Polyamic Acid-Graphene Oxide Nanocomposite for Electrochemical Screening of Antibiotic Residues in Water

By

Siyabulela Hamnca

A thesis submitted in partial fulfilment of the requirements for the degree of

Magister Scientiae in Nanoscience

Faculty of Science

University of the Western Cape

Cape Town, South Africa

Supervisor: Prof PGL Baker

November 2015

KEYWORDS

Organic Compounds

Antibiotics

Water pollution

Electrochemical sensors

Electrochemistry

Polyamic Acid (PAA)

Graphene Oxide (GO)

Cyclic voltammetry (CV)

Square wave voltammetry (SWV)

Scanning Electron Microscopy (SEM)

Fourier Transform Infrared Spectroscopy (FTIR)

Norfloxacin

Neomycin

Limit of detection (LOD)

Limit of quantification (LOQ)

ABSTRACT

Pollution of water sources, aquifers and wetland systems caused by industry, agriculture, and municipally treated wastewater is a worldwide problem that contributes to the scarcity of clean and potable water. Rivers, channels, lakes, oceans, and ground water are often contaminated by a variety of organic substances that can affect aquatic life and threaten human health. Organic compounds such as antibiotics that are not effectively removed by modern day water treatment technology are a growing threat to water quality and health.

The emergence of antibiotics in the environment particularly aquatics have become a matter of concern as they may result in induction and spread of bacterial resistance which may be harmful to humans or animals. After administration, antibiotics for human use or their metabolites are excreted into the effluent and reach the sewage treatment plant (STP). Not all Antibiotics in sewage treatment plants are eliminated. Consequently they can pass through the sewage system and may end up in environmental and even potable water systems. Antibiotic residues have been reportedly found in places such as hospital wastewaters, wastewater treatment plants and surface waters all over the world with concentrations ranging from approximately 60-120000 ng/, 2-580 ng/L and 5-1300 ng/L respectively.

The current methods that are used to detect antibiotics can be quite expensive and time consuming due to sample preparation (necessary for detection of very low concentrations of antibiotics in water) and technology used in the instruments. Electrochemical sensors and biosensors are simple systems, with high selectivity and sensitivity for individual measurements and cost effectiveness. The development of composites based on conductive phases dispersed in polymeric matrices has led to important advances in analytical electrochemistry.

Polyamic acid and graphene oxide are both materials with well-defined electrochemistry and are easily processable in the design of various sensor formats. In this study we present a novel polyamic acid - graphene oxide (PAA/GO) electrode which was prepared for electrochemical screening of antibiotic residues in aqueous systems. Polyamic acid (PAA) and graphene oxide (GO) were successfully synthesized independently and characterized using SEM which was used to study the morphology of the PAA, FTIR spectroscopy to confirm chemical structures and functional groups as well as CV and SWV which were used to identify the unique electrochemical behavior of PAA and GO respectively. Polyamic acid-graphene oxide nanocomposite was prepared and characterized by CV, SWV, FTIR and SEM. The novel electrode (PAA/GO/SPCE) was prepared by electrochemically depositing PAA/GO (0.03 mg/mL) onto SPCE electrodes using 5 cycles between −1000 mV and 1000 mV at 50 mVs. The analytical performance of the electrochemical sensor towards detection of neomyxin and norlfoxacin was compared to standard Uv-vis spectroscopy method. The Uv-vis spectroscopy showed LOD of $1.61x10^{-5}$ M and $1.41x10^{-5}$ M for norfloxacin and neomycin respectively. The PAA/GO electrochemical sensor had a LOD of $3.37x10^{-7}$ M for norfloxacin and $1.066x10^{-6}$ M for neomycin. Sensitivity of the UV/vis method was comparable to electrochemical sensor sensitivity for neomycin and norfloxacin.

DECLARATION

I declare that **“Polyamic acid-Graphene Oxide Nanocomposite for Electrochemical Screening of Antibiotic residues in water.”** is my own work, that it has not been submitted before for any degree or assessment in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

Siyabulela Hamnca **November 2015**

Signature ………… ………………………………..

Supervisor: Prof. Priscilla G.L. Baker

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God for granting me the opportunity to pursue and complete my degree.

I express my sincere gratitude and appreciation to my supervisors Prof Priscilla G.L Baker and Prof Emmanuel Iwuoha, for their support, supervision, guidance and encouragement.

To the researchers in the Sensorlab, I thank you for welcoming me, helping to complete this project and your support throughout.

To my family ,my mother Mandiswa Constance Hamnca ,my grandmother Nowinisile Godlo-Hamnca, Hombakazi Masiza,Anga Hamnca,Yondela Hamnca Sifiso Hamnca, Siyabonga Hamnca, Betty Hamnca ,Moyisile Hamnca ,Sakhamzi Masiza ,Nkosamaduna Masiza,Nolitha Masiza ,to my late family members my grandfather Marixana Hamnca , Bulelani Hamnca , Mdyude Hamnca ,Nomsa Hamnca and Zolani Hamnca, I am grateful to all you for always standing behind me, for encouraging me and for being patient with me.

I would also like to acknowledge the Department of Science and Technology (DST), National Nanoscience Postgraduate Teaching and Training Program (NNPTTP), SensorLab and Chemistry Department for funding my studies.

DEDICATION

I dedicated this project to every single member of my family, especially:

My mother

Mandiswa Constance Hamnca

And

My son

Amkele Logan Mexen

CONFERENCES

Siyabulela Hamnca, Xolani Simelane, Meryk Ward, Emmanuel I. Iwuoha, Priscilla G.L. Baker. "Development of a Graphenated Polyamic Acid Sensor for Electroanalytical Detection of Anthracene". The 3rd international symposium on electrochemistry 26– 28 May 2014, UWC, Bellville– SOUTH AFRICA. (poster)

Siyabulela Hamnca, Lisebo Phelane, Priscilla G.L Baker and Emmanuel Iwuoha "Electrochemical detection of Neomycin and Norfloxacin at novel polymer composite electrode in aqueous systems". 42nd National Convention of the South African Chemical Institute held at the Southern Sun Elangeni Hotel in Durban, South Africa from 29th November 2015 – 4th December 2015.(invited oral)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

A	Ampere
Au	Absorbance units
AFM	Atomic Force Microscopy
$BaTiO_3$	Barium titanate
BO	Benzoxazole
BT	Benzethiozide
CAP	Chloramphenicol
CVD	Chemical vapor deposition
CV	Cyclic Voltammetry
CNFs	Carbon nanofibers
CNTs	Carbon Nanotubes
CTC	Chlortetracycline
DMF	Dimethyl formamide
DNA	Deoxyribonucleic acid
DPV	Differential-Pulse Voltammetry
DTA	Differential thermal analysis

EDC/NHS	1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide)/ N-hydroxysuccinimide
ELISA	Enzyme linked immunosorbent assay
ERGO	Electrochemically reduced graphene oxide
FETs	Field –Effect Transistors
FQs	Fluoroquinolones
FTIR	Fourier Transform infrared spectroscopy
GC	Gas Chromatography
GICs	Graphite intercalation compounds
GO	Graphene oxide
Gr	Graphene
HRTEM	High resolution electron microscopy
LC	Liquid chromatography
LEV	levofloxacin
LOD	Limit of detection
LOQ	Limit of quantification
MIP	Molecularly imprinted polymer
PAA	Polyamic acid

PAAGO	Polyamic acid –Graphene oxide
PANI	Polyaniline
PBS	phosphate buffer solution
PI	Polyimide
PPy	Pyrrole
SEM	Scanning electron Microscope
SPCE	Screen printed carbon electrode
STP	Sewage treatment plant
SWV	Square wave voltammetry
UV	Ultraviolet
Vis	Visible
WWTPS	Wastewater treatment plants

Chapter1

This chapter introduces conducting polymers and provides a background about polyamic acid (PAA) as a conducting polymer. This chapter also presents the review of nanocomposites and techniques which are used to characterize the nanocomposites.

1.1 Polymers and Polymer nanocomposites

Polymers are light weight, corrosion-resistant materials with a wide variety of industrial and military uses. There three classes of polymers which are thermoplastics .thermoset and elastomers. Polymers are not good electrical conductors and electrical conductivity is desired in various polymer applications. Conducting polymers are polymers with a highly π conjugated polymeric chain. Typical conducting polymers include polyacetylene (PA), polyaniline (PANI), polypyrrole(PPy), polythiophene (PTh), poly(para-phenylene) (PPP), poly(phenylenevinylene) (PPV), polyfuran (PF), etc. (Xiaofeng Lu,2011).These polymers are reportedly stable under ambient conditions. These polymers have a wide variety of applications particularly polypyrrole films which have technological applications in the areas energy conversion, solar energy and electrochromic displays.

Composite materials are typically formed when at least two distinctly dissimilar materials are mixed to form a monolith (individual components consist of a single material). The emergence of the nanoscience field has made it possible to use a nanomaterial as one of the materials in the composite and it becomes a nanocomposite .Polymer nanocomposites are materials in which nanoscopicinorganic particles, Typically 10-100 nm at least one dimension, are dispersed in an organic polymer matrix in order to dramatically improve the performance properties of the polymer. Polymer nanocomposites show substantial property enhancements at much lower loadings than polymer composites with conventional micron-scale fillers (such as glass or carbon fibers), which ultimately results in lower component weight and can simplify processing, Furthermore, the multifunctional property enhancements made possible with nanocomposites depending on the type of matrix or a nanofiller may create new applications of polymers such as automobile, Food packaging, Cosmetics, Gas barriers, electronics, flame retardants and environmental applications (Winey and Vaia,2007).

