) nanoparticles exhibit attractive properties in many applications,

including luminous material, catalysts, electronic ceramics, and glass polisher. There are many reports on the synthesis of CeO_2 nanoparticles, yet the use of CeO_2 nanoparticles modified electrodes as electrochemical sensors has rarely been reported (Wei1 Y, et al., 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter consists of the following:

- Materials: Information on all the materials used.
- Methodology

3.2 Materials

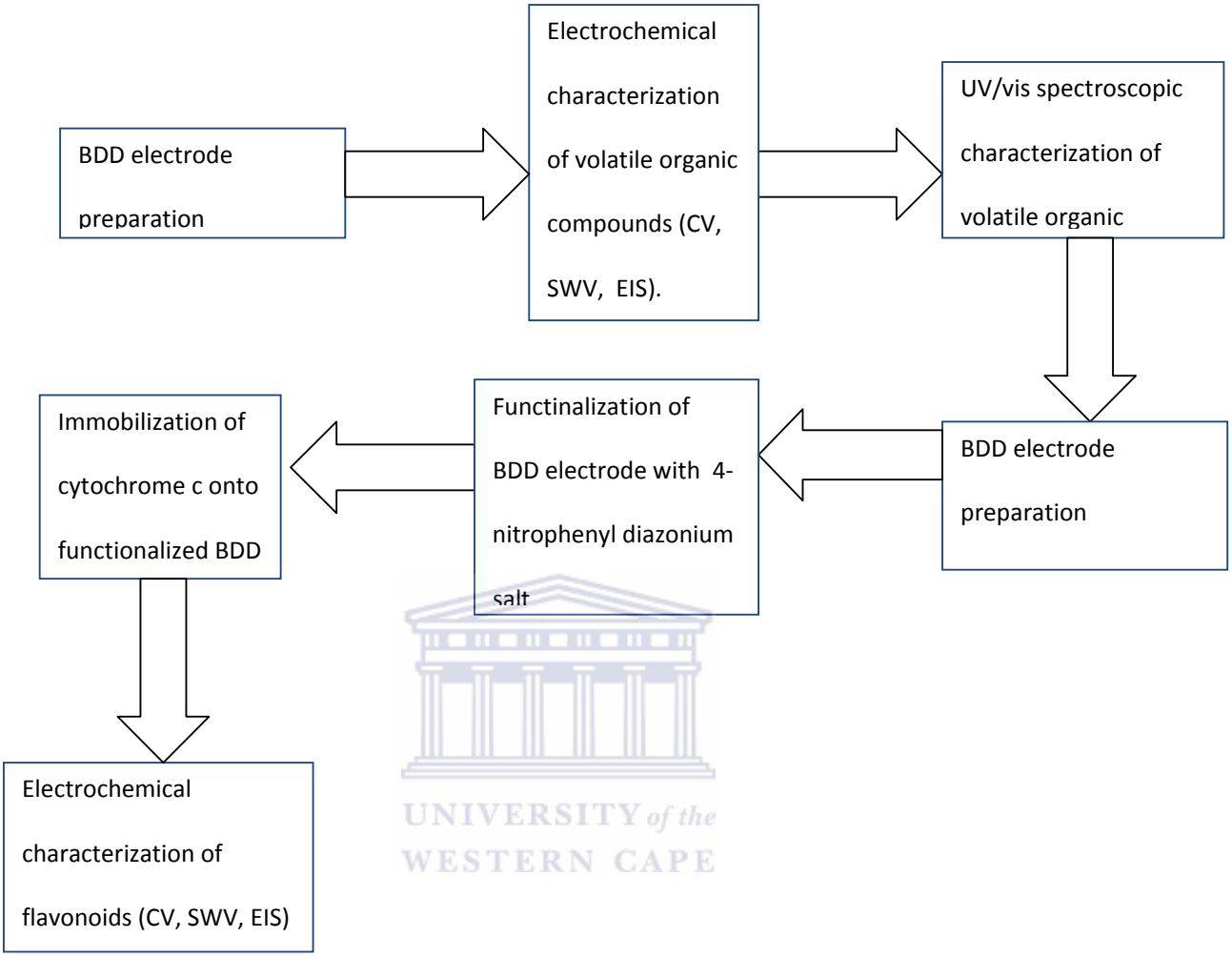
Ultra pure water (nuclease free) with resistivity 18.2 MΩ using a Millipore Synergy water purification system was used in all solution preparation. All reagents were of analytical grade. A list of the materials used is presented in the table below:

Table 3.1 List and source of materials used

Material	Source	Other information
Ultra pure water	Millipore Synergy purification system	Water Resistivity 18.2 MΩ

Benzaldehyde	Sigma-Aldrich
Nitrobenzene	Sigma-Aldrich
m-cresol	Sigma-Aldrich
Cytochrome c	Sigma Aldrich
4-nitrophenyl diazonium salt	Sigma Aldrich
Quercitin	Sigma Aldrich
Catechin	Sigma Aldrich
Rutin	Sigma Aldrich
Buffer components:	Sigma Aldrich
Na_2HPO_4, KH_2PO_4	
Boron-doped diamond electrode	Windsor Scientific Services
Ag/AgCl (3 M Cl-)	BASi
Pt auxiliary electrode	BASi

Research design



3.3 Methodology

3.3.1 Electrode and solution preparation

Boron-doped diamond electrode was used throughout this work. The commercial BDD electrode was activated upon arrival by cycling the electrode in vigorously stirred HNO_3 solution from -1500 mV to 2500 mV respectively. The cleaning method used was predominantly mechanical polishing using alumina powder of size 1, 0.3 and 0.05 micron respectively.

Solutions were prepared using general analytical procedures and formula such as equations 3.1 and 3.2 for preparation and dilution of solutions respectively.

$$\text{mass} = (\text{molar mass} \times \text{Molarity} \times \text{volume})/1000 \qquad \textbf{eqn. 3.1}$$

$$C_1 V_1 = C_2 V_2 \qquad \textbf{eqn. 3.2}$$

3.3.2 Standardization of the BDD electrode

Electrode surface are bound to change after each experiment due to contamination from adsorbed species. To ensure reproducibility, electrochemical measurements are taken after

each cleaning step. The cleaning is repeated until the surface can be said to be clean judging from the voltammetric data obtained in the respective electrolyte.

3.3.3 Voltammetry

Voltammetric techniques are characterized by the application of a potential *E* to an electrode and the monitoring of the resulting current *i* flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time *t* (Kounaves SP, et al., 2008). Thus, voltammetry can be broadly defined as the exploration of the three-dimensional space that relates potential (*E*), current (*i*), and time (*t*) (Bard AJ, et al., 2000). The history of voltammetry began with polarography at the dropping mercury electrode (DME) as far back as 1922 through the experimental work of the Czech chemist Jaroslav Heyrovsky for which he received the 1959 Nobel prize. Heyrovsky could obtain information about the nature of the species in solution that were reduced at the mercury drop by measuring current while the potential of the electrode was changed. In 1925, Heyrovsky and Shikata (Heyrovsky J, et al., 1925) developed an automatic instrument to photographically record *i–E* curves and called it a polarograph. This term now means voltammetry at the DME. The polarograph was one of the first automated recording analytical instruments and ushered in the field of instrumental analysis (Bard AJ et al., 2000). At the onset, an electrochemical cell usually consists of a working electrode and reference electrode. The working electrode (WE) facilitates the transfer of electron to and from the electrolyte while the reference electrode (RE) is used as a standard to indicate the potential of the working electrode. Using this two-electrode set up, it is extremely difficult for an

electrode to maintain a constant potential while passing current to counter redox events at the working electrode. To solve this problem, a third electrode called auxiliary or counter electrode (AE) has to be introduced to pass or sink all the current needed to balance the current observed at the WE. Thus in a three electrode system, the RE only acts as a reference in measuring and controlling the working electrodes potential and at no point does any current pass through it. A three electrode electrochemical cell configuration was used in this research as depicted below:

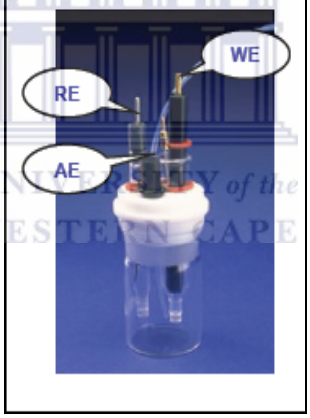


Figure 3. 1: An electrochemical cell consisting of working electrode (WE), reference electrode (RE) and auxillary electrode (AE)

Since the pioneering work of Heyrovsky, voltammetry has undergone rapid improvement. The term "voltammetry" was first introduced in 1940 to describe experiments in which the current as a function of potential at a solid working electrode is measured (Kolthoff IM, et al., 1940) and this field of study has undergone tremendous changes to date. For more on the

history of voltammetry the reader may consult a report by Bard and Zoski and the references therein (Bard AJ, et al., 2000). The basic components of a modern electroanalytical system for voltammetry are a potentiostat, computer, the electrochemical cell. The following are among the leading manufacturers of electrochemical workstations Bioanalytical Systems, CH Instruments, Eco Chemie, Zanher electrik, Radiometer, Brinkman Instruments (Metrohm) etc. The general theory of voltammetry is based on the effect of the applied potential and the behavior of the redox current which are described by several well-known laws. As summarized by Kounaves the applied potential controls the concentrations of the redox species at the electrode surface C^0_O and C^0_R and the rate of the reaction k^0 as described by the Nernst or Butler–Volmer equations, respectively. In the cases where diffusion plays a controlling part, the current resulting from the redox process (known as the faradaic current) is related to the material flux at the electrode–solution interface and is described by Fick's law. The interplay between these processes is responsible for the characteristic features observed in the voltammograms of the various techniques. For a reversible electrochemical reaction (that is, a reaction so fast that equilibrium is always re-established as changes are made), which can be described by equation 3.3,

$$O + ne^- \rightarrow R \qquad \textbf{eqn. 3.3}$$

The application of a potential *E* forces the respective concentrations of O and R at the surface of the electrode i.e. C^0_O, C^0_R a ratio in compliance with the Nernst equation:

$$E = (E^0 - RT \ln C^0_0)/nF\, C^0_R \qquad \textbf{eqn. 3.4}$$

Where R is the molar gas constant (8.314 J mol–1K–1), T is the absolute temperature (K), n is the number of electrons transferred, F = Faraday constant (96,485 $Cmol^{-1}$), and E^0 is the standard reduction potential for the redox couple. A change in the applied potential affects the ratio of C^0_O, C^0_R at the electrode surface since it is at equilibrium, and the ratio will adjust to satisfy the Nernst equation. A shift in the potential applied toward the negative will cause reduction while a positive shift will cause oxidation.

Apart from the actual electron transfer that occurs at the electrode interface, mass transport can also determine the faradiac current or general electrochemical rate. This is why we say an electrode process can be kinetically controlled or diffusion controlled. Diffusion, which is one of the means of mass transport (the others are migration and convection) is usually governed by Fick's law, which states that the flux of matter Φ is directly proportional to the concentration gradient and is given by

$$\Phi = -D_0 (dC_o/dx) \qquad \textbf{eqn. 3.5}$$

Where D_0 is the diffusion coefficient of O and *x* is the distance from the electrode surface.

Voltammetry has a variety of methods which include

- Potential sweep methods: linear and cyclic voltammetry
- Pulse methods: polarography, normal pulse voltammetry (NPV), differential pulse voltammetry and square wave voltammetry
- Controlled potential or current methods:
- Coulometry:

A brief overview of the predominating techniques used in this work will only be presented.

3.3.4 Cyclic Voltammetry

Cyclic voltammetry (CV) is one of the most widely used voltammetric techniques. In CV, the potential is ramped linearly at both forward and backward position at rates between 0.01–105 V/s, with the resulting current recorded as a function of potential (which is equivalent to recording current versus time). It is widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. A schematic CV plot shown below:

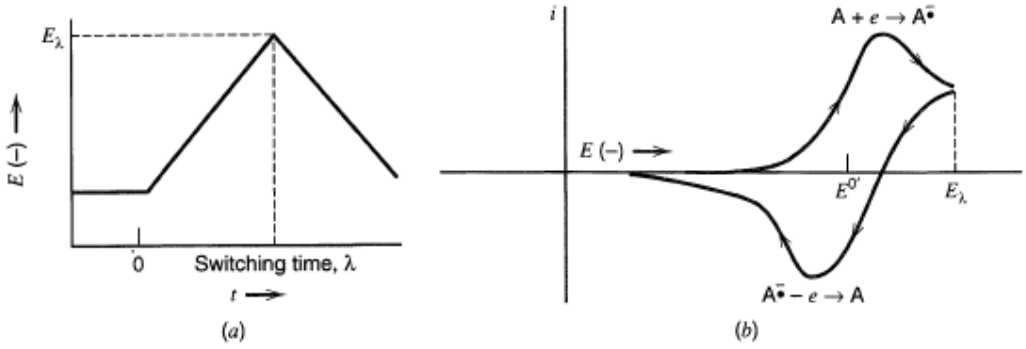


Figure 3. 2: (a) cyclic potential sweep, (b) resulting cyclic voltammogram

In a reversible system, diffusion is the main mode of transport and semi infinite linear diffusion conditions prevail. In cyclic voltammetry, the following are diagnostic of a reversible system Ipa/Ipc = 1. $\Delta Ep = 2.218RT/nF = 57/nmV$ at 298 K and it is independent scan rate v . Or Ep_c and Ep_a are independent of v. Randles-Sevcik equation is expressed by

$$Ip = (2.69 \times 10^5)n^{3/2}AD^{1/2}v^{1/2}C \qquad \textbf{eqn. 3.6}$$

Where Ip is the peak current, n is the number of electrons, D is the diffusion coefficient, A is the surface area of the electrode, C is the concentration of analyte, and v is the scan rate. The scan rate (v) defines the timescale of the experiment. For short timescales (high v), the diffusion-controlled current is increased over that for longer timescales (smaller v). This is due to the fact that the concentration gradient and the flux of product to the electrode increase with increasing v. This relationship is used to prove diffusion control of the current as

opposed to currents due to surface-bound or adsorbed redox. $|Ep - Ep_2| = 2|Ep - Ep_{1/2}| = 2.218(RT/nF)$.

3.3.5 Square Wave Voltammetry (SWV)

Figure 3.3 is typical square wave voltammogram showing the forward (i_f), reverse (i_r) and net (i_{net}) currents is shown below:

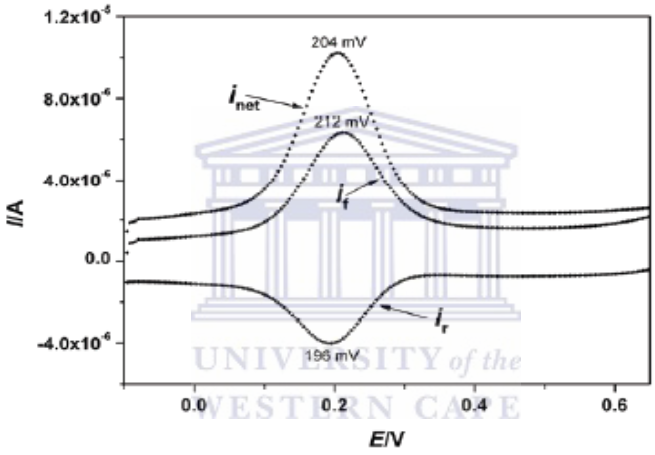


Figure 3. 3: square wave voltammogram

Though SWV was pioneered by Barker (Barker GC, 1952), it was the work of Osteryoung and co workers (Osteryoung, J, et al., 1986) that brought it to limelight. It has a slightly better sensitivity that DPV and can be used to study electrochemical processes at fast scan rates. The potential waveform above consists of a square wave superimposed on a staircase. The current at the end of the forward pulse, i_f , and the current at the end of the reverse pulse, i_r, are both registered as a function of the staircase potential, which is midway between the potentials corresponding to the forward and backward potential steps. The difference, i_{net}, (i_f-

i_r) is larger than each individual component in the region of the peak that is centred on the half-wave potential because if and i_r have opposite signs. This difference, effectively cancels the capacitive currents and thus higher scan rates are possible without background current interferences. This makes SWV a useful tool in kinetic study. SWV is characterised by four parameters: square wave period, τ, pulse width, tp = τ /2, step height, ΔEs and pulse height, ΔE_{sw} . The pulse width is related to the square wave frequency, $f = 1/(2t_p)$ and as the staircase step at the beginning of each cycle is ΔE_s it means that the effective scan rate is $\upsilon = \Delta E_s\, 2t_p = f\Delta E_s$. Peak Current is given by

$$\Delta ip = (nFAD^{1/2}\, C/\pi^{1/2}tp^{1/2})\, \Delta\, \psi_p \qquad \textbf{eqn. 3.7}$$

Experimentally, ΔE_s is usually kept constant while the frequency is varied.

3.3.6 Electrochemical impedance spectroscopy (EIS)

Macdonald, in his review (Macdonald DD, 2006), traced the foundation of electrochemical impedance spectroscopy (EIS) back to a scientist called Oliver Heaviside. Heaviside was the first person to define the term impedance. Electrochemical impedance spectroscopy is an excellent, non-destructive, accurate and rapid in situ technique for examining processes

occurring at electrode surfaces. A small amplitude ac (sinusoidal) excitation signal (potential or current), covering a wide range of frequencies, is applied to the system under investigation and the response (current or voltage or another signal of interest) is measured. This is in contrast to the 'usual' spectroscopic techniques where interactions of electromagnetic waves and materials are measured. The measurement of impedance is only valid when the system is linear – thus the need for the small amplitude of the excitation signals in EIS. The measurement should be carried out without significantly disturbing the properties being measured. Due to the wide range of frequencies used, the complex sequence of coupled processes such as, electron transfer, mass transport, chemical reaction, etc. can often be separated and investigated with a single measurement. It is routinely used in electrode kinetics and mechanism investigations, and in the characterization of batteries, fuel cells, and corrosion phenomena (Macdonald DD, 1990). It is also widely applied in the characterization of semiconductors, organic films and very recently biosensors. The application of EIS in biosensor is relatively new (Pejcic B, et al., 2006). A brief theory: From Ohm's law

$V = IR$ **eqn. 3.8**

Resistance is independent of frequency. AC current and voltage through a resistor are in phase with each other. Suppose we apply a sinusoidal potential excitation. The response to this potential is an AC current signal containing the excitation frequency and its harmonics

which is not in the same phase with the AC voltage. The resultant resistance in this case is called Impedance**.**

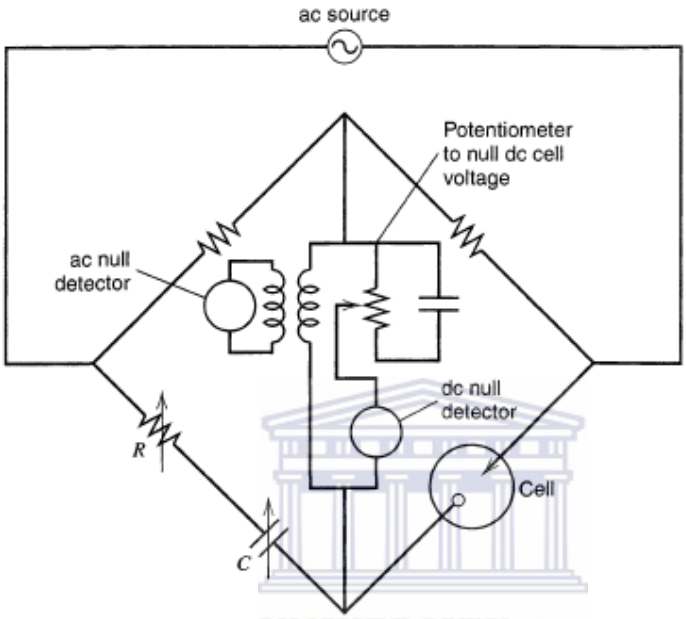


Figure 3. 4: A bridge circuit for measurements of electrochemical impedance.

3.4 General Experimental

3.4.1 Solutions

1 M sulphuric acid and 5 mM saline phosphate buffer solution (PBS) pH 7.2 containing Na_2HPO_4, KH_2PO_4 and was prepared, the buffer is referred to as phosphate buffer. 5 mM

solution of Benzaldehyde, Nitrobenzene and m-cresol was prepared in 0.1 M HCl. The stock solutions were stored at room temperature. 5 mM 4-Nitrophenyl diazonium salt solution was made by dissolving salt in 10 mL terabutylammonium-tetrafluoroborate (0.1M). 5 mg of cytochrome c from Saccharomyces cerevisiae from Sigma-Aldrich was dissolved in 5 mL of phosphate buffer. For immobilization only 1 mL from stock solution was used every time. 5 mM solution of Quercitin, Catechin and Rutin was prepared in of 5 mM phosphate buffer solution at pH 7.2. The stock solutions of cytochrome c and flavonoids was stored at 4 °C and not used when older than 4 weeks.

3.4.2 Electrochemical cell

A three electrode system was used to perform all electrochemical experiments. Boron-doped diamond (BDD) electrode with area 0.071 cm^2 was used as the working electrode; platinum wire as the counter electrode, and Ag/AgCl (3M Cl^-) as the reference electrode. All solutions were de-aerated by bubbling argon through it for 5 minutes.

CHAPTER FOUR

Electrochemistry of volatile organic compounds at unmodified boron doped diamond.

It is well known that the end products of the oxidation of volatile organic compounds at BDD electrodes are CO_2 and H_2O (Panizza M, et al., 2009). Hence the measurement of concentration dependent peak currents associated with electrochemically stable intermediate products, for the three volatile organic compounds (VOCs) investigated in this study, hold great promise for the development of BDD sensors as a tool for monitoring individual special of VOCs. BDD electrode was cleaned and polished as described previously in electrode preparation. The BDD electrode was cycled 5 to 10 times in 1 M H_2SO_4 (electrolyte) solution to obtain a low background current. The analytes benzaldehyde, nitrobenzene and m-cresol were added to 3 mL of the electrolyte in 10 µL aliquots, respectively.

4.1 Benzaldehyde

A potential range of -1700 mV to 2000 mV was chosen as the potential window for studying the electrochemistry of the volatile organic compounds (Figure 4.1).

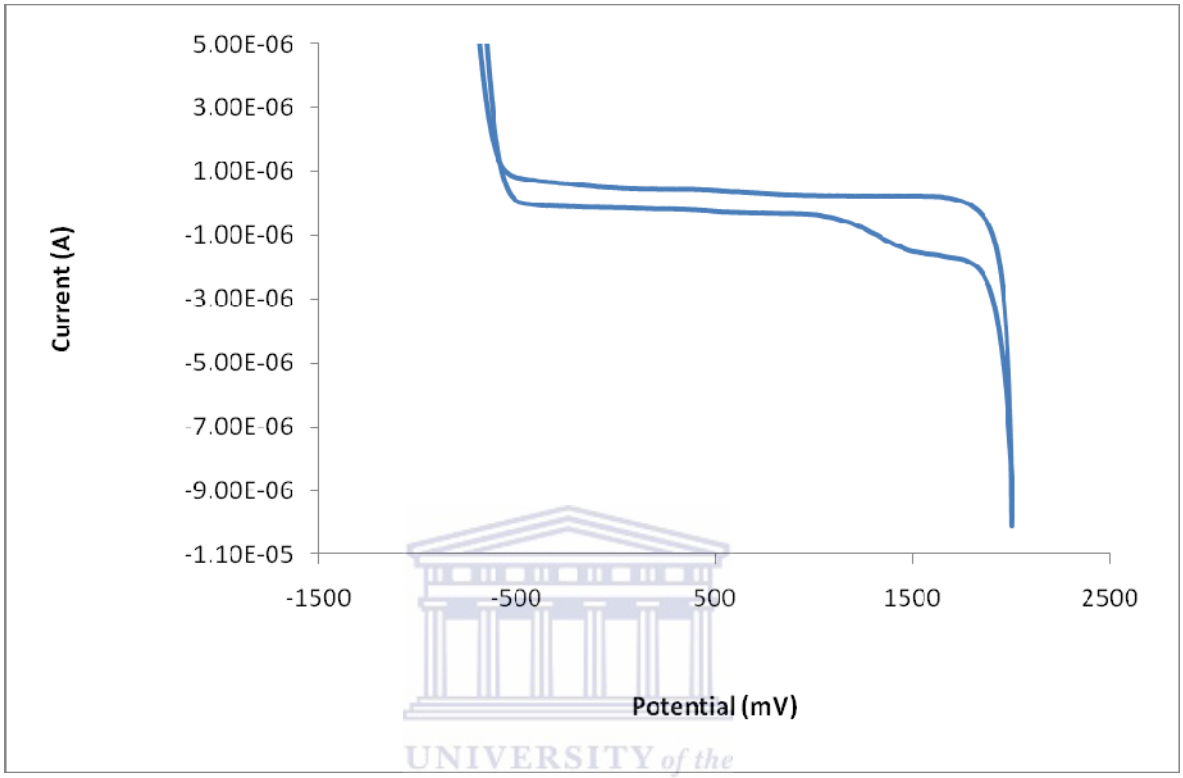


Figure 4.1: Cyclic Voltammogram of bare BDD electrode in H_2SO_4 at scan rate 200 mV/s.

Cyclic voltammetry (CV) of benzaldehyde at different concentrations and at fixed scan rate of 200 mV/s. The final concentrations of benzaldehyde in solution were 1.66×10^{-4} M, 3.31×10^{-4} M, 4.95×10^{-4} M, 6.58×10^{-4} M and 8.19×10^{-4} M, after each consecutive 10 uL aliquot addition. The oxidation of benzaldehyde showed two oxidation peaks i.e. Epa_1 at 320 mV and Epa_2 at 1600 mV and one reduction peak at -250 mV (Figure 4.2). Epa_1 was related to a unique intermediate for benaldehyde, before benzene ring opening reaction whereas Epa_2 was associated with complete degradation of benzaldehyde (Donald G, et al; 1975). Square

wave voltammetry (SWV) of benzaldehyde, confirmed the formal potential for the oxidation intermediate to be 465 mV (Figure 4.3).

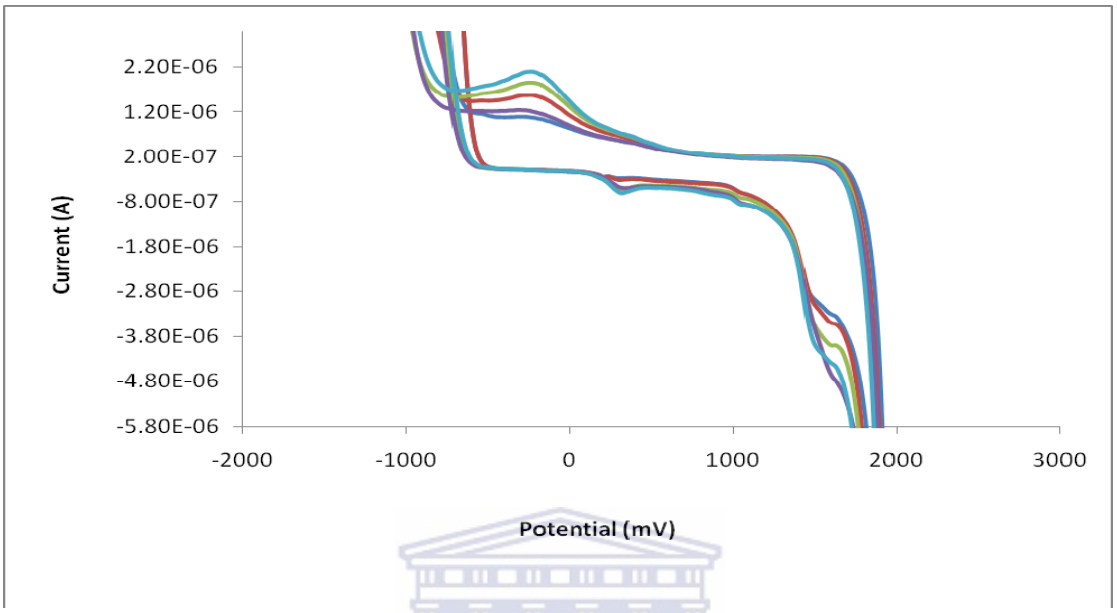


Figure 4.2: CV of benzaldehyde at different concentrations and fixed scan rate of 200 mV/s.

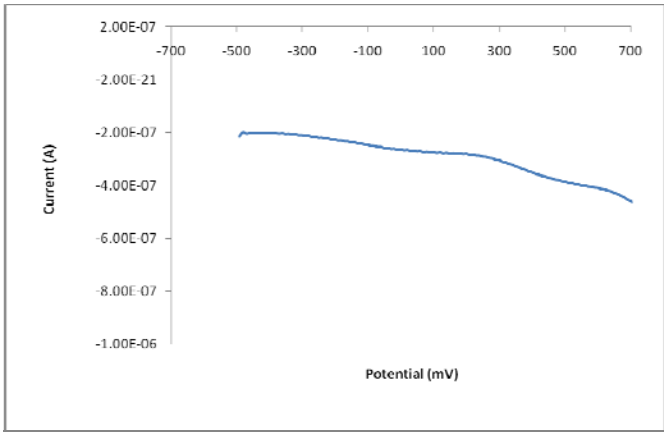


Figure 4.3: SWV of benzaldehyde at a frequency of 20 Hz and step potential of 10 mV.

A calibration curve for current response at Epa_1 as a function of increasing concentration was constructed and a linear relationship was observed correlation coefficient (R^2) of 0.86. Deviation from unity may be due to the rate of kinetics of electron transfer, or as a result of subsequent chemical reaction at the electrode surface (Figure 4.4.).

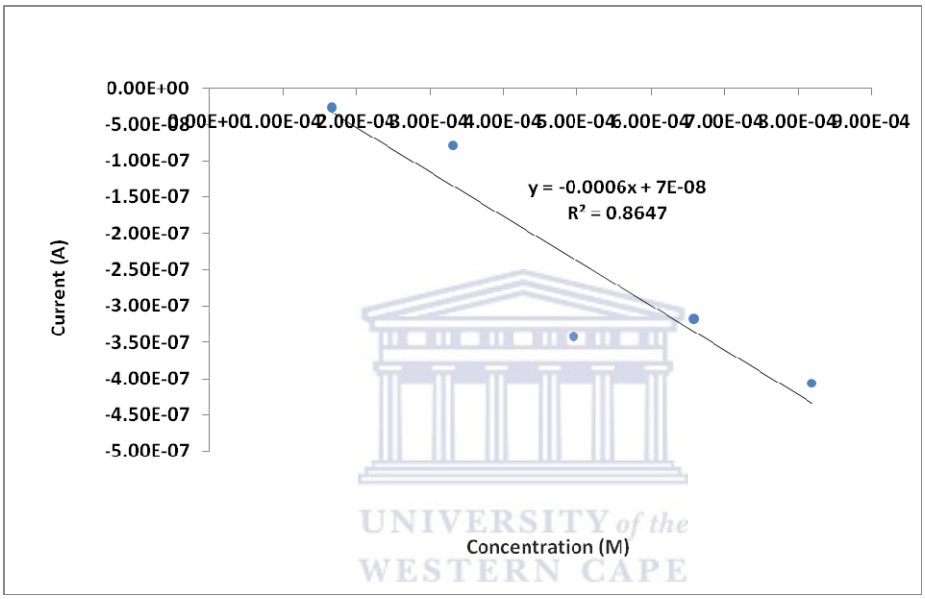


Figure 4.4: Calibration plot for benzaldehyde at bare BDD electrode.

Scan rate dependence of 8.19×10^{-4} M benzaldehyde at scan rates 150, 200, 250, 300, 350 and 400 mV/s was evaluated (Figure 4.5). A linear plot for anodic peak current versus square root of scan rate was obtained at these relatively fast scan rates, which confirms that the oxidation of benzaldehyde at BDD electrode is a diffusion controlled process (Figure 4.6).

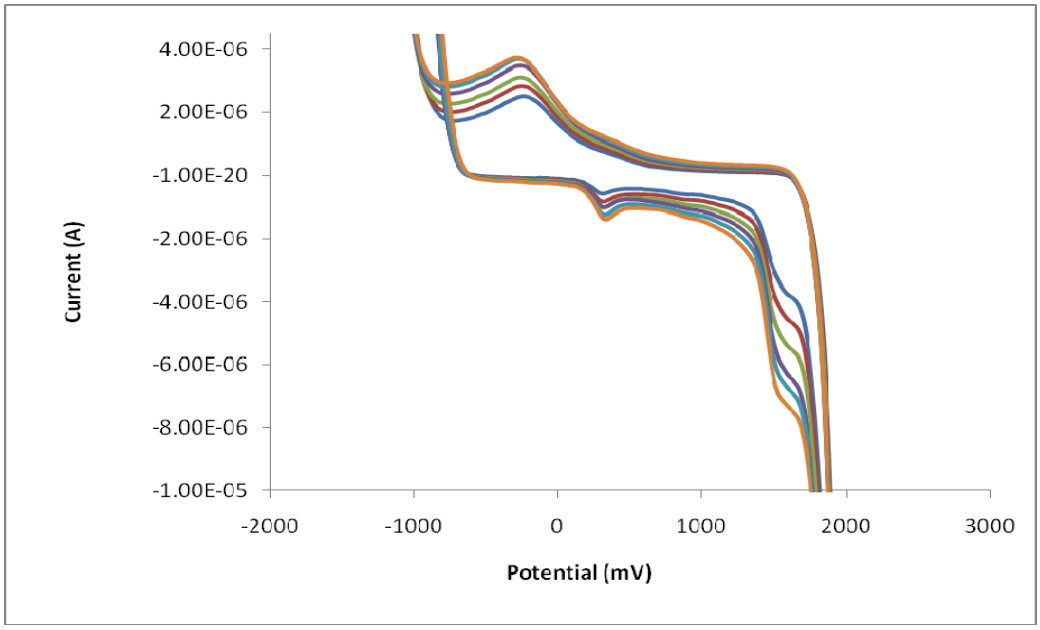


Figure 4.5: CV of benzaldehyde at different scan rates.

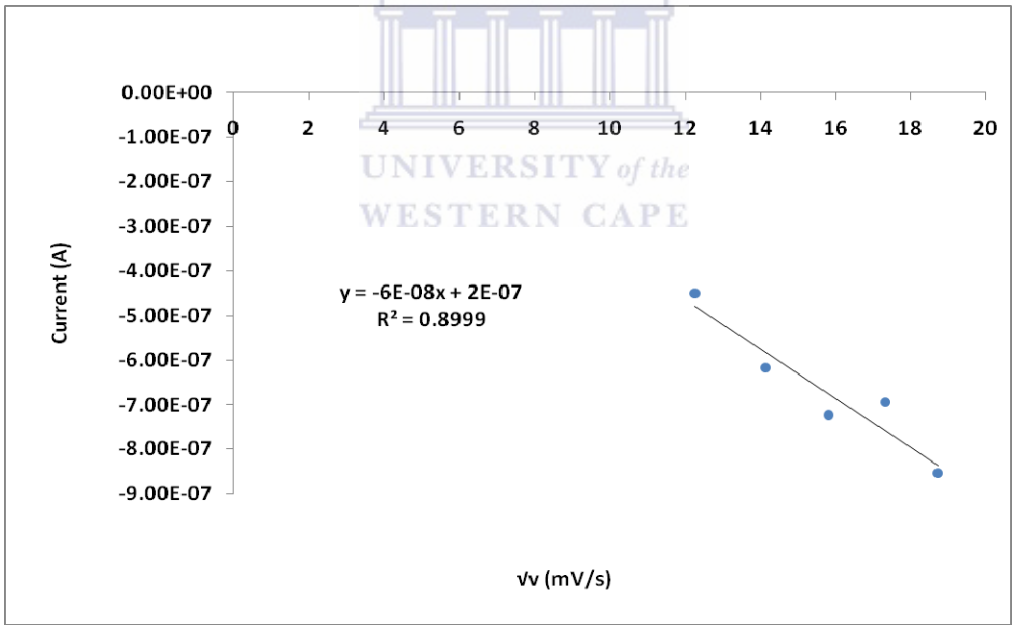


Figure 4.6: Peak currents as a function of square root of scan rate for benzaldehyde oxidation at BDD electrode.

The electron diffusion coefficient (De) was interpreted as a measure of the rate of electron transfer in species diffusing to the electrode interface and was calculated using Randle Sevcik

equation and the apparent rate constants (k^0) were evaluated using Nicholson treatment of data (Table 4.1). Electrochemcial kinetic parameters obtained are in good agreement for the fast rates of catalysis observed for benzaldehyde oxidation and are supported by values reported for chemical benzaldehyde oxidation, in literature (Lee DG, et al., 1975).

Table 4. 1:**The apparent rate constants and diffusion coefficients of benzaldehyde**

***v*/(mVs^{-1})**	**De/(cm^{2}s^{-1})**	**k/(cms^{-1})**
150	1.40×10^{-11}	4.41×10^{-4}
200	1.98×10^{-11}	6.06×10^{-4}
250	2.17×10^{-11}	7.09×10^{-4}
300	1.67×10^{-11}	6.81×10^{-4}
350	2.17×10^{-11}	8.37×10^{-4}

4.2 Nitrobenzene

Concentration dependent analysis of nitrobenzene at fixed scan rate of 200 mV/s was done and a suitable intermediate oxidation product was identified for the construction of a calibration curve. Two oxidation peaks, Epa_1 at 820 mV and Epa_2 at 1650 mV and two reduction peaks at -117 mV and -680 mV were observed (Figure 4.7).

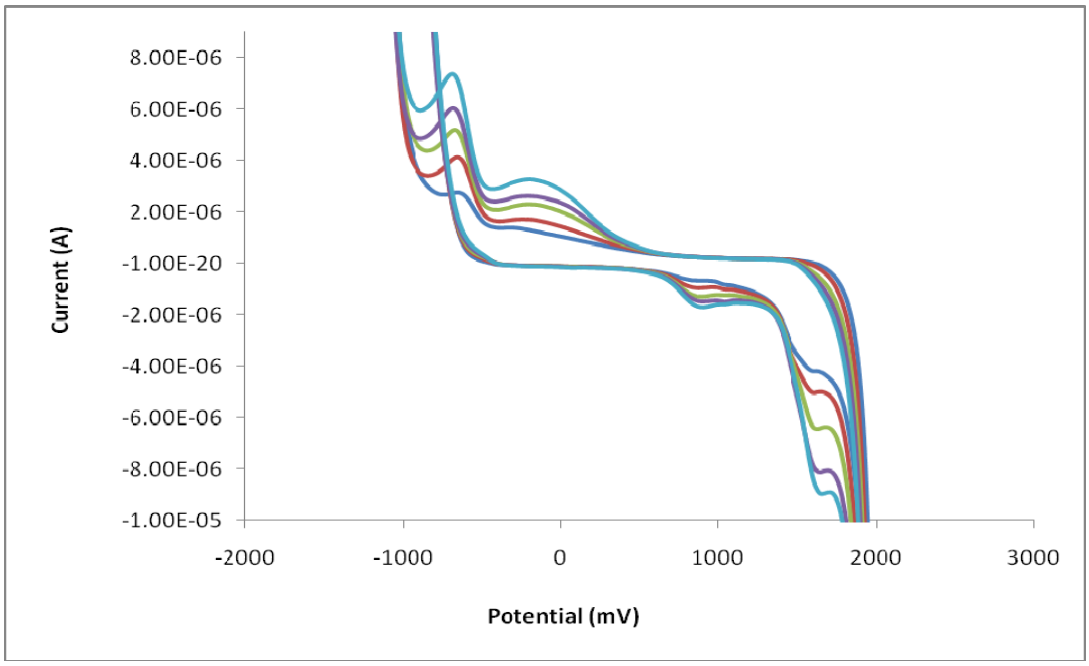


Figure 4.7: Cyclic voltammogram of nitrobenzene at different concentrations, scan rate of 200 mV/s.

The peak current due to the formation an oxidation intermediate at Epa_1 was associated with the monohydroxy intermediate formed from the oxidation of nitrobenzene and Epa_2 is the further oxidation of the intermediate on the electrode surface to CO_2 (Palmisano G, et al., 2007). Oxidative SWV of nitrobenzene confirmed the formation of a stable intermediate species with formal potential of 860 mV (Figure 4.8).

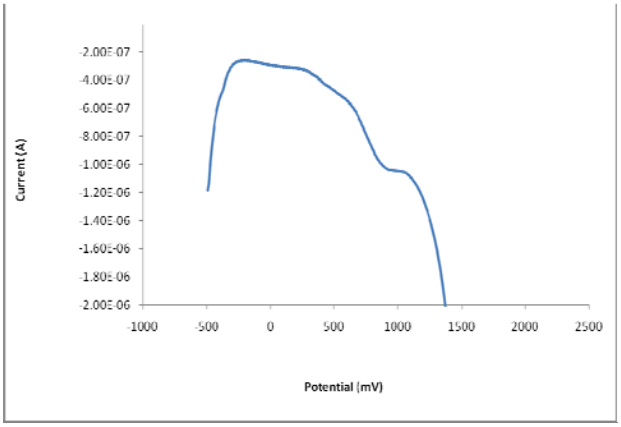


Figure 4.8: SWV of nitrobenzene oxidation at a frequency of 20 Hz and step potential of 10 mV.

The calibration plot constructed from CV data, showed linear response to increasing concentration over the concentration range investigated, with correlation coefficient (R^2) of 0.98 (Figure 4.9).