Polymer nanocomposites represent a new alternative to conventionally filled polymers, because of their nanometer sizes; filler dispersion nanocomposites exhibit markedly improved properties when compared to the pure polymers or their traditional composites. The enhanced properties include increased modulus and strength, outstanding barrier properties, improved solvent and heat resistance and decreased flammability.

To fully understand the properties of the polymer nanocomposites many scientists have been using different polymers along with different Nano fillers to form nanocomposites as to study the impact of Nano fillers on the overall properties of the nanocomposite. Nano fillers are the inclusions that have at least one dimension (length, width or thickness) in the nanometer size range that combine with a polymer matrix to form a polymer nanocomposite. The most common Nano fillers include montmorillonite organo clays, carbon nanofibers (CNFs), Carbon nanotubes (CNTs) and Metallic nanoparticles.

A study by Selampinar et al, 2007 focused on conducting polymer composites of polypyrrole and polyimide. The conducting composite of polypyrrole with a polyimide as the insulating matrix polymer was prepared using electrochemical synthesis technique The authors used FTIR,SEM and TGA to characterize the composite. They used Scanning Electron Microscope (SEM) to study the morphology of the composite films. The FTIR spectra were used by Selampinar and co-authors to be able to differentiate between the peaks of the pure PPy, PI/PPy and the washed Pi/PPy composite film. They used the TGA curve to study the thermal stability of PI/PPy electrolyte film and mechanical mixture of PI and PPy. Authors were able to show that the PI and the mechanical mixture show two weight loss pattern he latter being around570 °C whereas the electrolyte films showed greater temperature stability indicated by a sharp weight loss pattern at 670 °C. This research indicated the improved thermal stability of composite compared to individual materials (Selampinar et al, 2007).

Furthermore Wang and co-authors conducted a study based on characteristics of polyimide /Barium titanate composite films. It is has been reported that inorganic materials exhibit excellent thermally and other functional properties such as dielectric and magnetic properties. In this study the authors were interested in evaluated the characteristics of the polyimide $BaTiO_3$ composite films with various amounts of $BaTiO_3$.During the preparation of the composite they used a modifier 1-methoxy-2-proply acetate to disperse the $BaTiO_3$ particles in polyimide matrix. Using a Agilent 4284 LCR meter at the frequencies ranging from 10 KHz to 1 MHz, the authors were able to determine a dielectric constant of 45.6 and dielectric loss of 0.015 for the composite with 90%wt of $BaTiO_3$at the sweep frequency 10KHz. The authors discovered that water absorption of the composite drops to a level of 0.52% as the BaTiO3 contents reaches 10wt% in the composite (Wang et al,2009).

There different types of methods to prepare polymeric nanocomposites those include solution processing, melt processing, emulsion and in-situ polymerization. In Solution processing layered silicates can be easily separated and dispersed in an adequate solvent which the polymer is soluble. The polymer absorbs onto the delaminated sheets and when the solvent is evaporated, the sheets sandwich the polymer to form an ordered multilayered structure a polymer nanocomposite. Melt processing Nano filler is mixed with the polymer in the molten state in this case a solvent is not required.

In-situ polymerization it is where the monomers instead the polymer is used, a clay is added and an initiator is used then the polymerization occurs. The formed polymer chains will grow and separate the clays and enter the interlayer space then the polymer-clay composite is formed. Emulsion polymerization a hydrophobic monomer escapes from water and enters the drops formed by molecules of emulsifier then the polymerization takes inside the drops to form the polymer nanocomposite.

1.1.1 Polyamic acid (PAA)

Aromatic polyamic acids are generally synthesized by reaction of tetra carboxylic anhydrides and diamines (Suzuki, 1986). Polyamic acids have amides and carboxylic acid groups in the polymer backbone. Polyamic acid is a well-known precursor of polyimides exhibiting cation complexing properties (Padavan and wan et al, 2010: Andreescu et al, 2005)]. It is widely reported that the complexing power of polyamic acid is higher than that of the polyimide due to the fact they have carboxylic groups that have polyfunctional behavior, because of the polyfunctional behavior it is understood that polyamic acid (PAA) can create new possibilities of making new polyfunctional nanomaterials (Sadik et al, 2010). These properties of polyamic acid create an opportunity for the implementation of new nanomaterials which can be used to develop a novel class of nanosensors, biosensors, biochips (Duet al, 2010).

Hua et al. Synthesized polyamic acids (PAAs) containing benzothiozide (BT) and benzoxazole (BO). The used PAA/BO and PAA/BT to modify a gold electrode which was used to develop a biosensor platform with superior performance for the detection of hydrogen peroxide (Hua et al, 2011). Recently Hess et al developed a biosensor platform based on polyamic acid. The authors were able to demonstrate that the carboxylic acid end of the PAA can be modified using EDC/NHS chemistry and use to immobilize biomolecules on sensor surface (Hess et al, 2014).

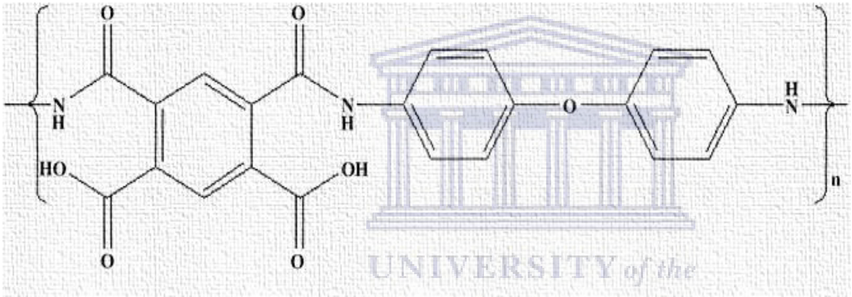


Figure 1.1: Chemical structure of Polyamic acid (PAA)

1.2 Graphene oxide and graphene based polymer composites

Chemically derived graphene oxide (GO) is an atomically thin sheet of graphite that has traditionally served as a precursor for graphene, but is increasingly attracting chemists for its own characteristics. It is covalently decorated with oxygen-containing functional groups either on the basal plane or at the edges so that it contains a mixture of sp^2- and sp^3-hybridized carbon atoms. In particular, manipulation of the size, shape and relative fraction of the sp^2-hybridized domains of GO by reduction chemistry provides opportunities for tailoring its properties optoelectronics, conductivity and mechanical strength (Loh et al, 2012: Marcano et al, 2010).

There are several methods which have been used to prepare graphene which include micromechanical cleavage, chemical vapor deposition, Epitaxial growth, Solvothermal synthesis, exfoliation of graphite intercalation compounds (GICs) and chemical oxidation-reduction methods. Chemical vapor deposition (CVD) and epitaxial growth often produce tiny amounts of large-size, defect-free graphene sheets. However, graphene obtained from the CVD and epitaxial growth method is not suitable for polymer nanocomposites because fabrication of nanocomposites requires a large amount of graphene sheets preferably with modified surface structure. Among these methods, micromechanical cleavage is more reliable and effective method to produce high quality graphene. However, this approach is limited by its low production yield.

Graphene has emerged as a subject of enormous scientific interest due to its exceptional electron transport, mechanical properties, and high surface area. It has been proven that when incorporated appropriately, these atomically thin carbon sheets can significantly improve physical properties of host polymers at extremely small loading (Kim et al, 2010).The superior properties of graphene compared to polymers are reflected in graphene-based polymer composites. Graphene-based polymer composites show superior mechanical, thermal, gas barrier, electrical and flame retardant properties, compared to the neat polymer (Das and Prusty, 2013).

The specific development of polymeric nanocomposites based on conventional polymers and conductive carbon based materials has drawn much attention as a route to obtain new materials with new structural and functional properties superior to those of the pure components and of previous nanocomposite systems with other fillers. Control of the size, shape and surface chemistry of the reinforcement materials are essential in the development of materials that can be used to produce devices, sensors and actuators based on the modulation of functional properties such conductivity, optical activity etc. Haque et al, 2012 presented an electrochemically reduced graphene oxide (ERGO)-based electrochemical immunosensing platform for the ultrasensitive detection of an antigen by the sandwich enzyme `linked immunosorbent assay (ELISA) protocol. In this study ERGO was proved to increase the sensitivity of the electrochemical immunosensor (Haque et al, 2012).

Primar and co-authors conducted a study based on the comparative toluene sensor behavior of polyaniline (PANI) and graphene/polyaniline nanocomposite (C-PANI) films. The sensing behaviors of the films were analyzed different temperatures (30, 50 and 100°C) for 100ppm toluene in air. The authors found that the nanocomposite C-PANI films have a better overall toluene sensing behavior in terms of sensor response (Primar et al, 2013).

1.2.1 Preparation methods of graphene based polymer nanocomposites

Preparation methods of graphene polymer nanocomposites include in-situ intercalative polymerization, solution intercalation and melt intercalation. In-situ intercalative polymerization in this method a graphene or modified graphene is first swollen within the liquid monomer. A suitable initiator is added and polymerization is initiated either by heat or radiation. Solution intercalation, in this method the polymer or pre-polymer is soluble in the solvent system and graphene or modified graphene layers are allowed to swell. Graphene or modified graphene is dispersed in a suitable solvent like water, acetone, chloroform, tetrahydrofuran (THF), dimethyl formamide (DMF) or toluene then the polymer adsorbs on to the delaminated sheets and finally the solvent is evaporated

Melt intercalation, in this method a graphene or modified graphene is mixed with the polymer matrix in molten state. A thermoplastic polymer is mixed mechanically with graphene or modified graphene at elevated temperatures using conventional methods like extrusion and injection molding. The polymer chain are then intercalated or exfoliated to form nanocomposites (Das and Prusty, 2013).