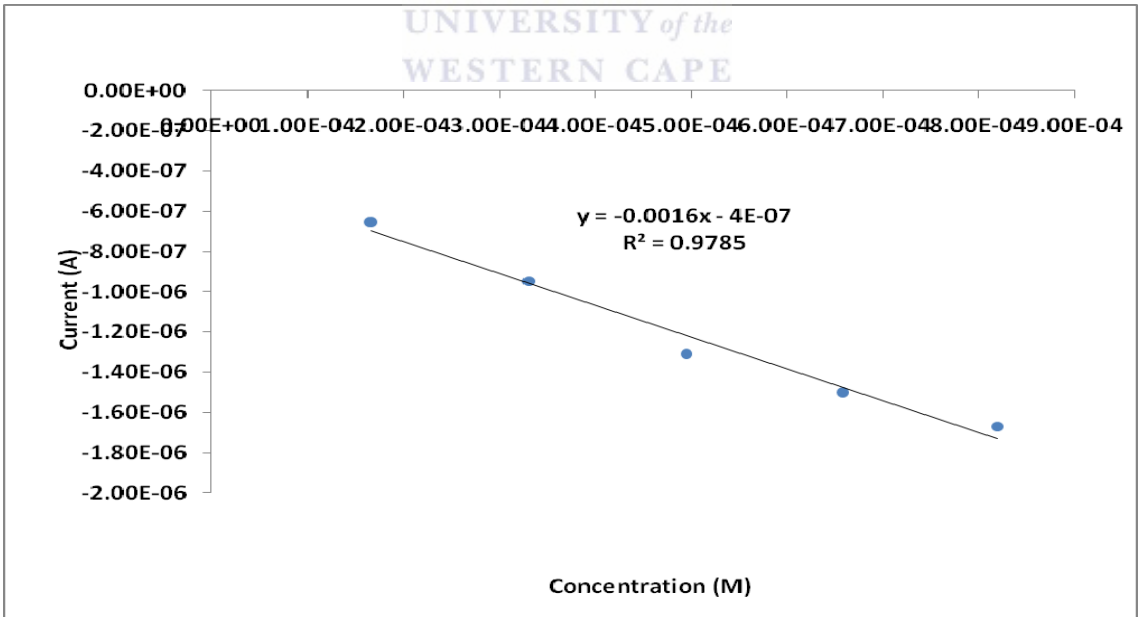


Figure 4.9: Peak currents as a function of concentration for nitrobenzene oxidation at unmodified BDD electrode.

Scan rate dependence at 150, 200, 250, 300, 350 and 400 mV/s and fixed concentration of 8.19×10^{-4} M (Figure 4.10), resulted in a higher degree of linearity compared to benzaldhyde electrochemical oxidation (Figure 4.11) with correlation coefficient (R^2) of 0.94, at relatively high scan rate.

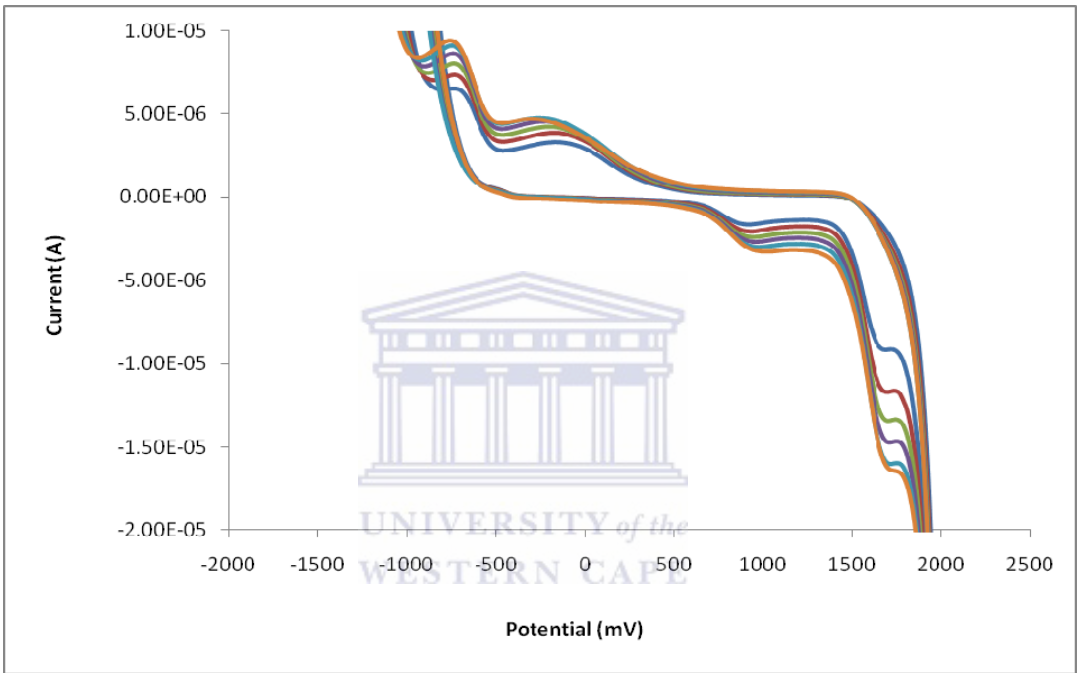


Figure 4.10: Cyclic voltammogram of nitrobenzene at different scan rates; 150, 200, 250, 300, 350 and 400 mV/s.

The higher degree of linearity was interpreted as faster electron transfer and improved catalysis for nitrobenzene compared to benzaldehyde. The improved catalysis could be

attributed to the electron rich nitrogen centre behaving as an active site for electro-oxidation reaction resulting in minimal secondary reactions that could retard the rate of catalysis.

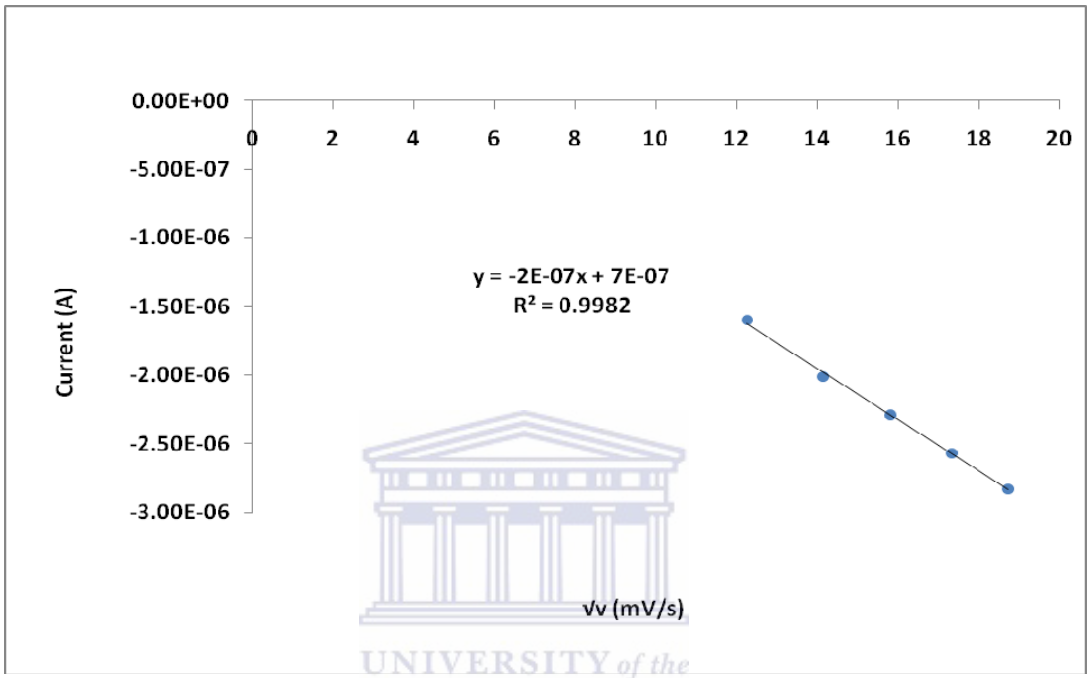


Figure 4.11: Peak currents as a function of square root of scan rate for nitrobenzene oxidation at unmodified BDD electrode.

The De, and apparent rate constants were evaluated as before. Good correlation between kinetic parameters measured here and those reported for electrocatalytic oxidation for nitrophenols at alternative anodes in literature, were observed (Table 4.2) (Liu H, et al., 2008)

Table 4. 2:**The apparent rate constants and diffusion coefficients of nitrobenzene**

***v*/(mV/s)**	**De/(cm^2s^{-1})**	**k/($cm\ s^{-1}$)**
150	1.75×10^{-10}	1.56×10^{-3}
200	2.09×10^{-10}	1.97×10^{-3}
250	2.19×10^{-10}	2.25×10^{-3}
300	2.29×10^{-10}	2.52×10^{-3}
350	2.37×10^{-10}	2.77×10^{-3}

4.3 m-cresol

Cyclic voltammetry of m-cresol at different concentrations 1.66×10^{-4} M, 3.31×10^{-4} M, 4.95×10^{-4} M, 6.58×10^{-4} M and 8.19×10^{-4} M and fixed scan rate of 200 mV/s was evaluated. Two oxidation peaks were observed i.e. Epa_1 at 1199 mV and Epa_2 at 1600 mV and one reduction peak at -80 mV (Figure 4.12). A deviation from the standard experimental procedure for the VOC analysis was employed for concentration dependent oxidation for m-cresol. The optimised experimental conditions employed for m-cresol electro-oxidation included an additional controlled polishing step, since m-cresol showed a tendency to adsorb onto the BDD electrode surface.

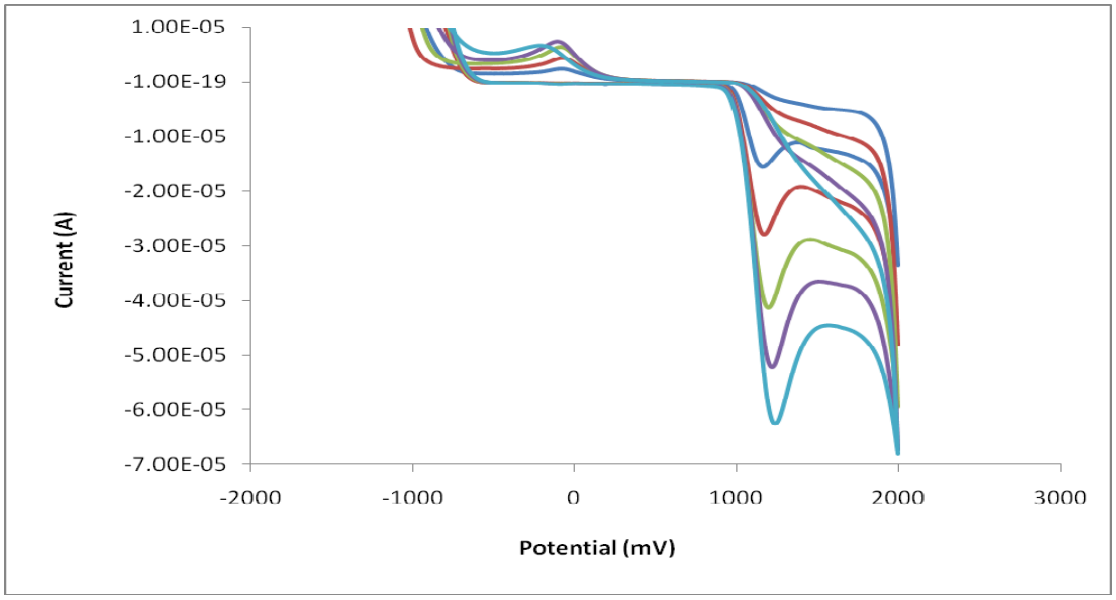


Figure 4.12: Cyclic voltammogram of m-cresol at different concentrations and fixed scan rate of 200 mV/s.

SWV of m-cresol confirmed the formation of a stable oxidation intermediate for which the formal potential was confirmed to be 1190 mV (Figure 4.13).

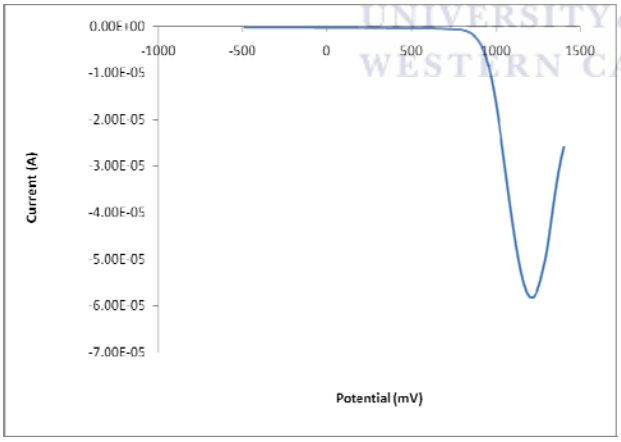


Figure 4.13: SWV of 8.19×10-4 M of m-cresol at frequency of 20 Hz and step amplitude of 10 mV.

A calibration curve of Ipa_1 vs concentration showed that current increased directly proportional to concentration with correlation coefficient (R^2) of 0.99 (Figure 4.14).

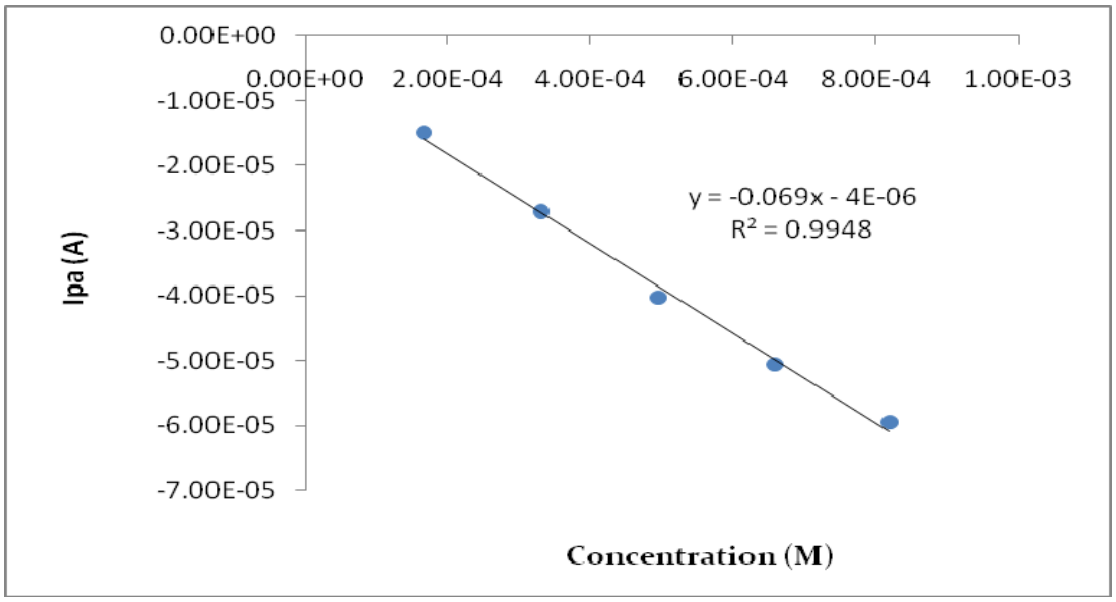


Figure 4.14: Peak currents as a function of concentration for m-cresol at unmodified BDD electrode.

Scan rate dependent CV of m-cresol for a fixed concentration (8.19×10^{-4} M) confirmed the highest rate of electrocatalysis of all three VOCs evaluated (Figure 4.15). A plot of Ipa_1 versus square root of scan rate showed good linearity ($R^2 = 0.94$).

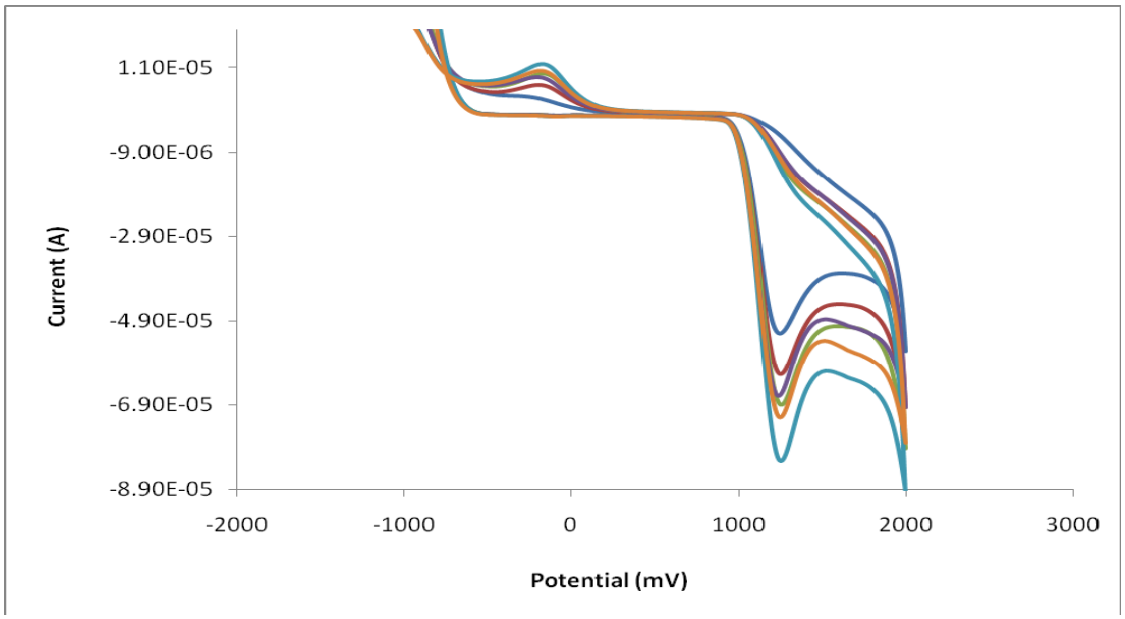


Figure 4.15: Cyclic voltammogram of m-cresol at different scan rates; 150, 200, 250, 300, 350 and 400 mV/s.

The fastest rate of electrochemical oxidation was observed for m-cresol. However the adsorption of m-cresol onto the BDD electrode surface (which is not observed for benzaldehyde and nitrobenzene) serves as an identification of at least one of the species that may complicate individual VOC determination. Adsorption of the oxidation intermediates results in fouling of typically used anodes for electrolysis and hampers efficient quantification analysis (Tomizawa S, et al., 2006).

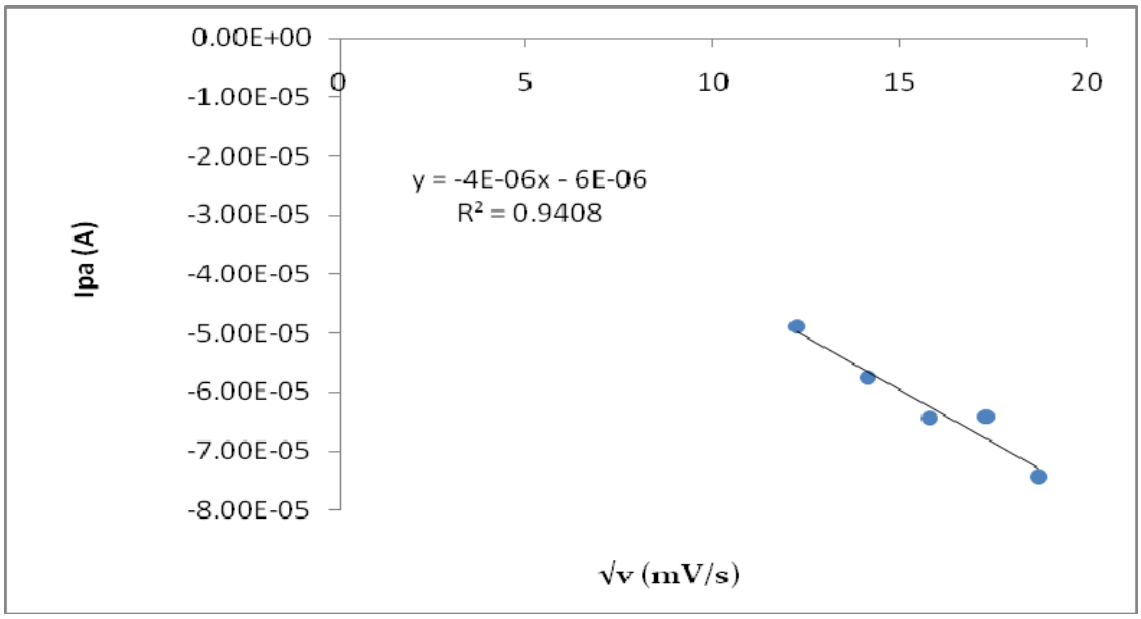


Figure 4.16: Peak currents as a function of square root of scan rate for m-cresol at unmodified BDD electrode.

Good correlation between kinetic parameters measured here and those reported for electrocatalytic oxidation of m-cresol using alternative methods of analysis, were observed (Table 4.3). (Tomizawa S, et al., 2006).

Table 4. 3: The apparent rate constants and diffusion coefficients of m-cresol

***v*/(mV/s)**	**De/(cm^2s^{-1})**	**k/(s^{-1})**
150	1.62×10^{-7}	4.7×10^{-2}
200	1.69×10^{-7}	5.6×10^{-2}
250	1.72×10^{-7}	6.3×10^{-2}
300	1.40×10^{-7}	6.2×10^{-2}
350	1.76×10^{-7}	7.6×10^{-2}

In order to evaluate the response of the bare BDD electrode's to the organic substrates as an electrochemical sensor, the sensitivity and detection limits were calculated (Table 4.4).