1.2.2 Characterization techniques of graphene based polymer nanocomposites

Different techniques are used to characterize graphene and graphene based polymer nanocomposites which include AFM, XPS, HRTEM, SEM TGA and DTA. Atomic force microscopy (AFM) is suitable for the definitive identification of the heights of Few-layered sheets. X-ray photoelectron spectroscopy (XPS) is employed to determine the elemental composition and bonding energy of the few-layered graphene materials. High-resolution transmission electron microscopy (HRTEM) is used to demonstrate the morphology of the thin –

film particles of layered graphene. Scanning Electron Microscope (SEM) is a technique which has the ability to provide detailed information of the graphitic materials morphology. Thermo gravimetric analysis (TGA) and Differential thermal analysis (DTA) used to study the thermal behavior of the graphene materials and the thermal stability of the graphene based polymer nanocomposite.

This work will focus on the polyamic acid/graphene oxide nanocomposite for electrochemical screening of environmental pollutants in water. These environmental pollutants will include norfloxacin and neomycin.

1.4 Problem statement

Antibiotics are very useful pharmaceuticals which are used to treat infectious diseases in humans and animals. Antibiotics can be more or less extensively metabolized by humans and animals. Large quantities of drugs that do not meet production specifications, antibiotics that have expired or prescribed antibiotic courses which have not been completed are at risk of ending up in municipal wastewater. Municipal wastewater screening and treatment systems often fail in complete removal of these antibiotic residues and they end up back in our potable waters. After administration, antibiotics for human use or their metabolites are excreted into the effluent and reach the sewage treatment plant (STP). The non-metabolized fraction is excreted as a still-active compound. Not all Antibiotics in sewage treatment plants are eliminated. This can lead them to pass through the sewage system and may end up back in the environment. Residual amounts of these antibiotics can reach surface waters, groundwater or sediments. These antibiotics in water may lead to induction and spread of highly resistant bacterial strains, which makes future treatment using the same regime of treatment completely ineffective. There are standard methods which are used to detect and quantify these antibiotic residues in water. These methods include liquid chromatography (LC) coupled to mass spectrometry (MS) LC coupled to tandem MS (LC-MS^2), single solid-phase extraction method coupled to liquid chromatography (LC) with positive electrospray tandem mass spectrometry detection, ultraviolet (UV) and radioimmunoassay (RIA). However these methods can be quite expensive and time consuming due to sample preparation (due to predicted low concentrations of antibiotics in water) and technology used. Electrochemical sensors and biosensors have emerged with advantages of being simple, uniform whole structures featuring direct transduction, high bio selectivity, high sensitivity, miniaturization electrical/ optoelectronic readout, continuous monitoring, ease of use, and cost effectiveness. User advantages include low price, reliability, no sample preparation, disposability, and clean technology. With these advantages portable electrochemical sensors can be very good in both the field and laboratory in detecting and quantifying these antibiotic residues. Electrochemical sensors have different modes of detection or measurement mode: electrochemical transduction or detection which includes amperometry, potentiometry, Surface charge using field-effect transistors (FETs) and conductometry. On the other hand, the main drawback of voltammetric techniques is their moderate selectivity, which limits their use for analysis of more complex samples containing more than one electroactive species, because in this case the possibility of interference by overlapping of voltammetric peaks is high. To solve this problem we aim to develop a multi-array electrochemical aptamer based biosensor which will be able to screen for more than one antibiotic residue, in one sample, with high selectivity and sensitivity and directly applied to real samples. To solve this problem we aim to develop a multi-array electrochemical sensor which will be able to screen for more than one antibiotic residue, in one sample, with high selectivity and sensitivity and directly applied to real samples.

This study is based on developing a PAA/GO based electroanalytical sensor for determination of the antibiotic residues (norfloxacin and neomycin).

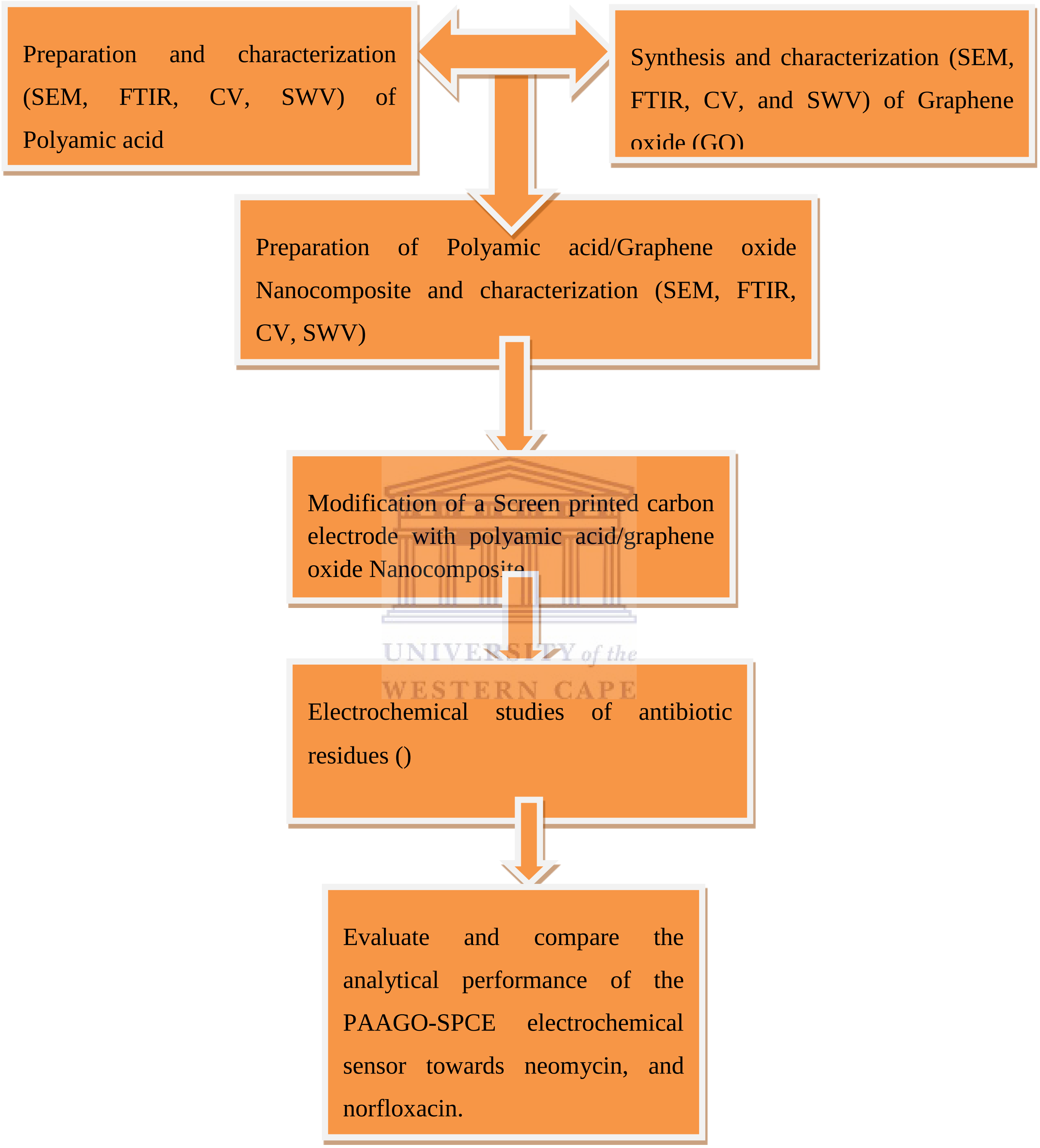
1.5 Aims and Objectives

Overall aim: low cost, sensitive and selective methods are required for effective detection and monitoring of antibiotics in municipal and environmental. In this study electrochemical sensors for the selective and sensitive determination of norfloxacin and neomycin will be developed using the novel PAA/GO nanocomposite.

Objectives of the study

- Synthesis and characterization of polyamic acid (PAA) for the development a novel polyamic acid/Graphene oxide nanocomposite.
- Evaluate the electrochemical behavior of polyamic acid (PAA) (CV and SWV) and Study the morphology SEM and chemical structure (FTIR) polyamic acid.
- Preparation of graphene oxide (GO) using the Hummers method with some necessary modifications produce laminar sheets of graphene oxide.
- Characterization of graphene oxide using, FTIR (chemical structure) and SEM (Morphology). Evaluating the chemical behavior of graphene oxide using electrochemical methods (CV and SWV).
- Preparation and characterization of PAAGO using FTIR to study the chemical structure of nanocomposite SEM to study the morphology of the nanocomposite and to study the electrochemical behavior of the nanocomposite using CV and SWV.
- Study the analytical performance of the selected antibiotics in aqueous medium at PAAGO-SPCE electrode in order to develop analytical protocol for antibiotic detection in real samples.

1.6 Conceptual diagram



Preparation and characterization (SEM, FTIR, CV, SWV) of Polyamic acid

Synthesis and characterization (SEM, FTIR, CV, and SWV) of Graphene oxide (GO)

Preparation of Polyamic acid/Graphene oxide Nanocomposite and characterization (SEM, FTIR, CV, SWV)

Modification of a Screen printed carbon electrode with polyamic acid/graphene oxide Nanocomposite

Electrochemical studies of antibiotic residues ()

Evaluate and compare the analytical performance of the PAAGO-SPCE electrochemical sensor towards neomycin, and norfloxacin.

1.7 Thesis scope

Thesis is divided into 6 chapters;

Chapter 1: Introduction of polymers and different types of conducting polymers including Polyamic acid (PAA). Review polymer based nanocomposite, graphene and graphene based nanocomposites with focusing on the characterization techniques which are used to characterize these nanocomposites.