Table 4. 4: Sensitivity and Detection limits of VOCs measures as individual organic compounds

Organic compound	Sensitivity	Detection limit
Benzaldehyde	6×10^{-4} A/M	4.64×10^{-5} M
Nitrobenzene	1.6×10^{-3} A/M	5.35×10^{-5} M
m-cresol	6.9×10^{-7} A/M	9.3×10^{-2} M

4.4. Detection of benzaldehyde, nitrobenzene and m-cresol in a mixture.

CV of benzaldehyde, nitrobenzene and m-cresol in one solution, using the same concentrations as for the individual measurement was performed at a fixed scan rate of 200 mV/s. The oxidation peaks for each electrochemical oxidation intermediate was clearly resolved it the cyclic voltammogram of the mixture (Figure 4.17).

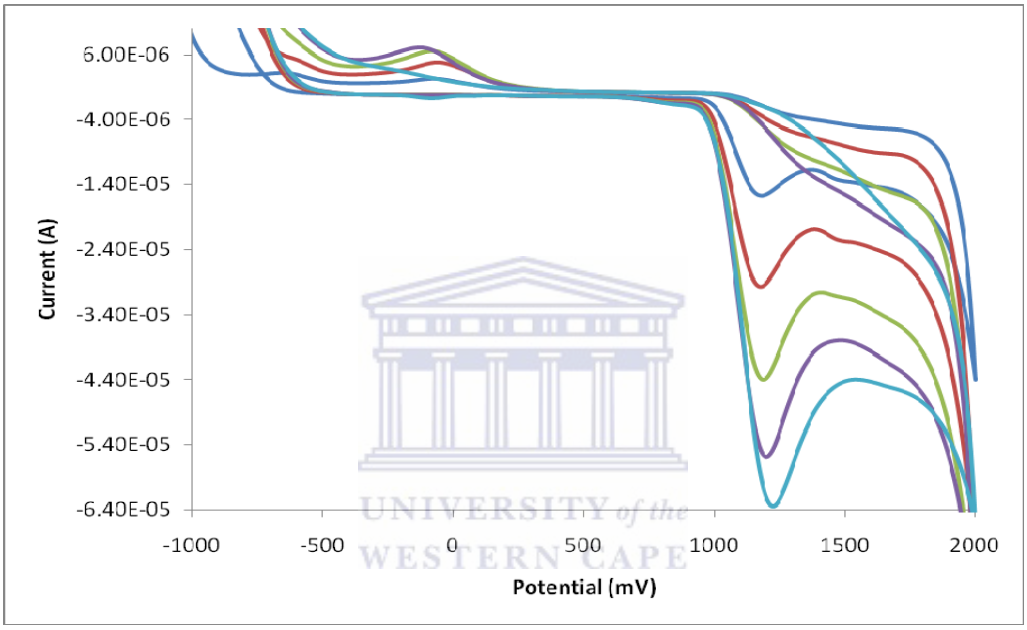


Figure 4.17: CV of a mixture of all three compounds at increasing concentrations.

Calibration plots were constructed for each of VOC from its oxidation intermediate peak potential determined from individual component analysis, as before. The slopes and linear regression coefficients calculated from calibration plots confirmed that the catalytic response for the individual VOC was in good agreement to the response observed for the VOCs as a mixture (Table 4.5)

Table 4. 5: Calibration plot data for each VOC in the mixture.

Organic compound	Epa, mV (oxidation intermediate)	Slope (μA/mM)	Limit of Detection (μM)	R^2
Benzaldehyde	330	1×10^{-4}	2.78×10^{2}	0.81
Nitrobenzene	820	1.6×10^{-3}	53.5×10^{1}	0.97
m-cresol	1192	7.1×10^{-2}	1.02	0.98

The slope was interpreted as sensitivity of the BDD electrode towards analyte and the limit of detection was measured as the intercept of the regression line with the x-axis. The BDD was found to be most sensitive towards m-cresol (7.1×10^{-2} μA/mM) and the lowest detection limit (1.02 μM).

4.5 UV/Vis Spectrosopy

UV absorption of all VOCs were measured at increasing concentrations, 1.66×10^{-4} M, 3.31×10^{-4} M, 4.95×10^{-4} M, 6.58×10^{-4} M and 8.19×10^{-4} M on a Nicolet UV/vis spetrometer using quartz cuvettes. Benzaldehyde showed one peak at wavelength 245 with a broad shoulder. This wavelength correlates with other literature values (Caralp F, et al., 1999). The UV absorption of nitrobenzene gave one peak at wavelength 245 nm. This wavelength is due

to the donor-acceptor bond of nitro groups (Gavrilov GA, et al., 1970). The UV absorption of m-cresol gave a peak at wavelength 280 nm (Oguchi T, et al., 2002)). However the UV absorption of benzaldehyde, nitrobenzene and m-cresol in one solution did not produce well resolved peaks, since the individual absorbance wavelengths were too close to one another (Figure 4.18). Therefore electrochemical oxidation studies at BDD have proven that VOCs may be determined as individual species and as a component within a mixture, with high sensitivity and low limits of detection. The resolution of individual peak potentials was good enough to perform individual analysis of compounds in a mixture. This is clearly not possible with UV/vis spectroscopy and thus electrochemical methods provide an effective and convincing alternative to VOC.

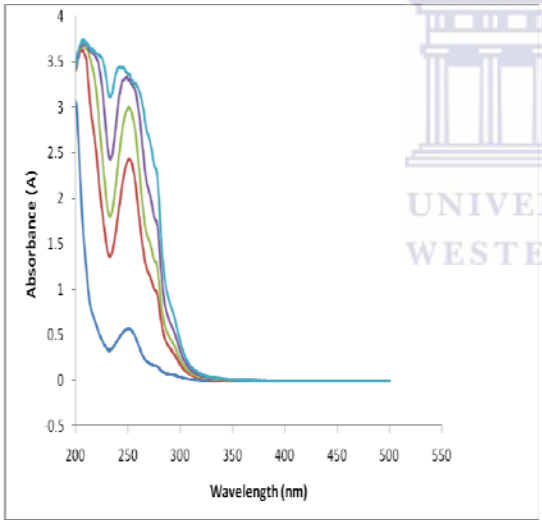
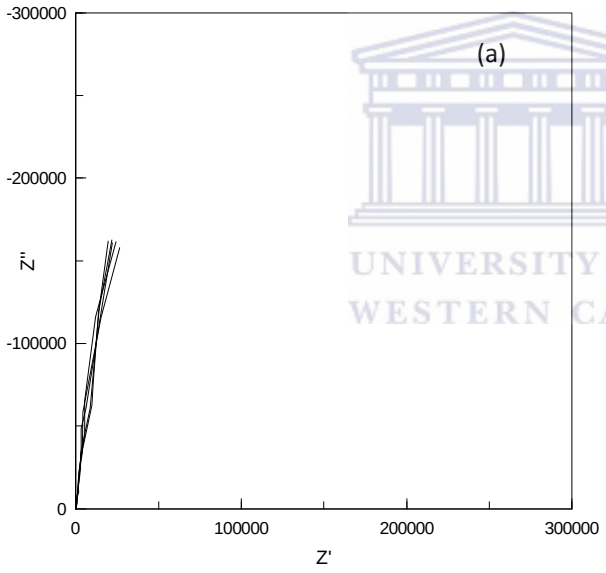


Figure 4.18 UV absorption spectra of benzaldehyde, nitrobenzene and m-cresol in one mixture, at different concentrations.

4.6 Electrochemical impedance spectroscopy

Impedance spectroscopy is a very useful technique to derive the capacitance and gives complementary frequency dependent information. The potential was kept fixed for each particular organic compounds corresponding to their formal potentials. The high frequency was 1000 Hz and the low frequency was 100 mHz. Complex plot data was fitted to an equivalent electrical circuit with a fitting error percentage below 15% considered acceptable.



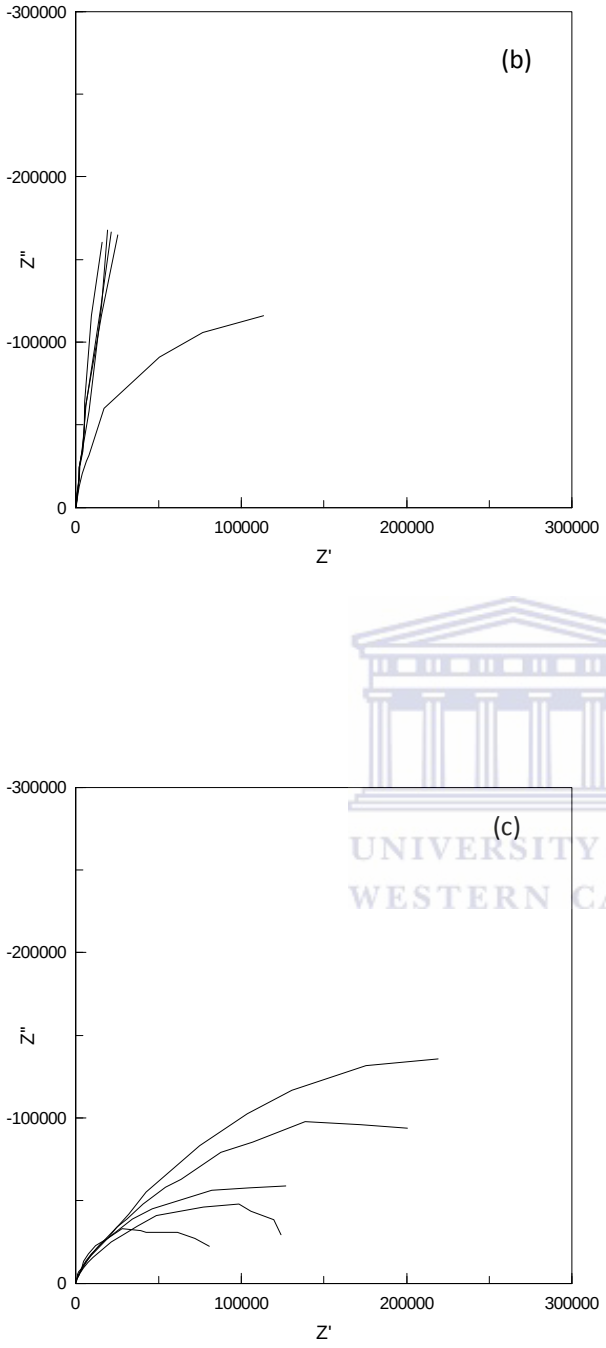


Figure 4. 18: Complex plot of (a) benzaldehyde(b) nitrophenol and (c) m-cresol at different concentrations at respective formal oxidation potential.

The data was modelled as a simple Randles circuit comprising representing solution resistance (Rs), interfacial capacitance as a constant phase element (CPE) and charge transfer resistance (Rct) (Figure 4.19).

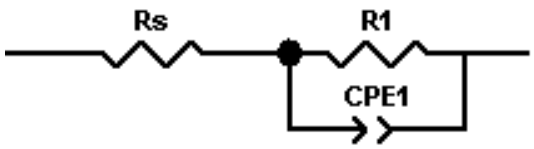


Figure 4.19: Randles circuit used for EIS data fitting.

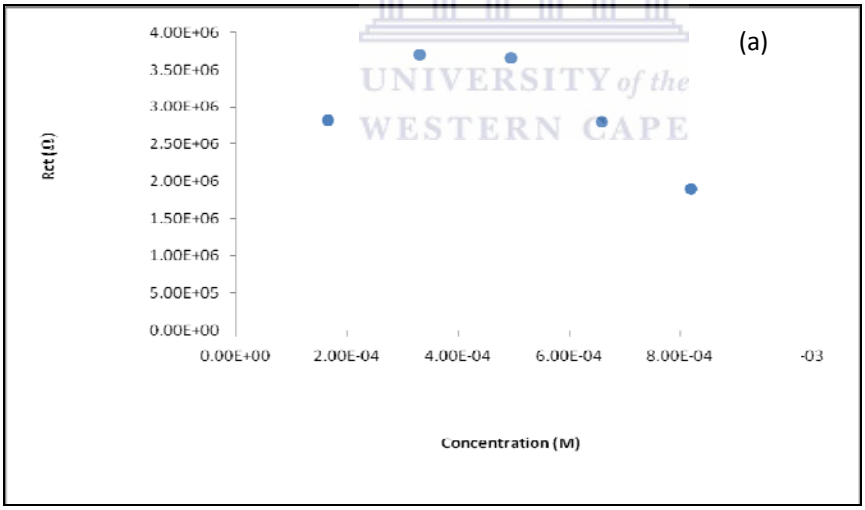
The results obtained from data fitting showed the largest observable trend in values associated with charge transfer resistance. The solution resistance remains fairly constant throughout, since the electrolyte and VOC concentrations in all experiments were kept the same. The constant phase element is an indication of the capacitive nature of the interface. Data obtained for benzaldehyde, nitrobenzene and m-cresol are consistent and no significant change associated with increased concentration is observed. This is an indication that adsorption at the electrode interface is not a major contributing factor to the efficient degradation of the VOC's studied (Tabel 4.6).

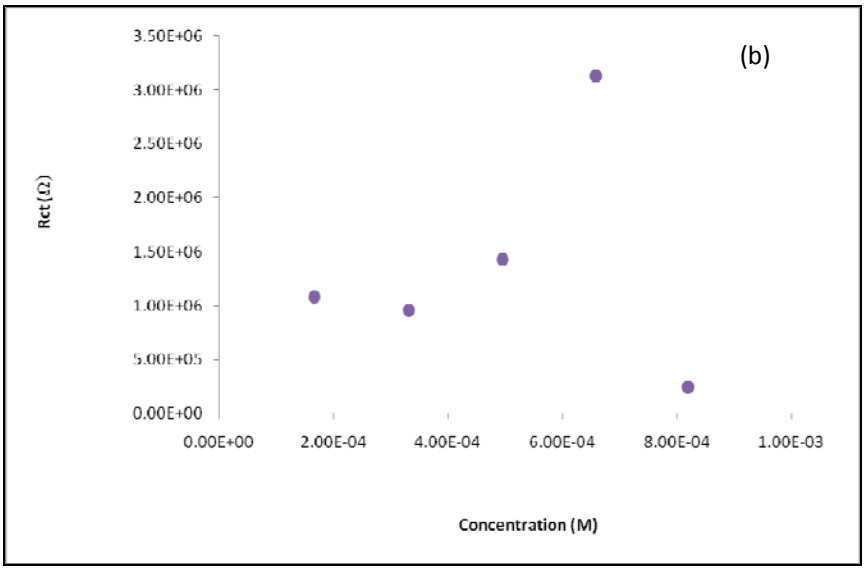
Table 4. 6:Equivalent electrical circuit fitting data for benzaldehyde, nitrophenol and m-cresol

Benzaldehyde					
Concentration	1.66×10^{-4} M	3.31×10^{-4} M	4.95×10^{-4} M	6.58×10^{-4} M	8.19×10^{-4} M
Rs	141	117	111	110	114
CPE-T	4.85×10^{-7}	4.91×10^{-7}	5.12×10^{-7}	5.25×10^{-7}	4.88×10^{-7}
CPE-P	0.952	0.945	0.936	0.931	0.949
Rp	2.82×10^{6}	3.70×10^{6}	3.66×10^{6}	2.80×10^{6}	1.90×10^{6}
Nitrophenol					
Concentration	1.66×10^{-4} M	3.31×10^{-4} M	4.95×10^{-4} M	6.58×10^{-4} M	8.19×10^{-4} M
Rs	126	122	106	96	91
CPE-T	4.56×10^{-7}	4.48×10^{-7}	4.63×10^{-7}	4.99×10^{-7}	4.86×10^{-7}
CPE-P	0.955	0.956	0.950	0.937	0.940
Rp	1.08×10^{6}	9.58×10^{5}	1.43×10^{6}	3.13×10^{6}	2.46×10^{5}
m-cresol					
concentration	1.66×10^{-4} M	3.31×10^{-4} M	4.95×10^{-4} M	6.58×10^{-4} M	8.19×10^{-4} M
Rs	145	113	-186	-205	-593

CPE-T	4.56×10^{-7}	4.48×10^{-7}	4.63×10^{-7}	4.99×10^{-7}	4.86×10^{-7}
CPE-P	0.955	0.956	0.950	0.937	0.940
Rp	1.08×10^{6}	9.58×10^{5}	1.43×10^{6}	3.13×10^{6}	2.46×10^{5}

The charge transfer resistance trend was evaluated as a function of concentration and was observed to have a maximum concentration response sepcific to each VOC investigated. The Rp maximum was related to sensitivity of the BDD electrode in much the same way as the slope of calibration plots in voltmmatric analysis. Unmodified BDD electrode showed a Rp maximum for benzaldehyde (0.4 µM), nitrobenzene (0.7 µM) and for m-cresol (0.7 µM) (Figure 4.20).





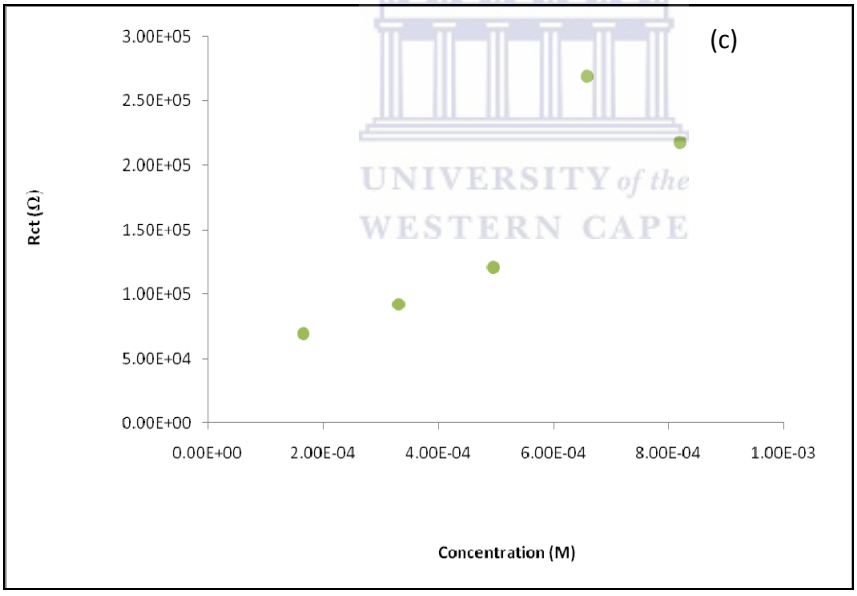


Figure 4.20: Charge transfer resistance as a function of (a) benzaldehyde, (b) nitrophenol and (c) m-cresol at different concentrations at respective formal oxidation potential .

The sensitivity of the unmodified commercial BDD sensor to these volatile organic compounds (VOC's) was determined as the fractional resistance change, ΔR/R, of the sensor. This is defined as

$$\Delta R/R = [\ |\ R_g - R_0\ |\ /\ R_0\ ] \times 100\ \%$$

Where R_g is the maximum resistance of the sensor following exposure to VOC's and R_0 is the initial resistance.

Table 4. 7: Fractional resistance of VOC's

Organic compound	**Sensitivity as fractional resistance, %**	**Concentration , µM**
Benzaldehyde	54	0.4
Nitrobenzene	69	0.7
m-cresol	91	0.7

EIS data confirmed the superior sensitivity of the BDD sensor to electrochemical oxidation intermediate of m-cresol as a fractional resistance difference of 91%. This is in good agreement with the sensor performance evaluated using fixed frequency voltammetric techniques.

4.7 Conclusion

Cyclic voltammetry, square wave voltammetry, electrochemical impedance spectroscopy was showed as suitable techniques for the determination of each unique intermediate formed from the oxidation of VOC's. The bare BDD electrode provides a sensitive sensor for benzaldehyde, nitrobenzene and m-cresol. The sensor was able to measure each intermediate product of the VOC's individually as well as in a mixture. The BDD sensor was found to be most sensitive towards m-cresol (7.1×10^{-2} μA/mM) and the lowest detection limit (1.02 μM).

CHAPTER FIVE

Cytochrome c modified boron-doped diamond for the detection of flavonoids

5.1 4-Nitrophenyl diazonium salt modified BDD

After the BDD electrode was cleaned and polished, 10 µL of a 5 mM 4-nitrophenyl diazonium salt solution was drop coated onto BDD electrode surface. The drop coated electrode was exposed to UV/light of wavelength 365 nm (VILBER LOURMAT) for a maximum of five minutes. After UV-curing the electrode was washed with acetone and water to remove excess drop coating solution that was not affixed to the BDD electrode surface during curing process. Cyclic voltammetry (CV) was used to characterize functionalized BDD electrode in a potential window of 600 mV to -600 mV and at different scan rates i.e. 50, 100, 150, 200, 250 and 300 mV/s (Figure 5.1). CV was able to confirm that the diazonium salt layer formed a stable electroactive layer that could be used for the immobilisation for the biomolecule. Scan rate dependent CV of the 4-nitrophenyl diazonium salt modified BDD electrode in 1 M KCl/HCl (pH < 1) confirmed typical electrochemistry of bound diazonium salt electrochemical interface **(**Gooding J, 2008; Beissenhirtz M, et al., 2003).

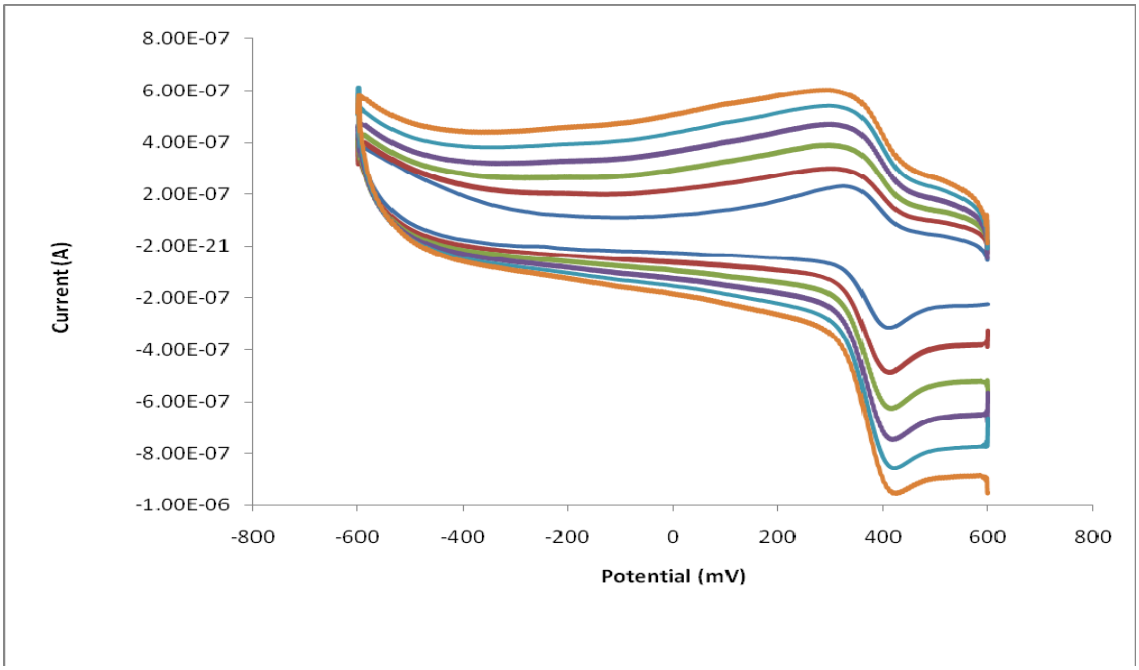


Figure 5. 1: Cyclic Voltammogram of 4-nitrophenyl diazonium salt in 1 M solution KCl/ HCl at pH < 1 and different scan rates.

5.2 Preparation of the Cytochrome c modified BDD

After the functionalization of BDD electrode with 4-nitrophenyl diazonium salt, immobilization of cytochrome c was carried out. Immobilization procedure involved cycling from 800 mV to -300 mV at a scan rate of 50 mV/s for 30 cycles (peak at -450 decreases, until it remained constant at a current of 2×10^{-7} A) in a stock solution of cytochrome c prepared as 5mg cytochrome c in 5 mL PBS (Figure 5.2) After immobilization of cytochrome c, the electrode was rinsed with distilled water and used immediately or stored in phosphate buffer (PBS, pH=7).

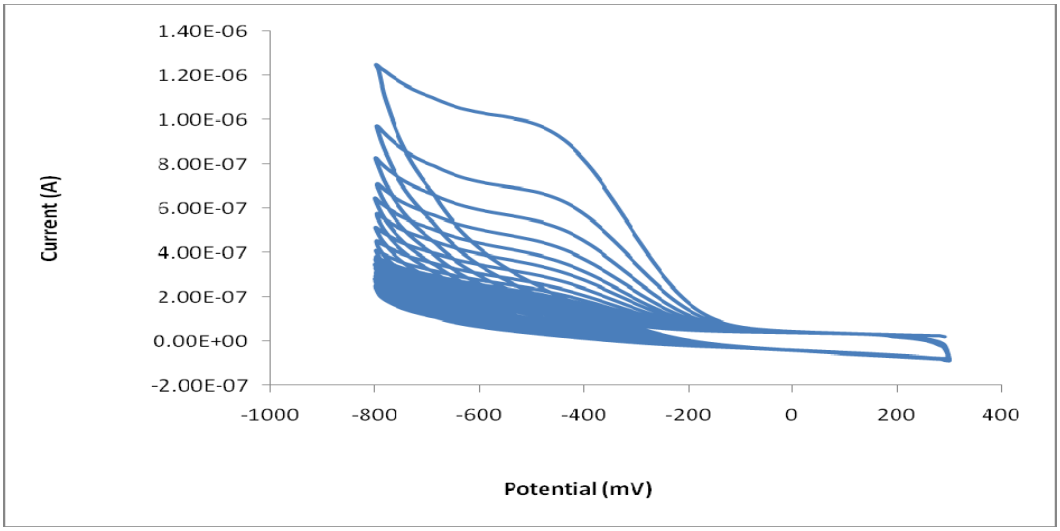


Figure 5. 2: Immobilization of cytochrome c using CV at 30 cycles, scan rate 50 mV/s and potential range of 300 to –800 mV.

Electrochemical characterization of cytochrome c modified electrode was done using CV in 5 mM PBS solution. Throughout electrochemical characterization the working pH was kept at physiological pH so as to protect the integrity of the cytochrome c (Figure 5.3).

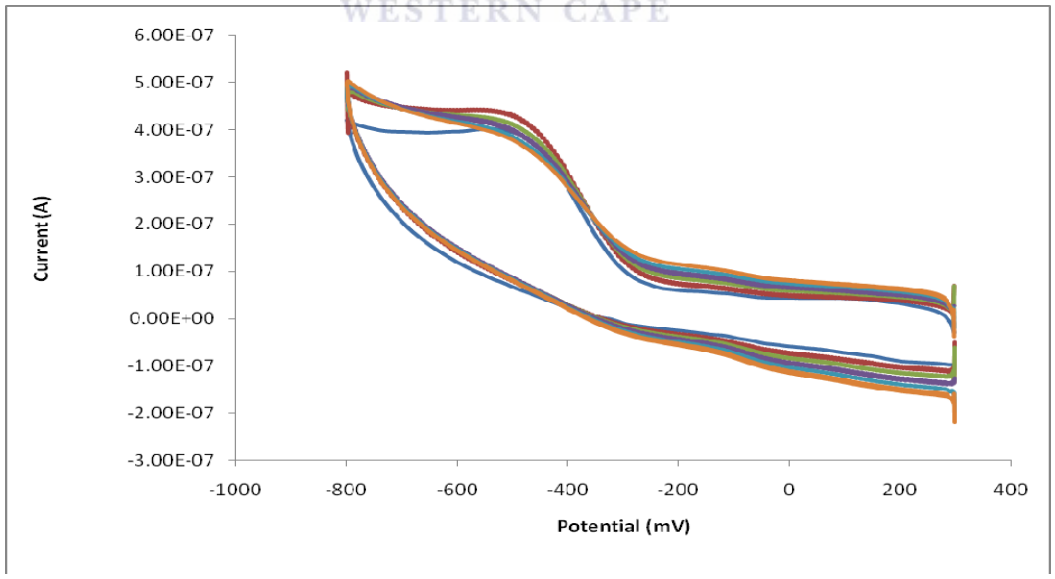


Figure 5. 3: CV of cytochrome biosensor at scan rates; 50, 60, 70, 80, 90 and 100 mV/s in 5 mM PBS.

SWV showed two distinct reduction peaks closely associated with the presence of Cu and Fe actives sites in the protein (Figure 5.4), (Hess et al, Analytical Letters, in press). CV and SWV confirmed the successful immobilisation of an electroactive cytochrome c layer onto a 4-nitrophenyl diazonium salt modified BDD electrode.

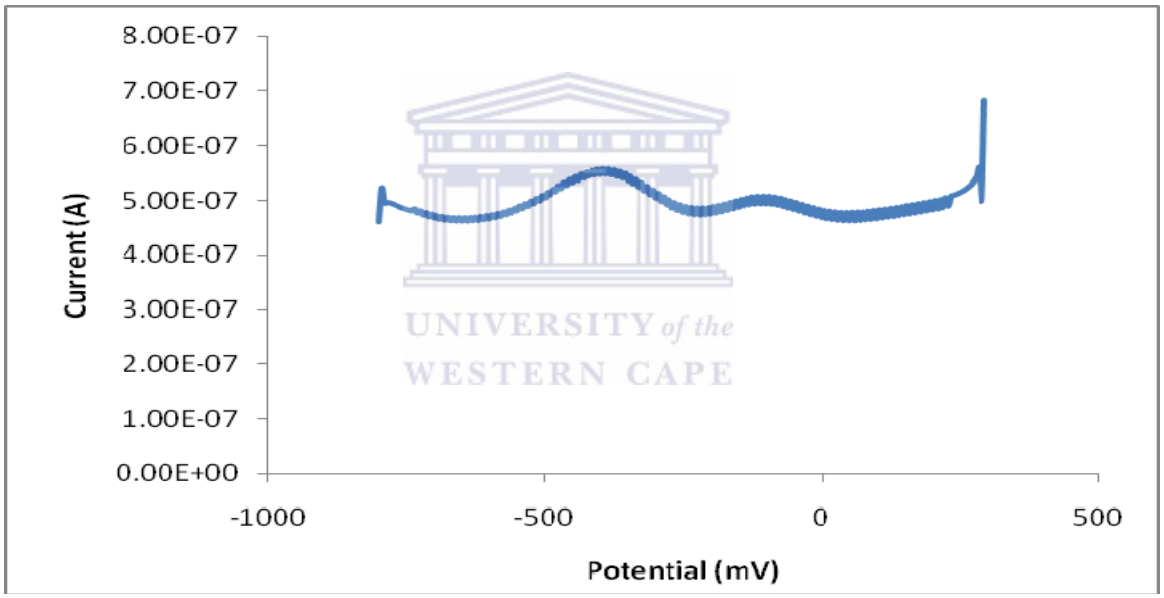


Figure 5. 4: Square Wave Voltammetry of Cytc/DZS BDD electrode at scan rate of 100 mV/s in 5 mM PBS.

Scanning electron microscopy (SEM)

The SEM images show a clear differentiation in surface morphology after each step of the modification of the commercial BDD electrode. The commercial BDD electrode has a polycrystalline structure with electronically heterogeneous characteristics. Therefore polishing and activating steps are required to improve surface electronic homogeneity and also selective functionalization. The covalent attachment of 4-nitophenyl diazonium salt onto the BDD electrode resulted in a laminar morphology of somewhat uneven distribution, but complete coverage of the BDD disk electrode. EDAX analysis based on K spectral lines confirmed the presence of at least 14% nitrogen, which was attributed to the aryl NO_2 groups, thus confirming the formation of diazonium salt layer (Figure 5.5). Incubation in cytochrome c solution produced a biosensor surface with distinctly different morphology confirming the attachment of the cytochrome c (Figure 5.6). The surface appears covered in a network of woven threads, superimposed on the laminar BDD-diazonium salt modified substrate. In the EDAX analysis of this final layer, the absence of nitrogen is significant since it suggests complete coverage of BDD-diazonium salt with cytochrome c.

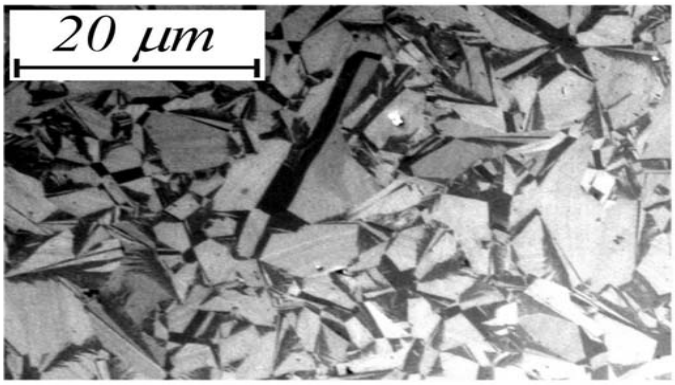


Figure 5.5 (a): Commercial boron-doped diamond

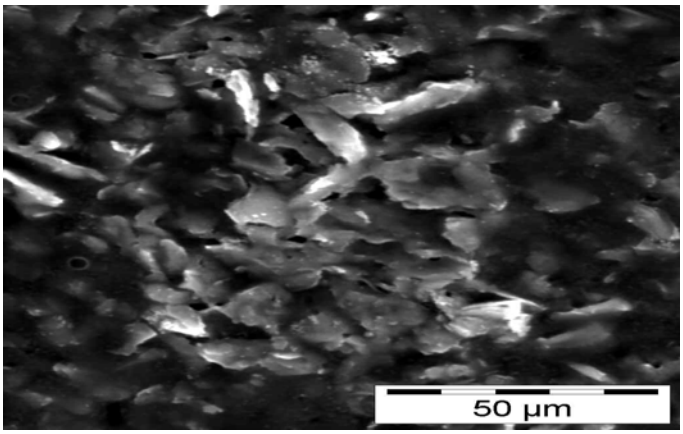


Figure 5.5 (b): Scanning electron microscopy of 4-nitrophenyl diazonium salt only.

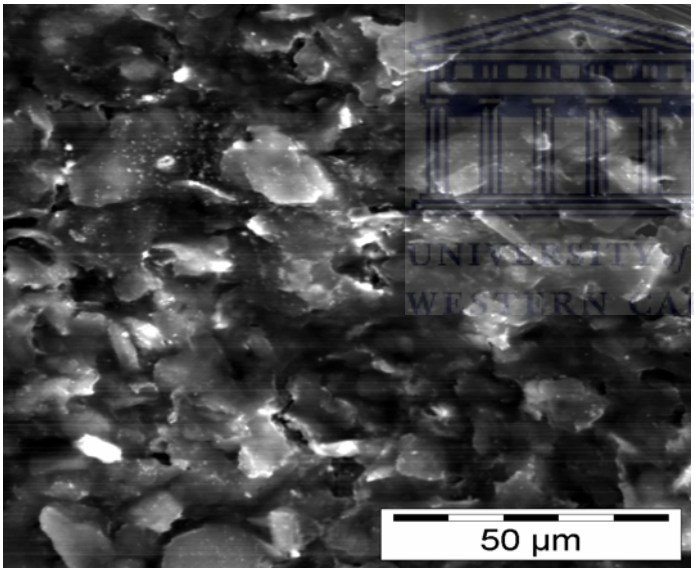


Figure 5. 5: Scanning electron microscopy of 4-nitrophenyl diazonium salt and cytochrome c.

5.2 Quercitin

The cyclic voltammogram (CV) of quercitin in 5 mM PBS (pH 7.2) at different concentrations and at fixed scan rate of 100 mV. The final concentration of quercitin in solution was 4.99×10^{-6} M, 9.98×10^{-6} M, 1.49×10^{-5} M, 1.99×10^{-5} M and 2.49×10^{-5} M, after consecutive 2 µL aliquot additions. The CV shows a reversible reaction, with a reduction peak at 113 mV and an oxidation peak at 198 mV. Cytochrome c was observed to have an electrocatalytic effect on quercitin oxidation and reduction. As the reduction and oxidation peaks increase linearly with concentration, the intensity of the reduction peak of cytochrome c decreased and finally disappeared (Figure 5.7) The oxidation peak is associated with the oxidation of the catechol moiety at ring B (Scheme 5.1), and this corresponds to a two electron, two proton reversible reaction according to literature (Zho, A., et al., 2007). The reduction peak corresponds to the reduction of the oxidation product. SWV of quercitin (Figure 5.8 a and b), confirmed the two reversible redox peaks obtained in cyclic voltammetry and formal potential evaluated was 156 mV.

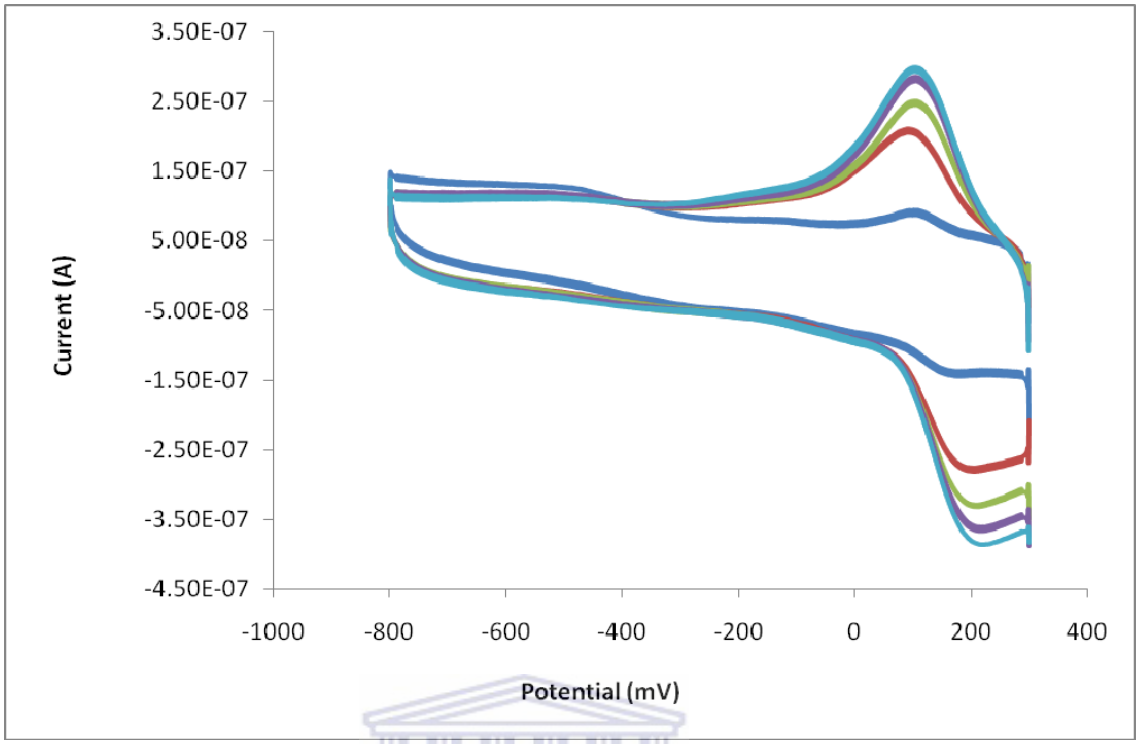
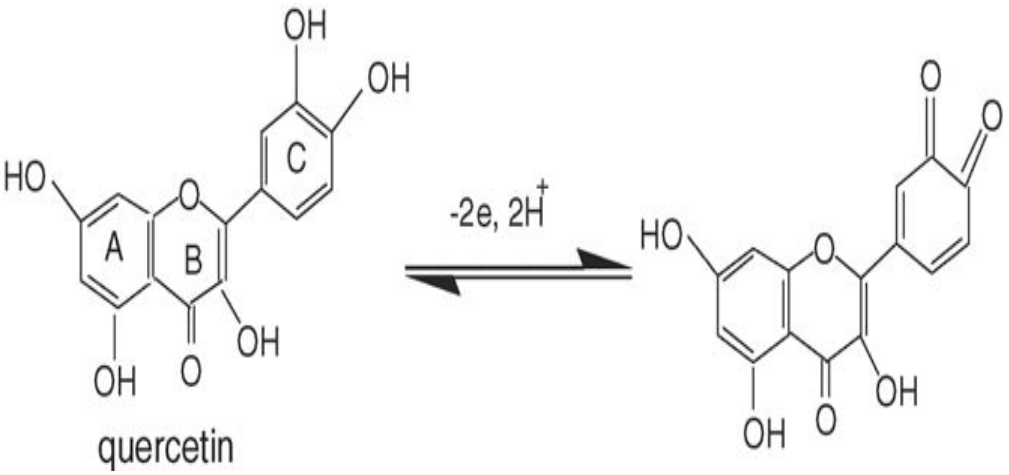
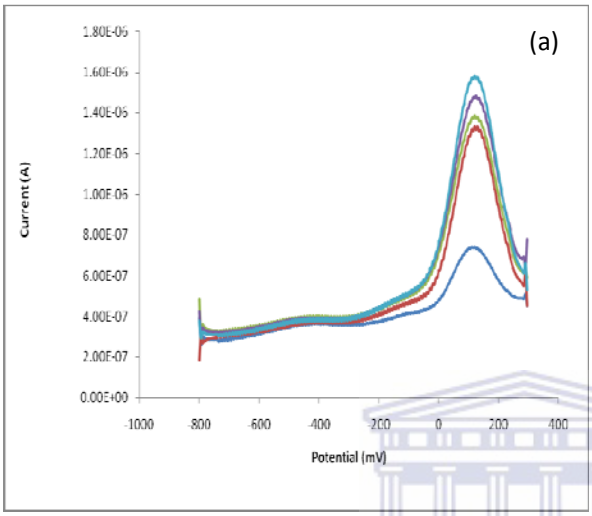


Figure 5. 6: CV of quercitin at different concentrations and fixed scan rate of

100 mV/s.