Chapter 2: This chapter takes a closer look at antibiotics and electrochemical sensors which are currently used to detect the selected antibiotics (norfloxacin and neomycin).

Chapter 3: This chapter provides the principle of operation and instrumental information for the methods used to characterize the transducer materials.

Chapter 4: Here we present the characterization of the transducer materials; this includes microscopic, spectroscopic and electrochemical characterization

Chapter 5: Evaluation of the spectroscopic behavior of norfloxacin and neomycin using Uv-vis analysis, as an indication of the sensitivity of spectroscopy based methods for detection of the selected antibiotics.

Chapter 6: Concludes by comparing the sensitivity and LOD of the PAA-GO nanocomposite on detecting antibiotic residues to literature studies, Uv-vis spectroscopy results and developing a protocol for antibiotic detection for detection on real samples.

Chapter 2:

This chapter provides a literature review on antibiotics and electrochemical sensors which are currently used to detect the selected antibiotics (Norfloxacin and Neomycin).

2.1 Antibiotic residues

An antibiotic can be defined as a chemotherapeutic agent that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoa. Antibiotics can be classified by either their chemical structure or mechanism of action. These chemicals can be grouped into different sub-groups such as ß-lactams, quinolones, tetracyclines, macrolides, sulphonamides and others. Antibiotics are drugs which can be either natural (Penicillin, tetracycline and macrolides) or synthetic (sulphonamides, quinolones etc.) (Kummerer, 2009). Antibiotics are very important pharmaceuticals which are used to treat infectious diseases on humans and they also used to in treat animals (veterinary medicine) (Chafer-Pericas, 2010). The emergence of antibiotics in the environment particularly aquatics have become a matter of concern. (Balcıoğlu, andÖtker, 2003: Baquero, 2008)

Residual amounts of these antibiotics can reach surface waters, groundwater or sediments. These antibiotics in water may lead to induction and spread of highly resistant bacterial strains, which makes future treatment using the same regime of treatment completely ineffective. Currently antibiotic resistance has outpaced the production of new antibiotics required for the treatment of the life threatening bacterial diseases in both humans and animals. For instance antibiotic *Salmonella* species are major causes of illnesses throughout the world. It is widely reported the major problem of the salmonella infection is worst where the water supply is contaminated with faecal material from humans and animals (Bbosa et al,2014 : Collignon ,2002) . The most common techniques which are currently used to detect and quantify antibiotics in water which include liquid chromatography (LC) coupled to mass spectrometry (MS) LC coupled to tandem MS (LC-MS^2) (Hernandez et al,2007 : Zawilla et al,2006), single solid-phase extraction method coupled to liquid chromatography (LC) with positive electrospray tandem mass spectrometry detection, ultraviolet (UV) and radioimmunoassay (RIA). Although GC has reportedly a high resolving power, antibiotics are polar compounds, insufficiently volatile or too thermally unstable to allow their direct determination using GC, so a previous derivatization step is necessary. As a consequence, LC has become the technique of choice, as it can allow determination of antibiotics with notable simplification in sample manipulation; hence the use of LC based techniques. LC-MS and LC-MS^2 has emerged as the most preferred techniques for the determination of antibiotics in aquatic environments (Hernandez et al, 2007). Commonly used methods for determining antibiotics typically include extraction for both clean-up and enrichment of aqueous samples, due to the predicted low concentration levels present in the aquatic environment.

Hernandez and co-authors did a study based on LC-MS or LC-MS2 for the determination of antibiotic residues in environmental waters. They focused mainly on quantification and confirmation of the analytes detected in samples. The authors evaluated different approaches for screening, quantification and confirmation of these compounds, giving special attention to dealing with the natural difficulties of confirming analytes with confidence at low-ng/L levels (Hernandez et al, 2007).

However these methods are methods are known be time consuming, expensive, require skilled operators, and lack the required selectivity. Over the years electrochemical based sensors and biosensors have emerged with advantages of being simple, uniform whole structures featuring direct transduction, high bio selectivity, high sensitivity, miniaturization electrical/ optoelectronic readout, continuous monitoring, ease of use, and cost effectiveness. User advantages include low price, reliability, no sample preparation, disposability, and clean technology. Hence, biosensors show the potential to complement both laboratory-based and field analytical methods for environmental monitoring (Badihi-Mossberg et al, 2007).

Zacco and co-authors conducted a study based on the electrochemical magneto immunosensor of antibiotic residues in milk. The immunological reaction for the detection of sulfonamides antibiotics was performed on the magnetic bead based on a direct competitive essay using a tracer with HRP peroxidase for the enzymatic labeling. The authors used a sensor made of graphite epoxy composite as the transducer for the electrochemical immunosensing. Using spiked milk samples, the authors were able to successfully evaluate the electrochemical performance of the immunosensor based on the magnetic beads. The determined detection limit for raw full cream milk was 1.44 µg/L (Zacco et al, 2007). This study will focus on electrochemical screening three antibiotics which are norflocaxin, neomycin in water systems.

2.1.1 Norfloxacin (NOR)

Norfloxacin (NOR)Norfloxacin (1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid) is a type of fluoroquinolone, fluoroquinolones are defined as a class of antibacterial compounds are used in both human and veterinary medicine, particularly this antibiotic is used to treat urinary infections (Espinosa-Mansilla et al,2005 : Gober et al,2012) . These compounds have been reportedly found in places such as hospital wastewaters, wastewater treatment plants and surface waters all over the world with concentrations ranging from approximately 60-120000 ng/, 2-580 ng/L and 5-1300 ng/L respectively. It is widely reported that the presence of this class of antibacterial compounds is a concern due to the possibility long term therapeutic doses may provide selective pressure of antibiotic resistant organisms. Fluoroquinolones (FQs) are known to suffer from degradation by UV irradiation. This degradations process is said to depend on the chemical and environmental conditions which include irradiation time, irradiation parameters and it is understood that structural photoproducts may be generated during the process and may affect the degradation process (Zhanget al,2008 : Gober et al,2012). High pressure liquid chromatography (HPLC) and mass spectrometric are the typical techniques which are used commonly used to study the degradation process of fluoroquinolones (FQs) such Norfloxacin and others (Wammer et al,2005:Zhang et al, 2014).

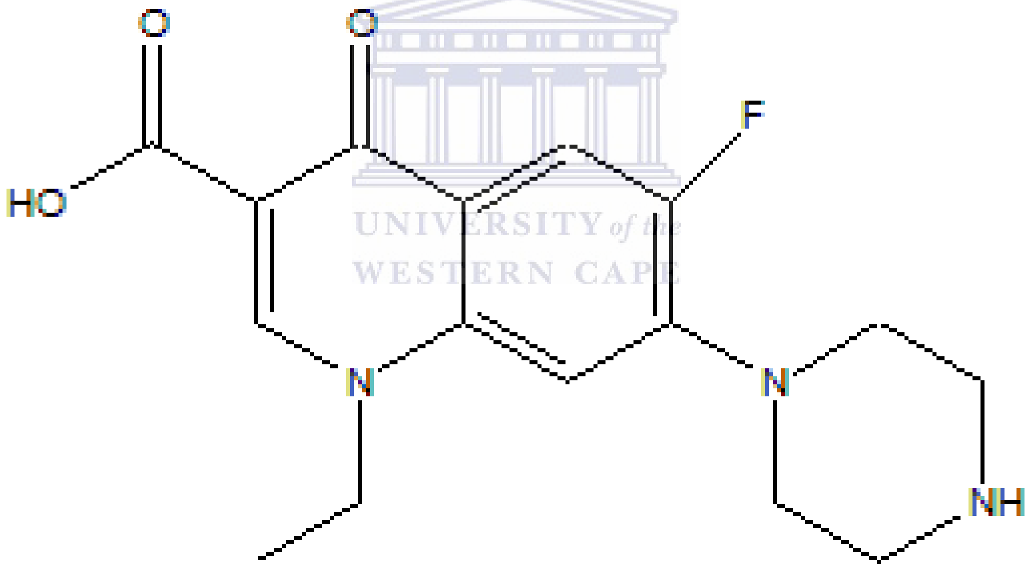


Figure 2.1: chemical structure of Norfloxacin (NOR)

HPLC is the most widely used technique to detect norfloxacin because of its selectivity and the ability to minimize interferences. However, it is time consuming due to sample preparations and extraction steps that need to be followed before analysis. HPLC requires a lot of solvents which increase cost and it is also a very expensive instrument that may require skilled operators and high maintenance. To overcome these disadvantages, there is a need for fast sensitive and cost effective methods for the successful determination of pharmaceuticals in water. Electrochemical methods have received considerable interest because of their low cost, high sensitivity, selectivity and their ability to minimize the interferences. However, the voltammetric response of norfloxacin is not satisfactory at the bare electrode. Many studies have indicated that polymer film modified electrodes show an enhanced response for the determination of various pharmaceuticals. In study we aim to develop a low cost, sensitive and selective polyamic acid-graphene oxide nanocomposite electrochemical sensor for the detection of norfloxacin.

2.1.2 Neomycin

Neomycin is an antibacterial compound which belongs to a group of broad spectrum aminoglycoside antibiotics. These antibiotics are well known anti agents that are used in clinical therapy of serious infections. Neomycin antibiotics are known for their ability to inhibit the growth of both gram negative and gram positive bacteria (Adams et al, 1996). These compounds are excreted by humans and animals through urine to hospitals wastewater and may end up in the wastewater treatment plant. The presence of these antibiotics may cause antibiotic resistant bacteria in environment particularly water systems which may cause harm to both humans and animals.