Scheme 5.1 Oxidation/reduction of catechol moiety of quercitin.



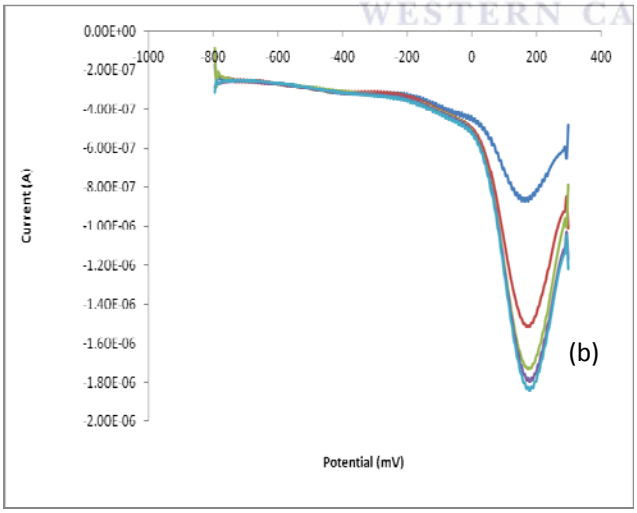


Figure 5. 7: (a) SWV of quercitin from 400 to – 800 mV at different concentrations at a frequency of 10 Hz and Es of 10 mV, (b) SWV of quercitin from -800 mV to 400 mV at different concentrations at a frequency of 10 Hz and Es of 10 mV

From CV data, calibration curve for current response at Epc and Epa as a function of increasing concentration was constructed, and a linear relationship was observed with correlation coefficients (R^2) of 0.92 and 0.94 (Figure 5.9 a and b). The sensitivity of cytochrome c BDD biosensor to quercitin was calculated at Ipc and Ipa (Table 5.1).

Table 5. 1: The sensitivity of the biosensor to quercitin

	Sensitivity (μA/mM)	Detection limit (μM)
Ipc	6.8	2.5
Ipa	7.8	1.79

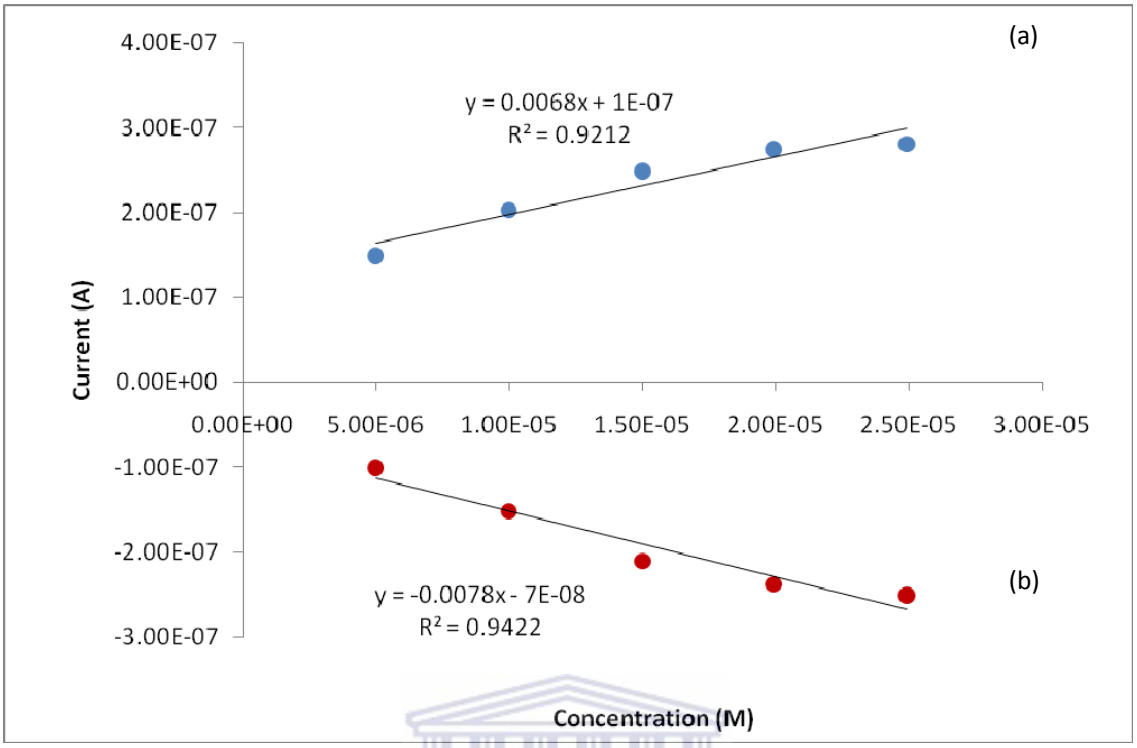


Figure 5. 8: (a) Cathodic and (b) Anodic peak currents as a function of concentration for quercitin at cytochrome c modified BDD electrode.

The cyclic voltammogram of quercitin at different scan rates; 80, 120, 140, 160, 180 and 200 mV/s (Figure 5.10). A linear plot for the cathodic and anodic peak currents versus square root of scan rate was obtained (Figure 5.11 a and b).

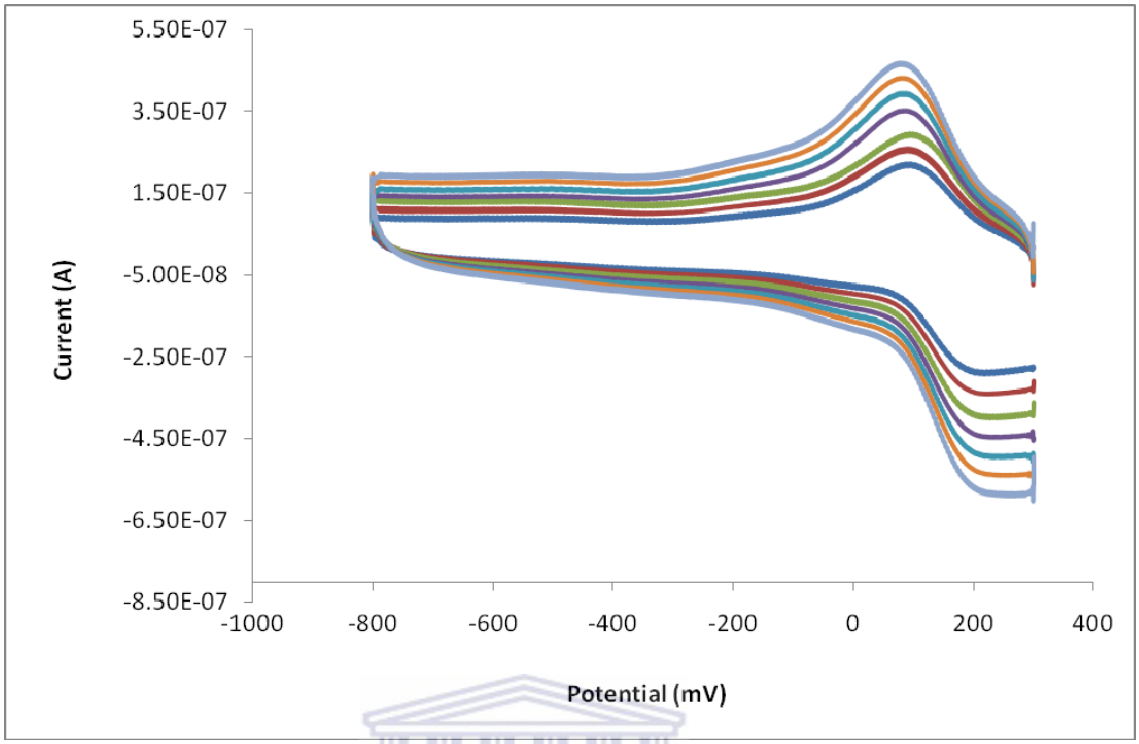


Figure 5. 9: CV of quercitin at different scan rates.

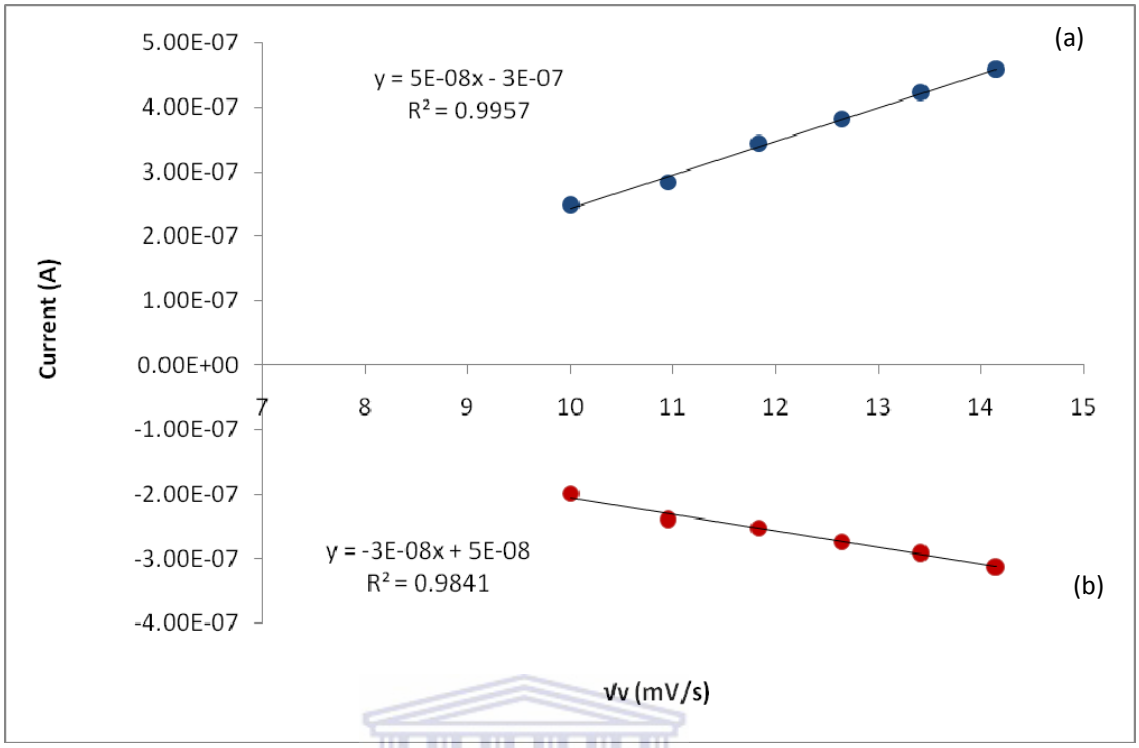


Figure 5. 10: (a) Cathodic and (b) Anodic peak currents as a function of square root of scan rate for quercitin at cytochrome c modified BDD electrode.

The peak currents (Ipc and Ipa) was directly proportional to scan rate with correlation coefficients (R^2) of 0.99 and 0.98. The De, was calculated using Randle Sevcik equation for reversible reactions and was found to be found 1.1×10^{-8} cm^2/s (Ipc) and 4.0×10^{-9} cm^2/s (Ipa) respectively.

5.3 Catechin

The cyclic voltammogram (CV) of catechin in 5 mM PBS (pH 7.2) at different concentrations is shown in figure 5.12. The CV of catechin showed a reversible peak at 298 mV (Epa), with a reduction peak at 110 mV (Epc).The same electrocatalytic effect of modifier cytochrome c was observed for catechin. The oxidation peak corresponds to the oxidation of the 3,4-dihydroxyl moiety at the B ring (catechol moiety) and the reduction peak corresponds to the reduction of the oxidation product (Scheme 5.2). The cytochrome c modified BDD electrode catalyses this oxidation/reduction reaction. This showed that the catechol moiety is more easily oxidized than the resorcinol group. The oxidation involves a two electron- two proton reversible reactions and forms o-quinone (Janeira P, et al. 2004).

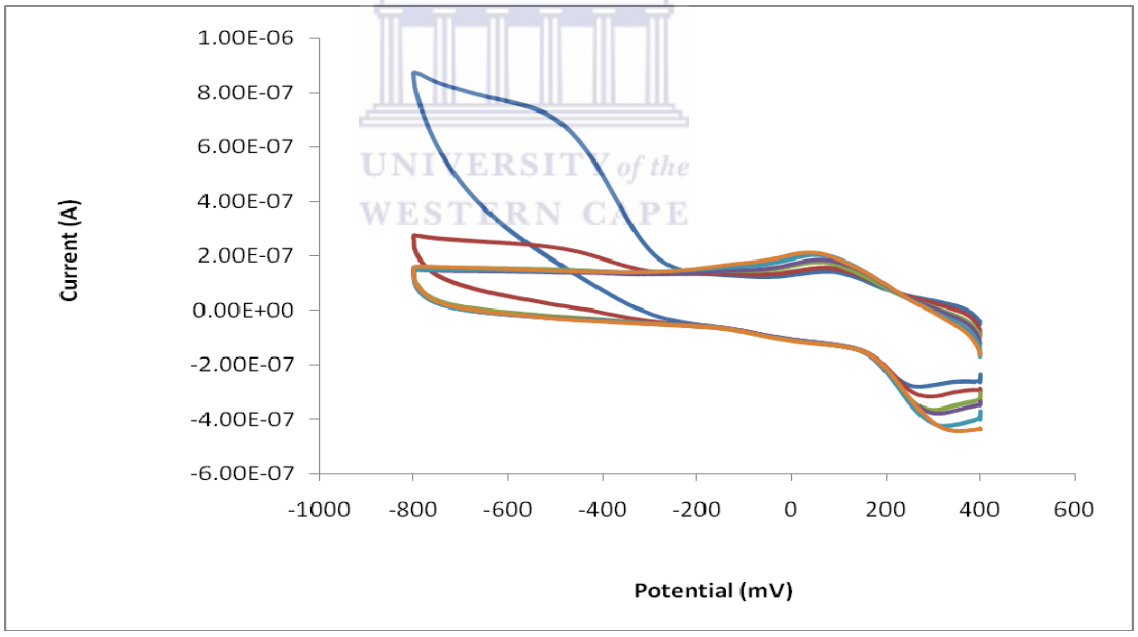
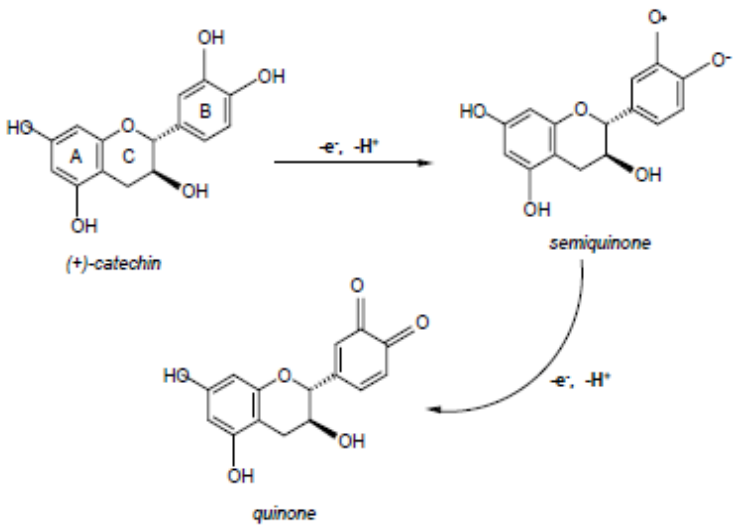
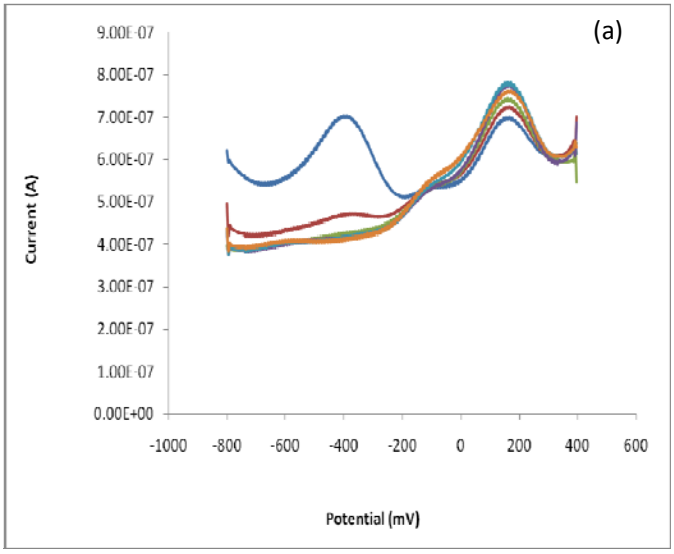


Figure 5. 11: Cyclic Voltammetry of catechin at different concentrations, 4.99×10^{-6} M, 7.49×10^{-6} M, 9.98×10^{-6} M, 1.25×10^{-5} M, 1.49×10^{-5} M and 1.74×10^{-5} M.



Scheme 5.2 Oxidation/reduction of catechol moiety of catechin.

Square wave voltammetry (SWV) was also used as it has the advatage of a greater speed of analysis, a lower consumption of electroactive species and reduced problems with blocking of the electrode surface in relation to other voltammetry methods. The formal potential was obtained from SWV was 204 mV (Figure 5.13 (a) and (b)).



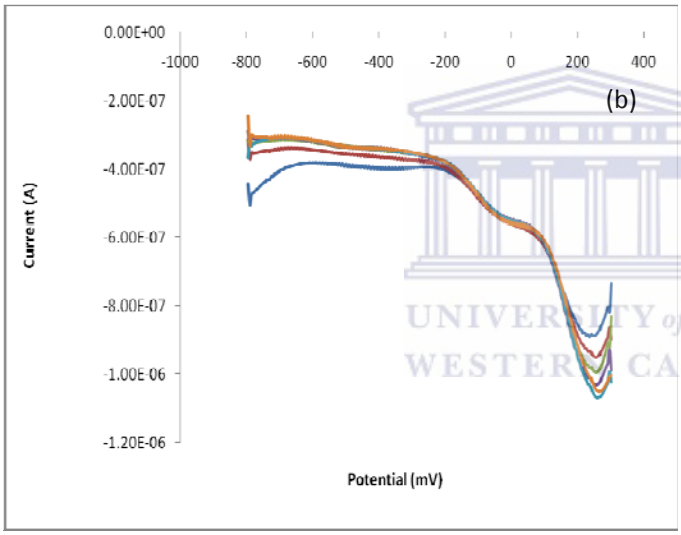


Figure 5. 12: (a) SWV of catechin (Epc) from 400 to -800 mV at frequency 10 Hz and Es of 10 mV, (b) SWV of catechin (Epa) from -800 to 400 mV at frequency 10 Hz and Es of 10 mV.

The calibration plot constructed from CV data, showed linear response to increasing concentration over the concentration range investigated with correlation coefficients (R^2) of

0.98 and 0.98 (Figure 5.14 (a) Cathodic and (b) Anodic peak currents). The sensitivity of cytochrome c BDD electrode to catechin was calculated at Ipc and Ipa (Table 5.2).

Table 5. 2: The sensitivity and detection limit of the biosensor to catechin

	Sensitivity (µA/mM)	Detection limit (µM)
Ipc	4.8	0.42
Ipa	12.7	1.02

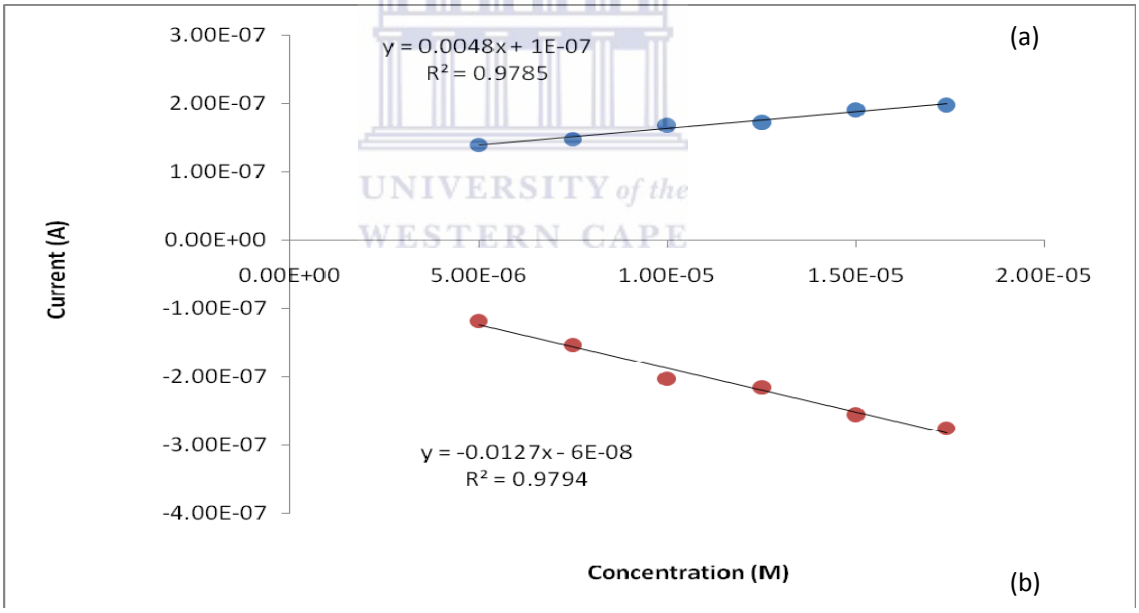


Figure 5. 13: (a) Cathodic and (b) Anodic peak currents as a function of concentration for catechin at cytochrome c modified BDD.