The most common techniques (including sample preparation and cleanup techniques) which are used for the determination and quantification of these pharmaceuticals include electrospray ionization tandem spectrometry (ESI-MS-MS), high performance anion chromatography with pulsed amperometric detection (Guan and Juan,2007), hydrophilic interaction chromatography mass spectrometry (HILIC-MS-MS) (Oertelet al, 2004). Oertel and co-authors did a study based on the determination of neomycin by LC-tandem spectrometry using hydrophilic interaction chromatography and solid phase extraction as additional preparation techniques. In this study the authors used 500ul of a biological sample where they were able to observe a limit of detection (LOD) of 100ng/ml (Oertel et al, 2004). Yuan and Guan, 2006 later used high performance anion chromatography with pulsed amperometric detection for determination of neomycin in water samples, the detection limit of neomycin was found to be 0.0027 ug/mL (Guan and Yuan, 2006). Even though chromatography based techniques remain the standard techniques used for the detection of these compounds many researchers have working on improving the detection limit using electrochemical studies. Li et al. conducted an electrochemical study of neomycin at DNA-modified gold electrodes. The electrochemical behavior of neomycin and the influence of Pb (II) were studied by using cyclic voltammetry, chronocoulometry and differential pulse voltammetry. The authors observed a detection limit of 2.0×10^{-8} M (Li et al, 2007). These findings suggest that electrochemical studies can improve the detection limit of these kinds of antibiotics

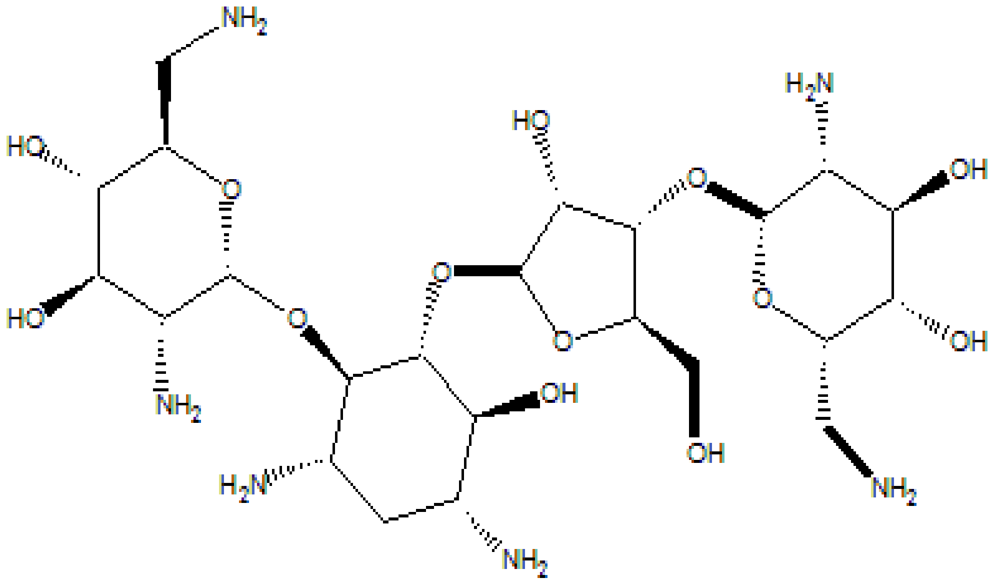


Figure 2.2: Chemical structure of Neomycin

The most widely used techniques which have been reported in the literature include ESI-MS-MS and HILIC-MS-MS are advanced techniques, good selectivity and sensitivity towards detecting neomycin have been demonstrated. HILIC-MS-MS technique requires solid phase extraction as an additional preparation technique which may take more time and increase cost of analysis. ESI-MS-MS uses solvents that are toxic or flammable. These disadvantages of the above mentioned techniques mean there is a need to develop new low cost, clean, fast and sensitivity techniques to detect neomycin and other pharmaceuticals in water. Electrochemical sensors remain popular choice due to the low cost, high sensitivity associated with using them. Not many studies have been done in developing new electrochemical sensors for detecting neomycin. In this thesis we demonstrate the feasibility to develop new electrochemical sensors based on PAA/GO for detecting neomycin in water.

2.2 Electrochemical sensors and biosensors

Sensors are small devices that incorporate a recognition element with a signal transducer. Such devices can be used for direct measurement of the analyte in the sample matrix. A chemical sensor can be defined as device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal (Yogeswaran and Chen, 2008). The chemical information, mentioned above, may originate from a chemical reaction of the analyte or from a physical property of the system investigated. Electrochemical sensors, in which an electrode is used as the transduction element, represent an important subclass of chemical sensors. Such devices hold a leading position among sensors presently available and have found a vast range of important applications in the fields of clinical, industrial, environmental, and agricultural analyses. Electrochemical consists of three types of transducers which are amperometric, potentiometric and conductimetric transducers.

Amperometry which is based on the measurement of the current resulting from the electrochemical oxidation or reduction of an electroactive species, Potentiometric measurements involve the determination of the potential difference between either an indicator and a reference electrode or two reference electrodes separated by a perm selective membrane, when there is no significant current flowing between them and The conductometric transducer is a miniature two-electrode device designed to measure the conductivity of the thin electrolyte layer adjacent to the electrode surface (Bühlmann et al ,1998 ; Janata, J, 1990 ;Janata ,2003 ; Jaffrezic-Renault, and Dzyadevych, 2008). Electrochemical sensors provide an opportunity to interface, at the molecular level, chemical or biological recognition events and electronic signal-transduction processes and also make it possible to analyse antibiotics in real samples, this claim is widely reported in the literature. Yan et al, 2012 successfully developed a novel electrochemical aptasensor for highly sensitive detection of Chloramphenicol (CAP) in honey based on target-induced strand release (TISR). CAP aptamer was immobilized on electrode and then hybridized with the complementary biotinylated detection probe to form aptamer/ DNA duplex. In the presence of CAP, the TISR resulted in the dissociation of biotinylated detection probe from the electrode (Yan et al, 2012).

2.3 Graphene-polymer composite based antibiotic electrochemical sensors

Over the years nanostructured materials have received huge of interest due to their remarkable properties and have been continuously used as efficient electron mediators for the fabrication of highly sensitive chemical and biosensor. The nanostructured nanomaterials include the carbonaceous materials such as carbon nanotubes (CNTs) and graphene (Gr). These nanomaterials have attracted a lot researches due to their electrical, thermal and physical properties. Graphene and carbon nanotubes have been particularly used in developing electrodes for electrochemical detection of organic contaminants such as antibiotics in aqueous systems. Monitoring water for hazardous pollutants is important and based on the need to protect the environment and public health from possible distribution of natural industrial organic contaminants, with the aim of providing clean and safe water for people and animals. The use of graphene in the development of antibiotic electrochemical sensors and biosensors is widely reported in the literature. Liu et al ,2013 fabricated a molecular imprinted polymer (MIP) sensor by directly eletropolymerizing monomer α-phenylenediamine in the presence of template chlortetracycline (CTC), based on controlled electrochemical reduction of graphene oxide (GO) at cathodic potentials. MIP sensor shows special recognition ability toward to template CTC during the rebinding process and performs a considerable enhancement of linear current response of CTC over the range of 10.0–500.0 µM (Liu et al, 2013). Furthermore Wang and co-authors developed a highly sensitive levofloxacin (LEV) sensor based on molecularly imprinted polymer (MIP) incorporation with graphene–Au nanoparticles (G-AuNPs). In this sensor, the authors reported that G-AuNPs dramatically promoted the electrooxidation of LEV on electrode while molecularly imprinted polypyrrole of LEV served as the recognition element (Wang et al, 2014).

Recently Li et al, 2014 developed electrochemical aptasensor modified with thionine (TH), graphene–polyaniline composite film (Gr– PANI), and gold nanoparticles (GNPs) were fabricated for a convenient and sensitive determination of kanamycin. The aptasensor showed a favorable analytical performance for kanamycin detection with a detection limit of 8.6×10^{-9} M.

Most of the sensors for detecting antibiotics which have been reported in the literature include biosensors, aptsensors, and molecular imprinted sensors demonstrate good selectivity and sensitivity. However the major problem associated with interferences, complex chemistry and cost of fabrication. These sensors work based on the affinity of the recognition element and the analyte, therefore this may cause interference in the signal produced by the transducer. Most importantly these biosensors ,aptasensors and molecular imprinted may be expensive due to the number of materials which are used to fabricate one sensor for a specific analyte whereas a chemical sensors can screen different analytes using one type of a sensor ,thus reducing costs. In this study we aim to develop a simpler and cost effective electrochemical sensor based on a PAA/GO nanocomposite for detecting antibiotics in water.

Chapter 3:

This chapter provides details and the use of the instruments and the operation principles of the techniques which were used to characterized the transducer materials.

3.1 Scanning electron microscopy (SEM)

The SEM analysis was conducted on a Hitachi S3000N Scanning Electron Microscope and Zeiss Auriga, High resolution (fegsem) field emission gun scanning electron microscope. An acceleration voltage of 5.0 kV was employed at various magnifications. SEM produces various signals which are secondary electrons (SE), back-scattered electrons (BSE), X-rays, specimen current, cathodoluminescence (CL), and transmitted electrons. The signals result from interactions of the electron beam with atoms at or near the surface of the sample. The SEM can produce very high resolution images of a sample, revealing details less than 1nm in size. Scanning electron microscopy is a kind of electron microscope which produces an image of a sample by scanning it with a focused beam of electrons in a raster scan pattern. The electrons interact with electrons in the sample, signals are produced that can be detected and provides information about the sample’s surface morphology, topography, composition, and other properties such as electrical conductivity.

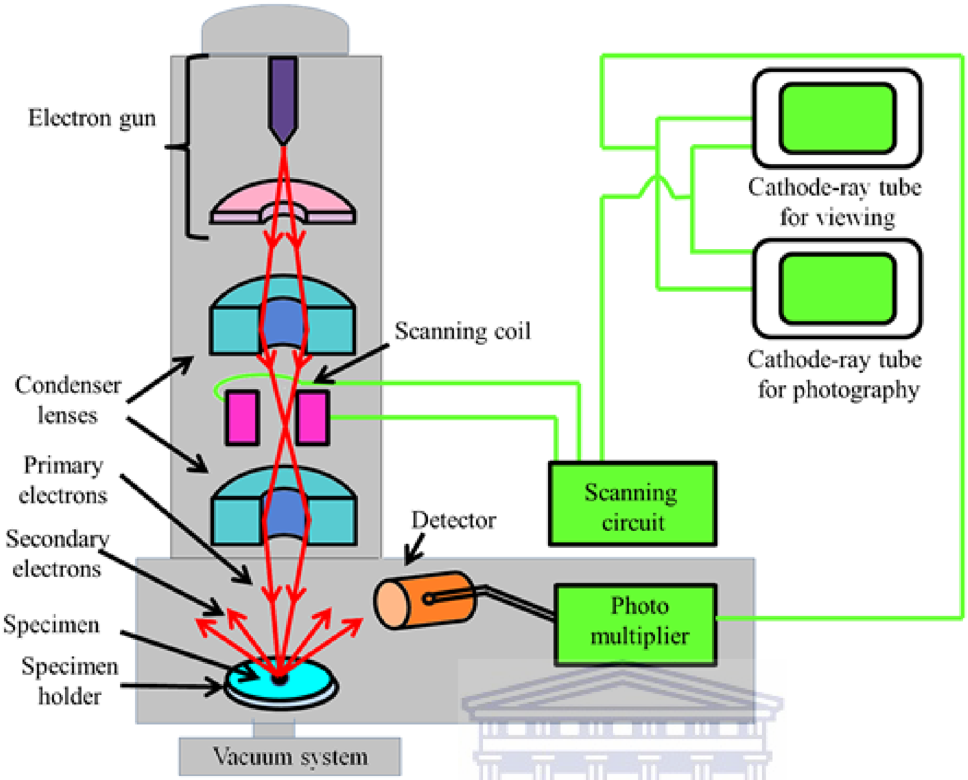


Figure 3.1: Schematic diagram of scanning electron (SEM)

Scanning electron Microscopy (SEM) will be used to study the morphology of graphene oxide, polyamic acid and the polyamic acid-graphene oxide nanocomposite. The morphology of polyamic acid as conducting polymer will provide information about where the graphene oxide can be incorporated into the polyamic acid structure to form the PAA/GO nanocomposite. The morphology of graphene oxide will confirm the successful synthesis of graphene layers and whether they can be entrapped by the expected clustered structure of the polyamic acid. PAA/GO morphology will confirm the formation of a nanocomposite with GO incorporated onto the clustered structure of polyamic acid.

3.2 Fourier Transform Infrared (FTIR) Spectroscopy

Spectroscopic characterization was performed on a PerkinElmer Spectrum 100, FT-. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted).

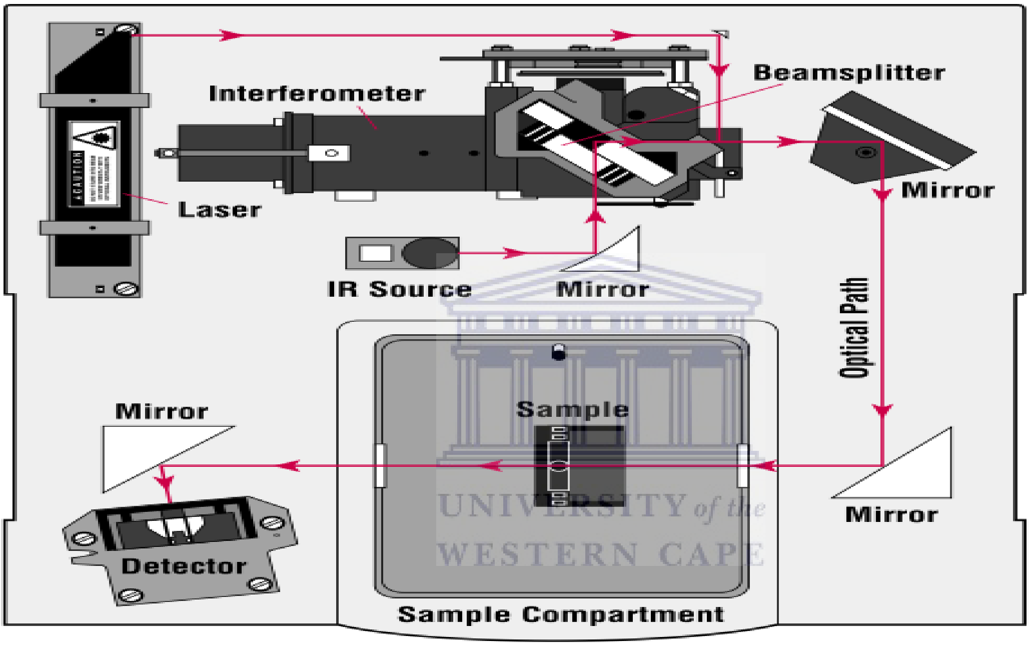


Figure 3.2: schematic diagram of Fourier transform infrared spectroscopy

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. With modern software algorithms, infrared is an excellent tool for quantitative analysis. This makes infrared spectroscopy useful for several types of analysis. IR spectrometer will used to study the structure and functional groups of chemically synthesized polyamic acid (PAA), Graphene Oxide (GO) and the structural and vibration changes of newly developed Polyamic Acid/Graphene Oxide nanocomposite

3.3 Voltammetric techniques (Square voltammetry and cyclic voltammetry)

Voltammetric techniques will be performed using PalmSens PTrace 4.4 electrochemical work station using a conventional three-electrode cell. Electrochemical analyses can be thought of in terms of two broad classes of measurement, one in which the potential that develops between two electrodes is measured (potentiometry) and another in which the current that flows between two electrodes is measured (amperometry). The working electrode was prepared by electrodepositing Polyamic Acid (PAA), Graphene Oxide (GO) and Polyamic Acid /Graphene nanocomposite onto a screen printed carbon electrode. Electrochemical methods allow researchers to study and determine both macro and trace amounts of inorganic and organic compounds in the assay of the basic substance and in the analysis of impurities respectively.

3.3.1 Cyclic voltammetry (CV)

Cyclic voltammetry (CV) experiments were carried using PalmSens electrochemical workstation (Bioanalytical Systems, USA.). Cyclic voltammetry (CV) is one of the most commonly used electrochemical techniques, and is based on a linear potential waveform; that is, the potential is changed as a linear function of time. The rate of change of potential with time is referred to as the scan rate. This electrochemical technique allows to us probe the mechanics of redox and transport properties of a system in solution. This is accomplished with a three electrode arrangement whereby the potential relative to some reference electrode is scanned at a working electrode while the resulting current flowing through a counter (or auxiliary) electrode is monitored in a quiescent solution. The technique is ideally suited for a quick search of redox couples present in a system; once located, Usually the potential is scanned back and forth linearly with time between two extreme values – the switching potentials using triangular potential waveform, a couple may be characterized by more careful analysis of the cyclic voltammogram.