Scan rate dependence at 100, 120, 140, 160, 180 and 200 mV/s and fixed concentration of 2.49 $\times 10^{-5}$ M (Figure 5.15) resulted in the same degree of linearity to quercitin oxidation as well as reduction (Figure 5.16 (a) and (b)), with the same correlation coefficients (R^2) i.e 0.99. This is an indication that the cytochrome c biosensor has the same electrocatalytic effect on both quercitin and catechin.

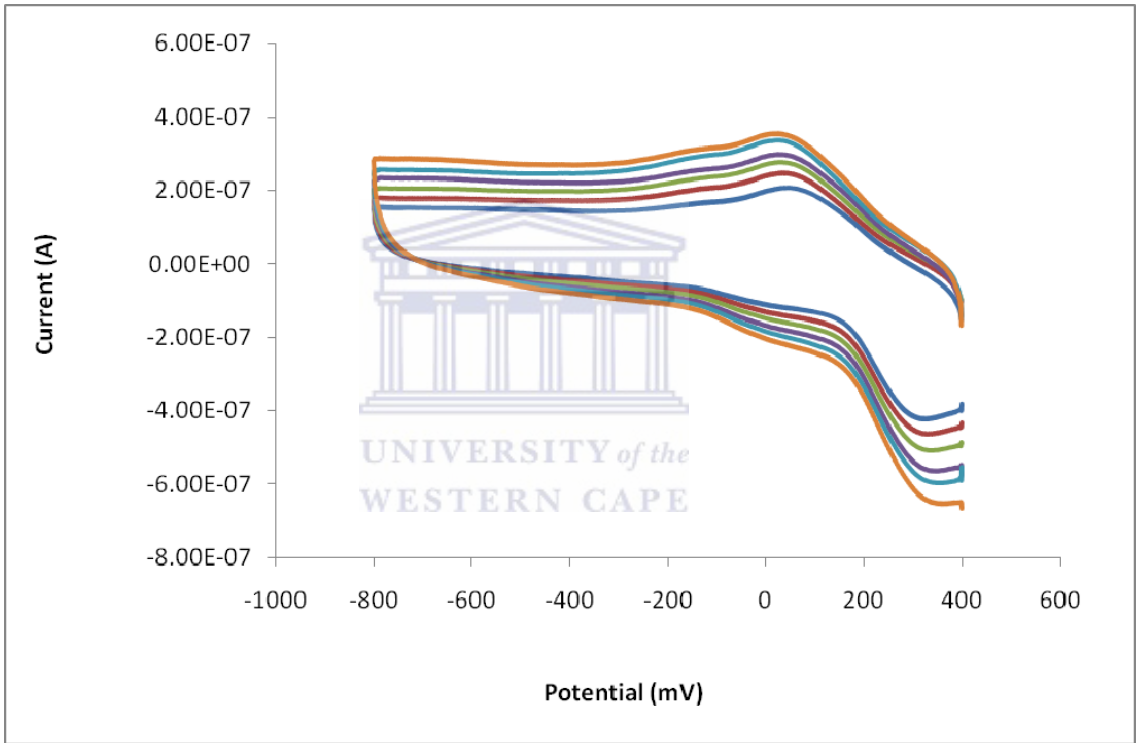


Figure 5. 14: Cyclic Voltammetry of catechin in 5 mM PBS at different scan rates ; 100, 120, 140, 160, 180 and 200 mV/s.

The same response for quercitin and catechin was observed due to the fact that both compounds have catechol moieties that are involved in the oxidation/reduction reactions as to the resorcinol group of rutin which also undergoes oxidation.

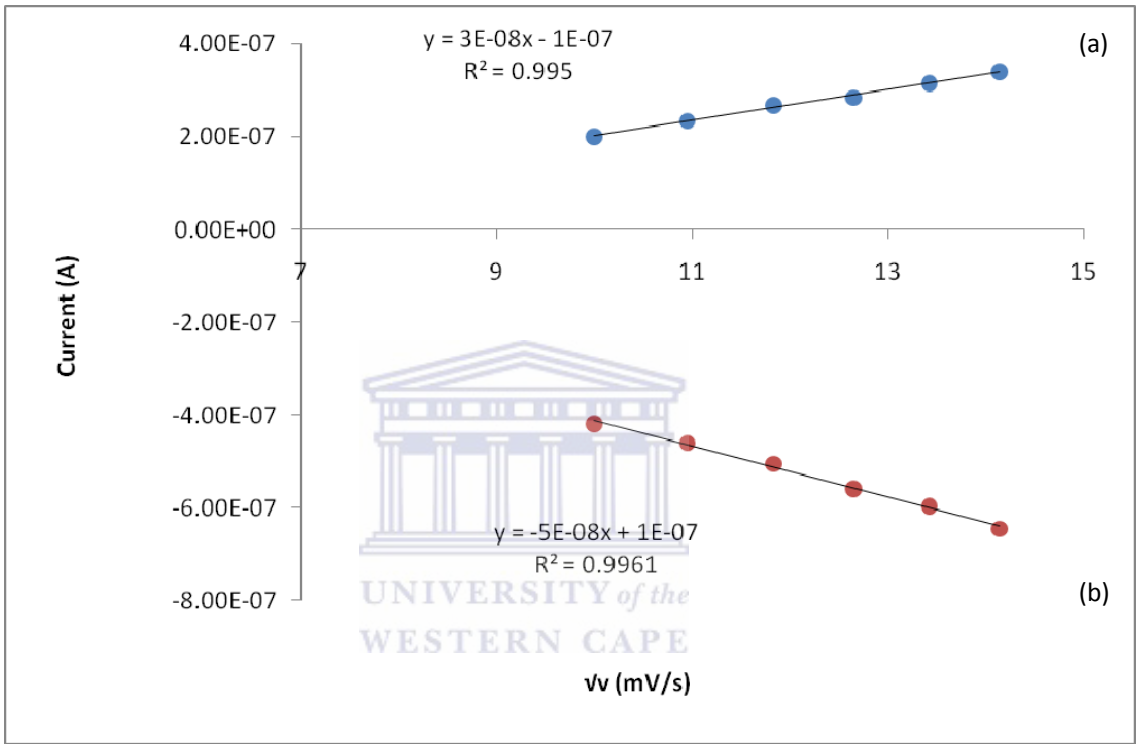


Figure 5. 15: (a) Cathodic and (b) Anodic peak currents as a function of square root of scan rate for catechin at cytochrome c modified BDD electrode.

The electron diffusion coefficient De, was evaluated as before and was calculated to be 1.1 × 10^{-8} for Ipc and 4.0 × 10^{-9} for Ipa respectively. From De, it can be seen that both quercitin and catechin diffuses at the same speed to the electrode interface and the modifier cytochrome c response is the same for both compounds.

5.4 Electrochemical characterization of Rutin

Concentration dependent analysis of rutin in 5 mM PBS (pH 7.2) at fixed scan rate of 100 mV/s was performed. Oxidation and reduction products were identified for the construction of calibration curves. CV showed two reversible reactions, with reduction peaks at 263 mV and -89 mV, and oxidation peaks at 304 mV and 80 mV (Figure 5.17). The two oxidation peaks are associated with the oxidation of hydroxyls groups and the reduction peaks are associated with the reduction of the oxidation peaks. Rutin has a catechol moiety 3,4-dihydroxy group in ring B, and resorcinol group 5,7-dihydroxy group in ring A. The oxidation of the catechol moiety occurs first and is associated with Epa_1. The oxidation process involves a two electron – two proton reversible reaction and forms o-quinone (Scheme 5.3), (Ghica and Brett, 2005). Epa_2 is associated with the oxidation of resorcinol group (Ghica and Brett, 2005; Xu et al., 2005). SWV confirmed two pairs of reversible redox peaks obtained from CV with formal potentials 285 mV for redox peaks Epc_1 and Epa_1, 89 mV for redox peaks Epc_2 and Epa_2 (Figure 4.18 a, b).

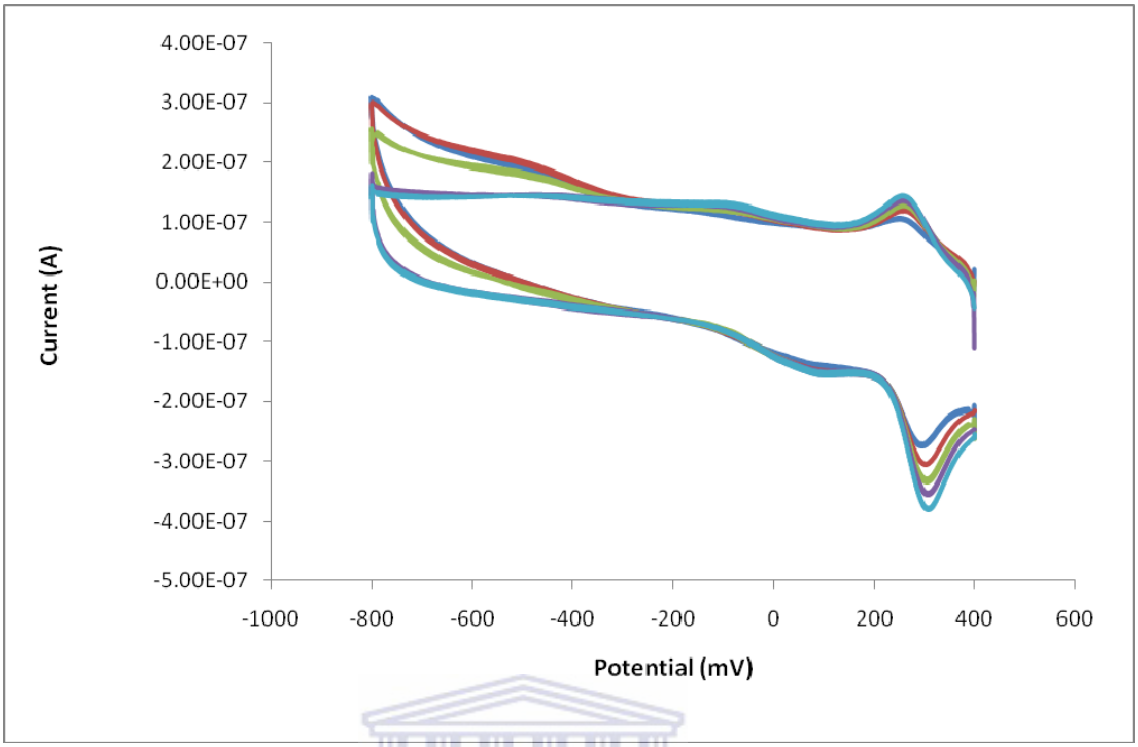
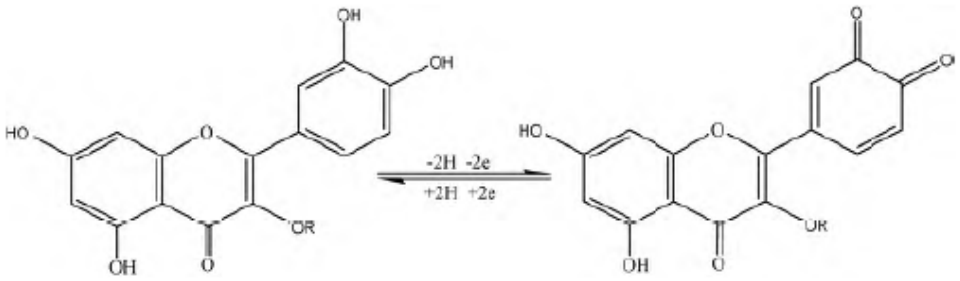
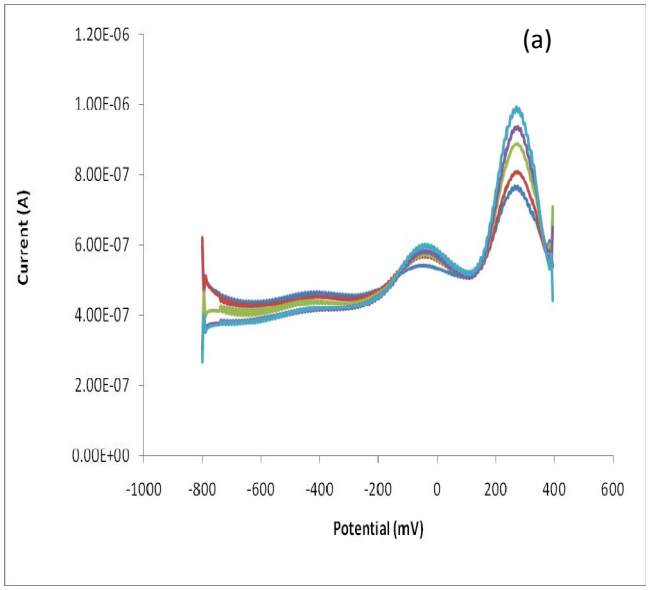


Figure 5. 16: CV of rutin at different concentrations, 9.98×10^{-5} M, 1.50×10^{-5} M, 1.99×10^{-5} M, 2.49×10^{-5} M, 2.98×10^{-5} M and 3.48×10^{-5} in 5 mM PBS.



Scheme 5.3 Oxidation/reduction catechol moiety of rutin.



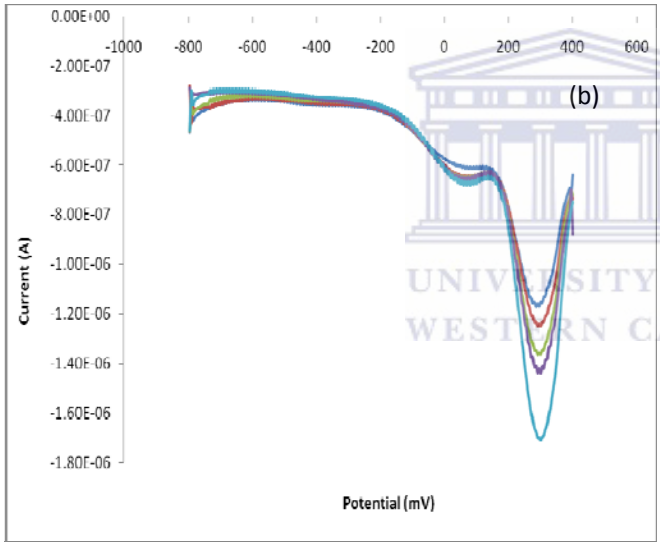


Figure 5. 17: (a) SWV of rutin from 400 to -800 mV at a frequency of 10 Hz and a step potential of 10 mV, (b) SWV of rutin from -800 to 400 mV at a frequency of 10 Hz and step potential of 10 mV.

A calibration curve for the current responses at Epa_1, Epa_2, Epc_1 and Epc_2 as a function of increasing concentration, was constructed and a linear relationship was observed with

correlation coefficients (R^2) of 0.93 for Epc_1 (Figure 5.19 a), 0.98 for Epa_1 (Figure 5.19 b), 0.98 for Epc_2 (Figure 5.19 c) and 0.81 for Epa_2 (Figure 5.19 d). Sensitivity and detection limits were evaluated at each oxidation and reduction peak (Table 5.3).

Table 5. 3: The sensitivity and detection limit of the biosensor to rutin

	Sensitivity (μA/mM)	Detection limit (μM)
Ipc_1	1.7	2.94
Ipa_1	5.8	1.27
Ipc_2	0.5	4.0
Ipa_2	0.8	11.24

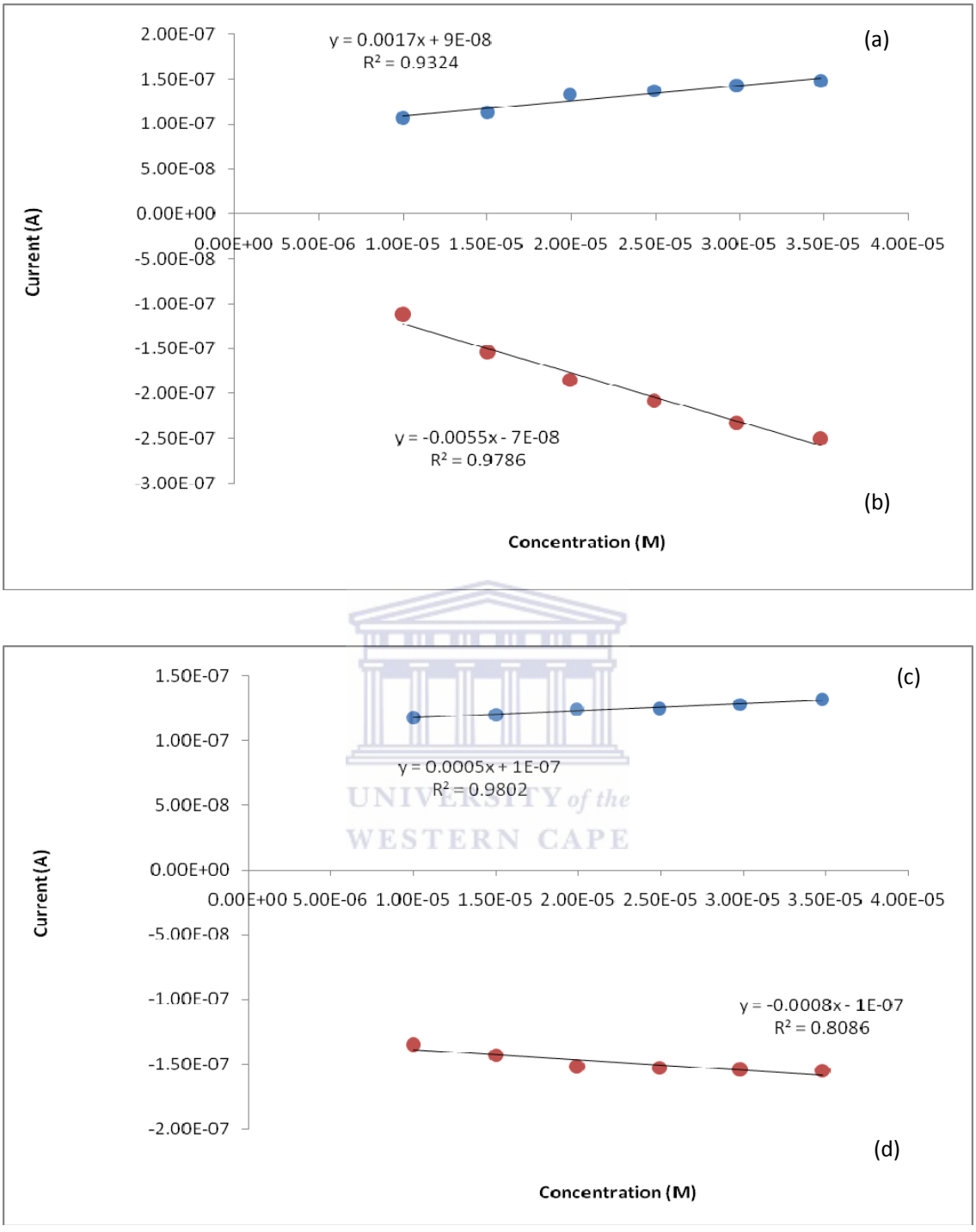


Figure 5. 18: (a) Cathodic (Ipc_1); (b) Anodic (Ipa_1); (c) Cathodic (Ipc_2) and (d) Anodic (Ipa_2) peak currents as a function of concentration for rutin at cytochrome c modified BDD electrode.

Scan rate dependent CV of rutin for a fixed concentration (3.48×10^{-5}M) showed both pair of reversible redox reactions having the same linearity with all four peaks having correlation coeffient (R^2) of 0.99, at high scan rates (Figure 5.20).

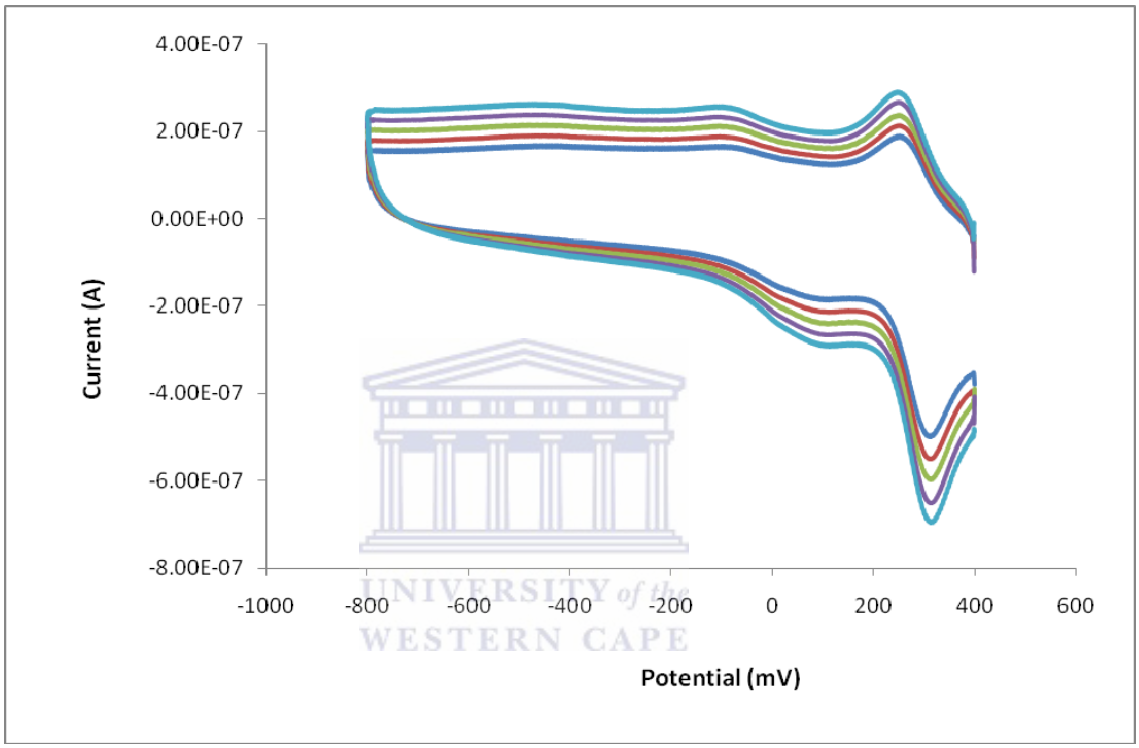
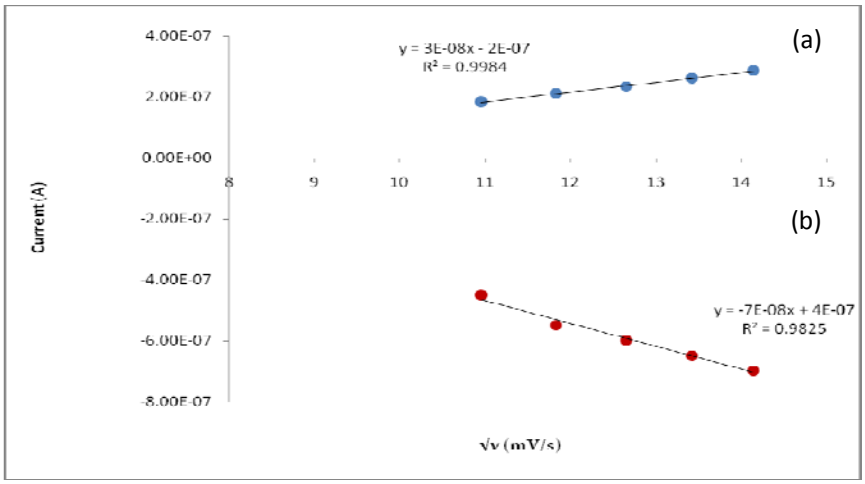


Figure 5. 19: CV of rutin at different scan rates; 120, 140, 160, 180 and 200 mV/s.

A calibration curve for Ipc_1, Ipa_1, Ipc_2 and Ipa_2 versus scan rate showed current increase directly proportinal to scan rate with correlation coeffients (R^2) of 0.99 for Ipc_1, 0.98 for Ipa_1, 0.99 for Ipc_2 and 0.99 for Ipa_2 (Figure 5.21 a, b, c and d).



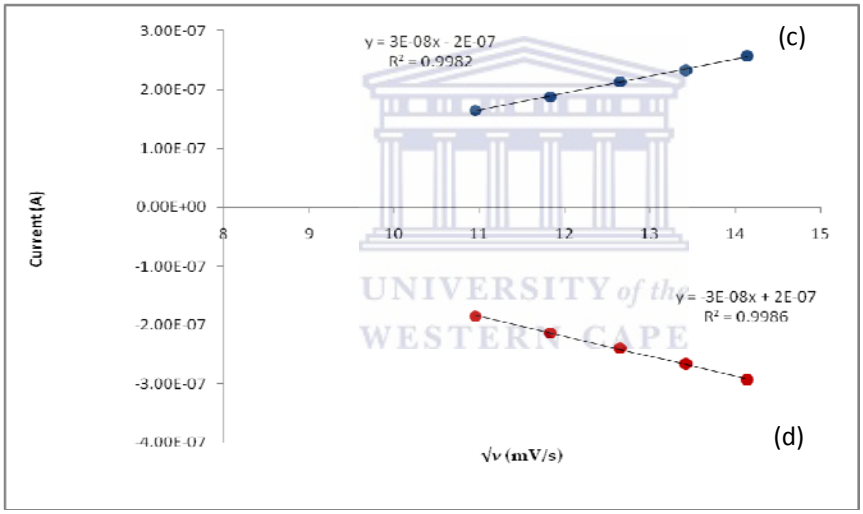
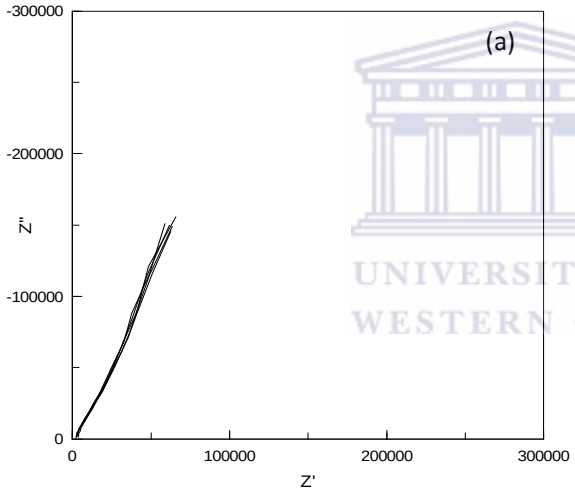


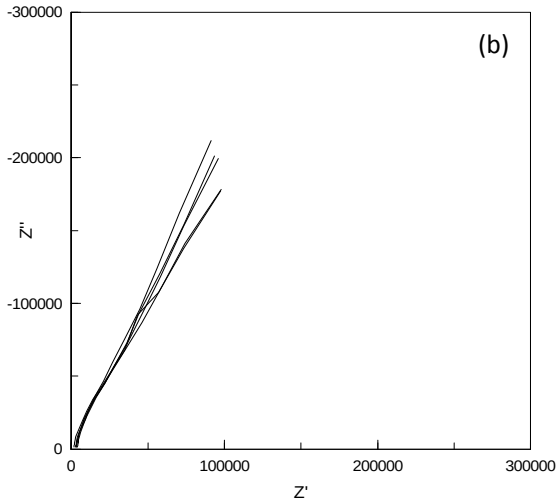
Figure 5. 20: (a) Cathodic (Ipc_1); (b) Anodic (Ipa_1); (c) Cathodic(Ipc_2), and (d) Anodic (Ipa_2) peak currents as a function of scan rate for rutin at cytochrome c modified BDD electrode.

The De for the first reversible redox reaction Ipc_1 and Ipa_1 was calculated to be 4.9×10^{-9} and 2.1×10^{-8} and for the second reversible redox reaction, Ipc_2 was 4.0×10^{-9} and Ipa_2 was 4.0×10^{-9}.

5.6 Electrochemical impedance spectroscopy

Impedance spectroscopy is a very useful technique to derive the capacitance and gives complementary frequency dependent information. The potential was kept fixed for each particular flavonoid corresponding to their formal potentials. The high frequency was 1000 Hz and the low frequency was 100 mHz. Complex plot data was fitted to an equivalent electrical circuit with a fitting error percentage below 15% considered acceptable.





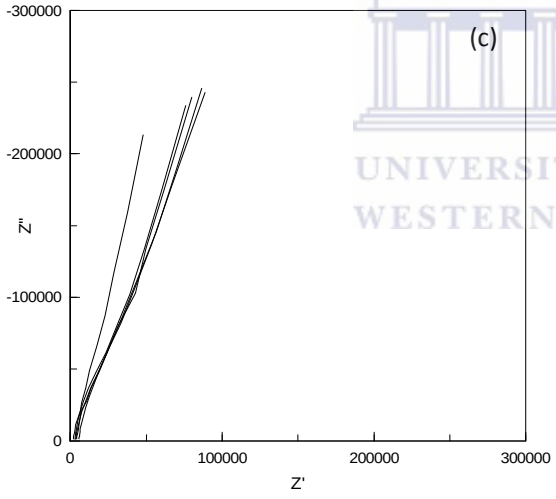


Figure 5. 21: Complex plot of (a) quecitin, (b) catechin and (c) rutin at different concentrations at respective formal oxidation potential .

The data was modelled as a simple Randles circuit comprising representing solution resistance (Rs), interfacial capacitance as a constant phase element (CPE) and charge transfer resistance (Rct) (Figure 5.23).

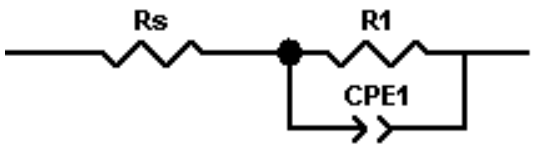


Figure 5. 22: Randles circuit used for EIS data fitting.

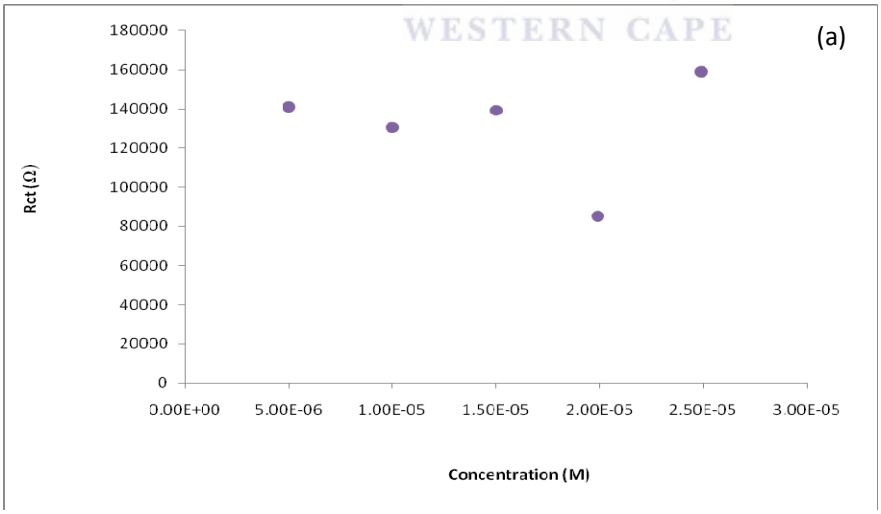
The results obtained from data fitting showed the largest observable trend in values associated with charge transfer resistance. The solution resistance remains fairly constant throughout, since the electrolyte (PBS) and flavonoids concentrations in all experiments were kept the same. The constant phase element is an indication of the capacitive nature of the interface. Data obtained for quercitin, catechin and rutin are consistent and no significant change associated with increased concentration is observed. This is an indication that adsorption at the electrode interface is not a major contributing factor to the efficient degradation of the flavonoid compounds studied (Table 5.4).

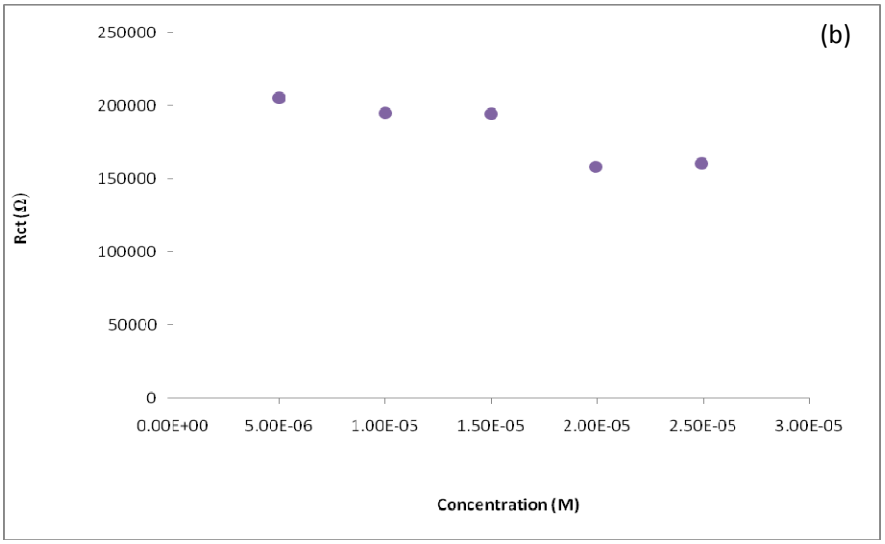
Table 5. 4: Equivalent electrical circuit fitting data for quercitin, catechin and rutin.

	4.99×10^{-6} M	9.98×10^{-6} M	1.49×10^{-5}	1.99×10^{-5} M	2.49×10^{-5} M
Quercitin					
Rs	3248	2279	1777	2163	2098
CPE-T	5.73×10^{-7}	6.38×10^{-7}	6.40×10^{-7}	4.70×10^{-7}	6.62×10^{-7}
CPE-P	0.843	0.827	0.829	0.871	0.821
Rp	1.41×10^{5}	1.30×10^{5}	1.39×10^{5}	8.52×10^{4}	1.59×10^{5}
Catechin					
Rs	3141	3704	2804	2471	1351
CPE-T	3.35×10^{-7}	3.47×10^{-7}	3.59×10^{-7}	3.39×10^{-7}	3.48×10^{-7}
CPE-P	0.909	0.903	0.897	0.909	0.907
Rp	2.05×10^{5}	1.95×10^{5}	1.94×10^{5}	1.58×10^{5}	1.60×10^{5}
Rutin					
Rs	5449	3889	2824	1926	1962
CPE-T	3.02×10^{-7}	3.42×10^{-7}	4.99×10^{-7}	4.83×10^{-7}	4.89×10^{-7}

CPE-P	0.926	0.909	0.912	0.920	0.916
Rp	2.97×10^5	3.59×10^5	8.79×10^5	8.67×10^5	1.02×10^6

The charge trasnfer resistance trend was evaluated as a function of concentration and was observed to have a maximum concentration response sepcific to each flavonoid investigated. The Rp maximum was related to sensitivity of the modified BDD electrode in much the same was as the slope of calibration plots in voltmmatric analysis. Modified BDD electrode showed a Rp maximum for quercitin (2.49×10^{-5} M), catechin (4.99×10^{-6} M) and for rutin (2.49×10^{-5} M) (Figure 5.24 a, b, c)





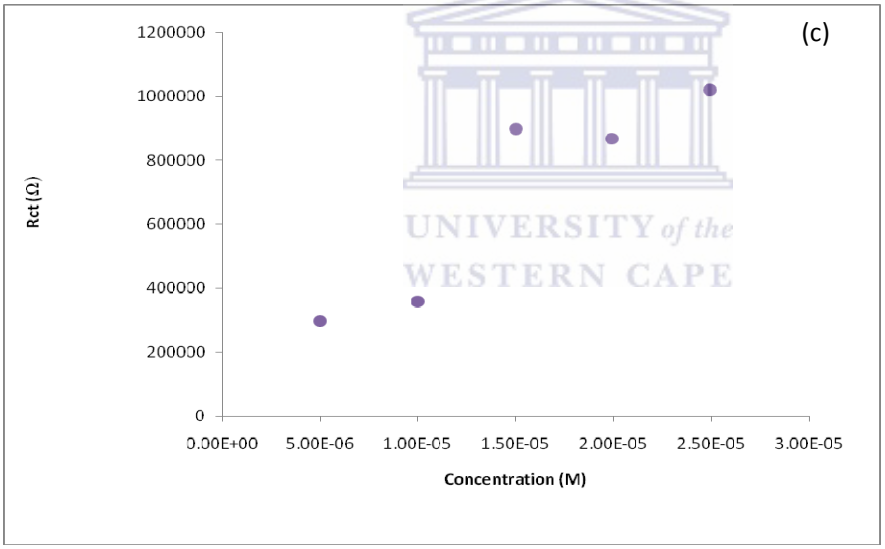


Figure 5. 23: Charge transfer resistance as a function of (a) quercitin, (b) catechin and (c) rutin at different concentrations at respective formal potential .

The sensitivity of the biosensor to these flavonoids was determined as the fractional resistance change, ΔR/R. This is defined as

$$\Delta R/R = [\ |\ R_g - R_0\ |\ /\ R_0\ ] \times 100\ \%$$

Where R_g is the maximum resistance of the sensor following exposure to flavonoids and R_0 is the initial resistance.

Table 5. 5: Fractional resistance of flavonoids

Flavonoid	Fractional resistance
Quercitin	56%
Catechin	40%
Rutin	100%

EIS data confirmed the superior sensitivity of the modified BDD electrode to electrochemical oxidation/reduction of rutin as a fractional resistance difference of 100%. This is in good agreement with the performance evaluated using fixed frequency voltammetric techniques.

5.7 Conclusion

Cytochrome c was immobilized onto a 4-nitrophenyl diazonium salt modified BDD electrode. The sensing range of the cytochrome c modified BDD electrode for flavonoids was from 4.99 μM to 25 μM. The chemical transformation of flavonoids was investigated by cyclic voltammetry, square wave voltammetry and electrochemical impedance spectroscopy. The oxidation/reduction reaction of these flavonoids was observed to be strongly related to their structure, which was made up of several free phenolic OH groups. The oxidation process of flavonoids in general, showed reversible reactions corresponding to oxidation of catechol moiety, namely 3,4-dihydroxyl group in ring B, followed by the complementary reduction peak.

CHAPTER SIX

OVERALL CONCLUSION

6.1 Conclusion

Bare BDD electrode was able to degrade each of the VOC's in sulfuric acid solution. It was also able to measure each intermediate product of the VOC oxidation. This study supports the use of cyclic voltammetry, square wave voltammetry and electrochemical impedance spectroscopy for simple and rapid identification of individual electrochemical intermediates as a basis for speciation of VOC, typically presenting as an analytical mixture. An unique oxidation intermediate for benzaldehyde was observed at 330 mV and was used in the construction of an appropriate calibration curve. The unmodified BDD electrode was able to measure benzaldehyde at a detection limit of 2.78×10^{2} µM and sensitivity of 1×10^{-4} µA/mM. At a peak potential of 820 mV the intermediate for nitrobenzene was observed, and was used to construct a calibration curve. The unmodified BDD electrode was able to measure nitrobenzene at a detection limit of 53.1×10^{1} µM and sensitivity of 1.6×10^{-3} µA/mM. The unique intermediate for m-cresol was observed at 1192 mV. m-cresol had the lowest detection limit 1.02 µM and sensitivity of 7.1×10^{-2} µA/mM. The intermediates identified were benzoic acid for benzaldehyde, nitrophenol for nitrobenzene and methylcatechol for m-cresol. The electron diffusion coefficient (De) was interpreted as a measure of the rate of electron transfer in species diffusing to the electrode interface and was

calculated using Randle-Sevick equation and the apparent rate constants (k^0) was evaluated using Nicholson equation.

Cytochrome c biocatalyst was prepared by functionalizing the BDD electrode with 4-nitrophenyl diazonium and then electrochemically immobilizing cytochrome c onto the functionalized electrode. A well defined pair of reversible peaks was obtained for quercitin (Epa: 198 mV and Epc: 113 mV) and catechin (Epa: 298 mV and Epc: 110 mV) and two pairs of reversible redox peaks for rutin (Epa_1: 304 mV, Epa_2: 80 mV, Epc_1: 263 mV and Epc_2: -89 mV) at cytochrome c biosensor. The experimental results obtained from voltammtery, were beneficial in elucidating the mechanism of electron transfer of quercitin, catechin and rutin. The data showed reversible process corresponding to the oxidation of catechol moiety in ring B for all the three flavonoids and the oxidation of the resorcinol group only for rutin. The cytochrome c modified BDD was most sensitive to rutin for both redox reactions as to quercitin and catechin. Charge transfer resistance, obtained from EIS data, was chosen as the parameter to express the rate of catalytic reduction as a fractional resistance difference percentage, at each respective analyte formal potential. Rutin displayed the highest fractional resistance (100%), which was in good agreement with the high sensitivity as measured by CV.

6.2 Future work

- Further structural analysis by SNFTIR.

- Further morphology investigation using atomic force microscopy (AFM) and scanning electrochemical microscopy (SEM) to measure areas of reactivity related to surface morphology.

- Application to real water samples to explore the effect of a real sample matrix on measurement sensitivity and selectivity.

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