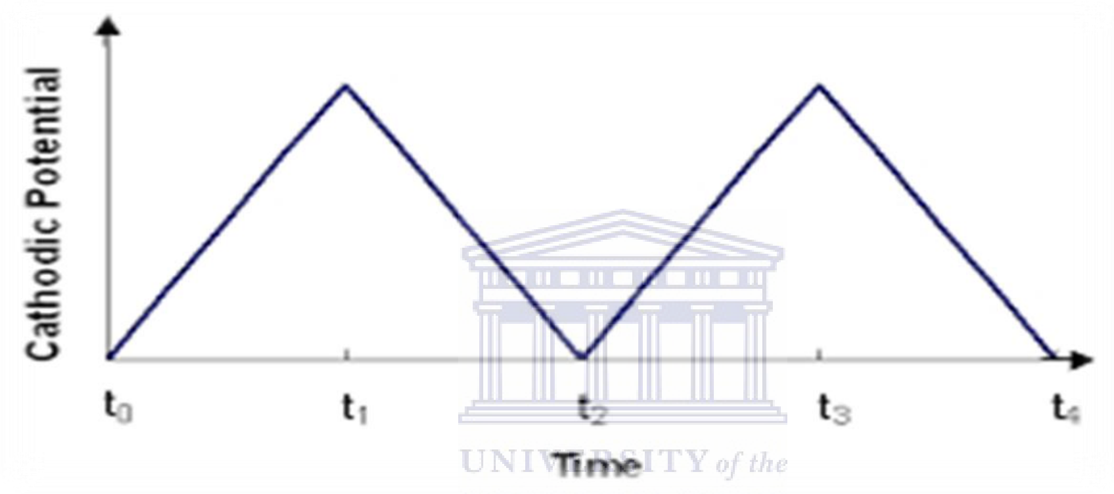


Figure 3.3: Cyclic voltammetry (CV) potential waveform

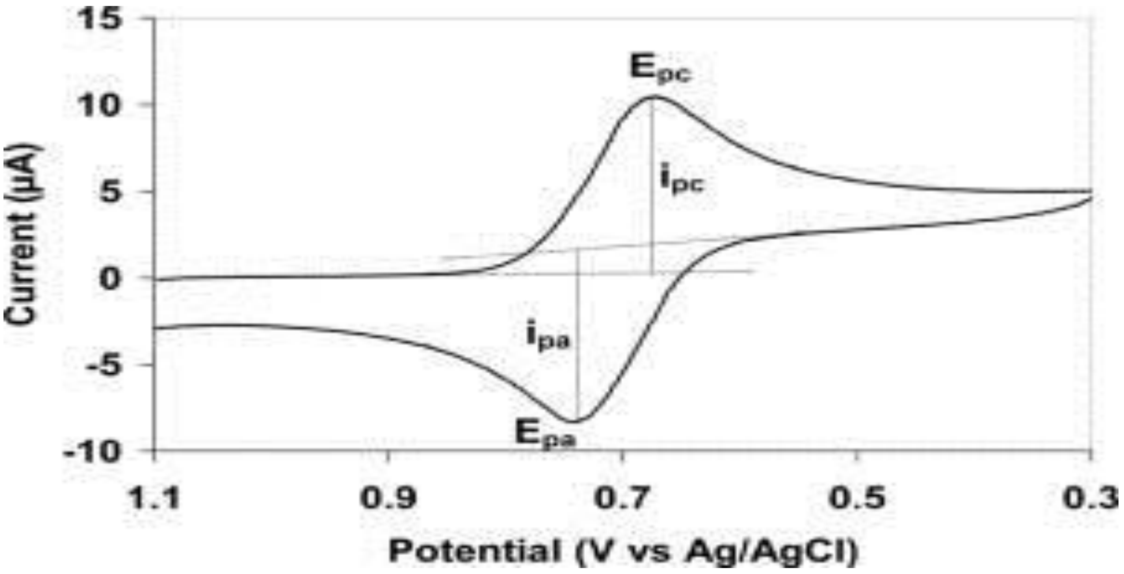


Figure 3.4: An example of cyclic voltammetry voltammogram

In this study CV is used for understand redox reactions of GO, PAA and PAAGO to obtain information about the chemical reactions that occurs CV will, thus will provide an information about the unique electrochemical behavior of PAA, GO and PAA/GO and electron kinetic differences between the individual components (PAA and GO) and PAA/GO nanocomposite.

3.3.2 Square Wave Voltammetry (SWV):

Square wave voltammetry (SWV) experiments were carried using PalmSens electrochemical workstation (Bioanalytical Systems, USA.) Voltammetric techniques, mainly Differential-Pulse Voltammetry (DPV) and Square-Wave Voltammetry (SWV), are by far the most used electrochemical techniques for analytical purposes since they clearly present high sensitivity making them very suitable for low-level quantifications. Square-wave voltammetry (SWV) is a powerful electrochemical technique suitable for analytical application, mechanistic study of electrode processes and electro kinetic measurements .The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude E_{sw} superimposed on a staircase waveform of step height DE, where the forward pulse of the square wave coincides with the staircase step. The net current, inet, is obtained by taking the difference between the forward and reverse currents (i_{for} – i_{rev}) and is centered on the redox potential.

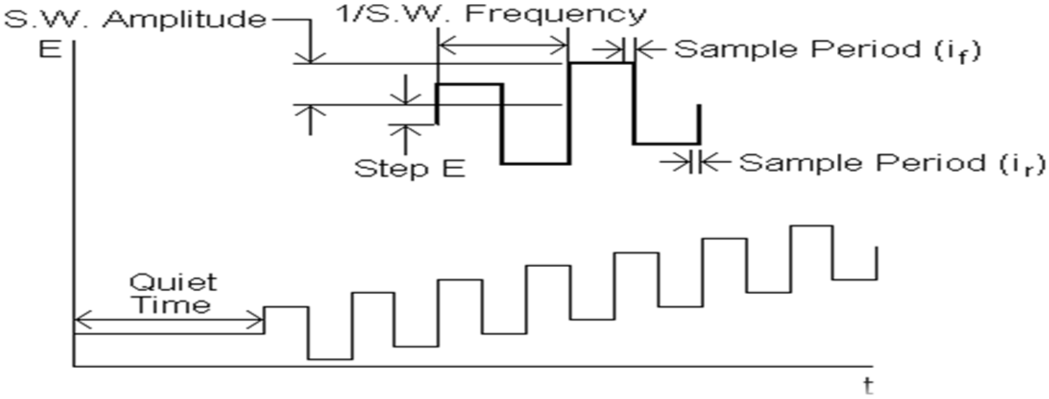


Figure 3.5: Potential vs time waveform of square wave voltammetry

Square-wave voltammogram of fast and reversible two step electrode reaction depends on standard potentials of individual electron transfers and on the parameters of SWV excitation signal. The peak height is directly proportional to the concentration of the electroactive species and direct detection limits as low as 10^{-8} M can be achieved. This electroanalytical technique is thus less sensitive to the effects of matrix interferences, a property which characterizes its success in electroanalysis.

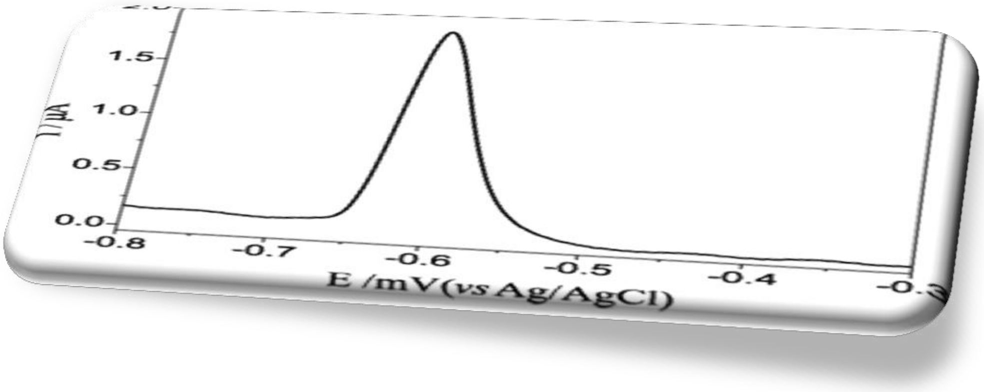


Figure 3.6: An example of a typical square wave voltammogram

Square wave voltammetry (SWV) will be used to detect the antibiotic residues in different concentrations to develop a standard calibration curve of the antibiotic residues. SWV is a highly sensitive technique compared to cyclic voltammetry (CV), thus will provide a highly sensitive response of norfloxacin and neomycin and the therefore will provide reliable analytical parameters such LOD, LOQ and the sensitivity of the novel electrode,

3.4 Ultraviolet Visible Spectroscopy

(UV-Vis): Uv-vis analysis will be performed on a NICOLET evolution 100 Uv-vis instrument. The molecular spectroscopy is the study of the interaction of electromagnetic waves and matter. The scattering of sun’s rays by raindrops to produce a rainbow and appearance of a colorful spectrum when a narrow beam of sunlight is passed through a triangular glass prism are the simple examples where white light is separated into the visible spectrum of primary colors. This visible light is merely a part of the whole spectrum of electromagnetic radiation, extending from the radio waves to cosmic rays. All these apparently different forms of electromagnetic radiations travel at the same velocity but characteristically differ from each other in terms of frequencies and wavelength. Ultraviolet (UV) and visible radiation comprise only a small part of the electromagnetic spectrum, which includes such other forms of radiation as radio, infrared (IR), cosmic, and X rays .Uv-vis (λ 200 - 800 nm) studies the changes in electronic energy levels within the molecule arising due to transfer of electrons from π- or non-bonding orbitals. It commonly provides the knowledge about π-electron systems, conjugated unsaturations, aromatic compounds and conjugated non-bonding electron systems etc. In this project Uv-vis was used for quantitative analysis of the antibiotic residues (norfloxacin, and neomycin) to develop standard calibration curves of the antibiotic residues and thus will provide analytical parameters (LOD, LOQ and sensitivity) that can be compared to the novel electrochemical sensor.

Chapter 4

This chapter presents the synthesis and preparation of the transducer materials. In this chapter we also discuss the characterization of the transducer materials; this includes microscopic, spectroscopic and electrochemical characterization.

4.1.1 Materials

The reagents 4, 4-oxydianiline (97 %) (ODA), 1, 2, 4, 5-benzenetetracarboxylic acid (96 %) (PMDA), tetrahydrofuran (99.9 %) acetonitrile (99 %) (ACN), Graphite powder <20 µm , Potassium permangate (99 %), Hydrogen Peroxide solution ultra ≥ 30 % ,sodium nitrite , sulfuric acid were all obtained from Sigma-Aldrich, South Africa. All chemicals were of analytical grade and were used without further purification. Deionized (ultra-pure) water with a resistivity of 18.2 MΩ/cm was purified by a Milli-QTM system (Millipore) and was used as reagent water for aqueous solution preparation.

4.1.2 Preparation of Phosphate Buffered saline (PBS)

A buffer solution of 0.1 M and pH 7 was prepared by dissolving 8.895 g of disodium hydrogen orthophosphate (Na_2HPO_4) and 7.81 g of sodium dihydrogen orthophosphate ($Na_2H_2PO_4$) separately in 500 mL deionized water. Then the salt solution was mixed according to the Henderson-Hasselbach equation to obtain the required pH level.

4.1.3 Synthesis of Polyamic Acid

The Polyamic Acid was synthesized in organic medium using 4.4 oxydianiline (ODA) and 1,2,4,5 –benzenetetra carboxylic acid (Pyromellic dianhydride, PMDA) precursors.

Briefly to prepare the Polyamic Acid (PAA), 2.0021g (0.01 mol) of ODA and 157 mL of ACN were stirred until the homogenous solution was formed. Then 50 mL of ACN containing 2.1812 g of PMDA (0.011 mol) was added in a drop wise manner for more than 1 hour and the solution was stirred for more 24 hours. The resulting yellowish precipitates were filtered through a membrane under suction and finally dried at room temperature. The amount of PAA obtained was 3.16 g, producing 76 % yield.

4.1.4 Synthesis of Graphene Oxide (GO)

Graphene oxide was synthesized from graphite powder according to Hummer's method, with some necessary modifications. Graphite powder (2 g) and sodium nitrite (1 g) were mixed with sulfuric acid (50 mL) in a dry clean conical flask and stirred at room temperature for 30 minutes, followed by subsequent mixing in an ice bath for 20 minutes. Potassium permanganate (7 g) was added gradually over 30 minutes period with constant stirring. The resulting solution was allowed to reach room temperature prior to being placed in a water bath set at 35 °C and left to stir for 2 hours. The flask was returned to the ice bath with constant stirring and 150 ml of ultra-pure water was added before the addition of approximately 5 mL hydrogen peroxide (H_2O_2) until the effervescence ceased. The flask was removed from the ice bath and allowed to stir at room temperature overnight and centrifuged for 20 minutes. Three successive washes with HCl mixed solvent (9:1 volume ratio) were followed by one with ultra-pure water. The resulting darkish product was dried for 48 hours in a vacuum oven.

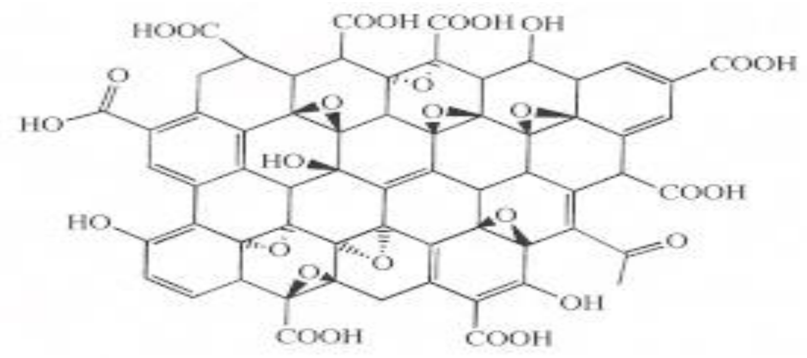


Figure 4.1: Chemical structure of Graphene Oxide (GO)

4.1.5 Preparation of Polyamic Acid –Graphene oxide (PAA-GO) Nanocomposite

Polyamic Acid (0.0534 g) and Graphene oxide (0.0536 g) were mixed with 3 mL of 0.1 M phosphate buffered saline (PBS) pH 7.2. The resulting solution (0.03mg/ml 1:1mass ratio) was then sonicated for 30 minutes.

4.2 Microscopic characterization.

4.2.1 Polyamic Acid (PAA)

For the SEM analysis : PAA was dissolved in 0.1 M PBS (0.03 g/mL solution) respectively and electrochemically deposited onto SPCE electrodes using 5 cycles between −1000 V and 1000 V at 50 mV/s. The analysis was performed on a Hitachi Model X-650 Scanning electron analyser coupled with Energy Dispersive X-ray analyser. Using conductive glue samples were placed on aluminium stubs and coated with a thin layer of carbon grids.

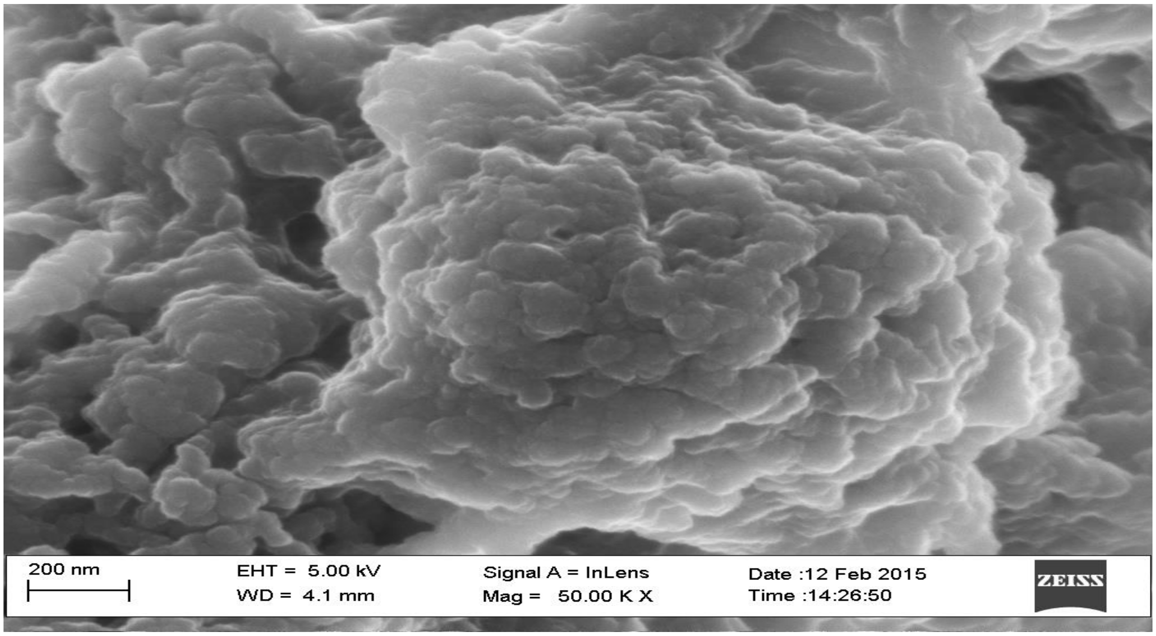


Figure 4.2 : SEM image of polyamic acid

The HR-SEM image of Polyamic Acid (Fig.3a) showed a cluster structure typical of polyamic acid. The clustered structure provides a high surface area for attachment of nanomaterials to produce nanocomposites with enhanced properties.

4.2.2 Graphene Oxide (GO)

The Graphene Oxide (GO) was characterized as a powder. The analysis was also performed on a Hitachi Model X-650 Scanning electron analyser coupled with Energy Dispersive X-ray analyser. Samples were placed on Copper grids for analysis on the SEM instrument.

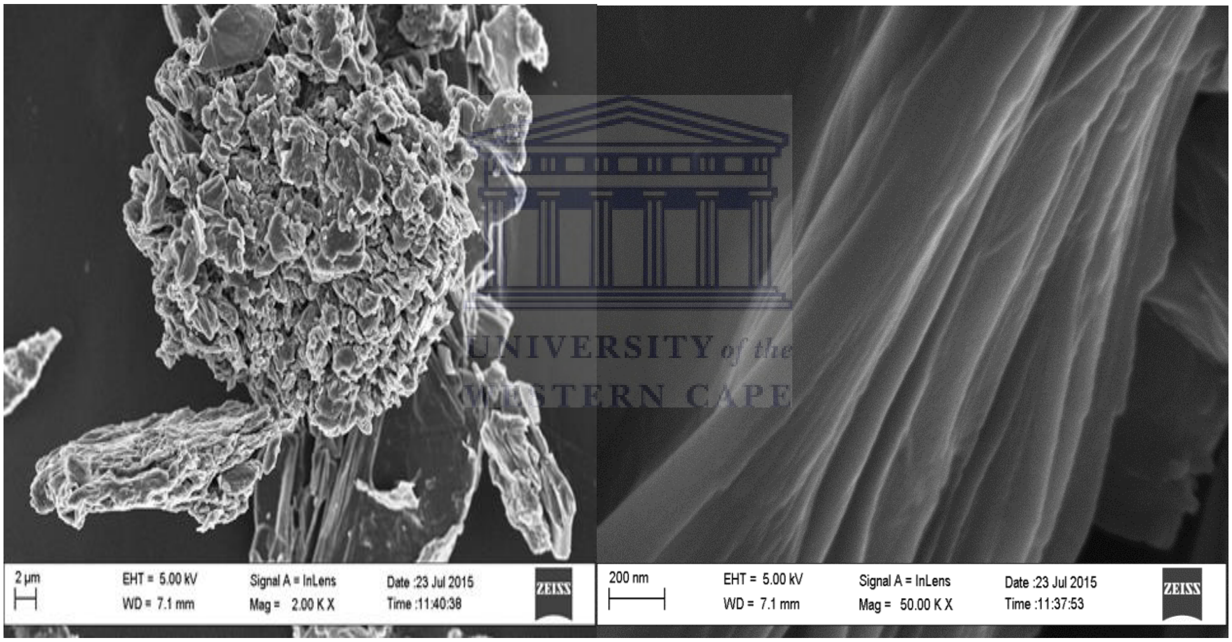


Figure 4.3: SEM of Graphene Oxide (GO)

HR-SEM image of Graphene Oxide (GO) (fig3b and c) showed the laminar wrinkled surface of graphene and the sheets stacked together to form a multi-layer structure. The geometric wrinkling arises from π–π interaction within sheets of GO, which not only minimizes the surface energy but also induces mechanical integrity and tensile strength, so that the film-forming ability is greatly improved. The wrinkled structure of GO sheets provides a large rough surface for further modification (Li et al, u2013).

5.2.3 Polyamic acid-Graphene oxide (PAA)

For SEM (PAA-GO) was dissolved in 0.1 M PBS (0.03 g/mL solution) respectively and electrochemically deposited onto SPCE electrodes using 5 cycles between −1000 V and 1000 V at 50 mV/s. The Graphene Oxide (GO) was characterized as a powder. The analysis was performed on a Hitachi Model X-650 Scanning electron analyser coupled with Energy Dispersive X-ray analyser. Using conductive glue samples were placed on aluminium stubs and coated with carbon.

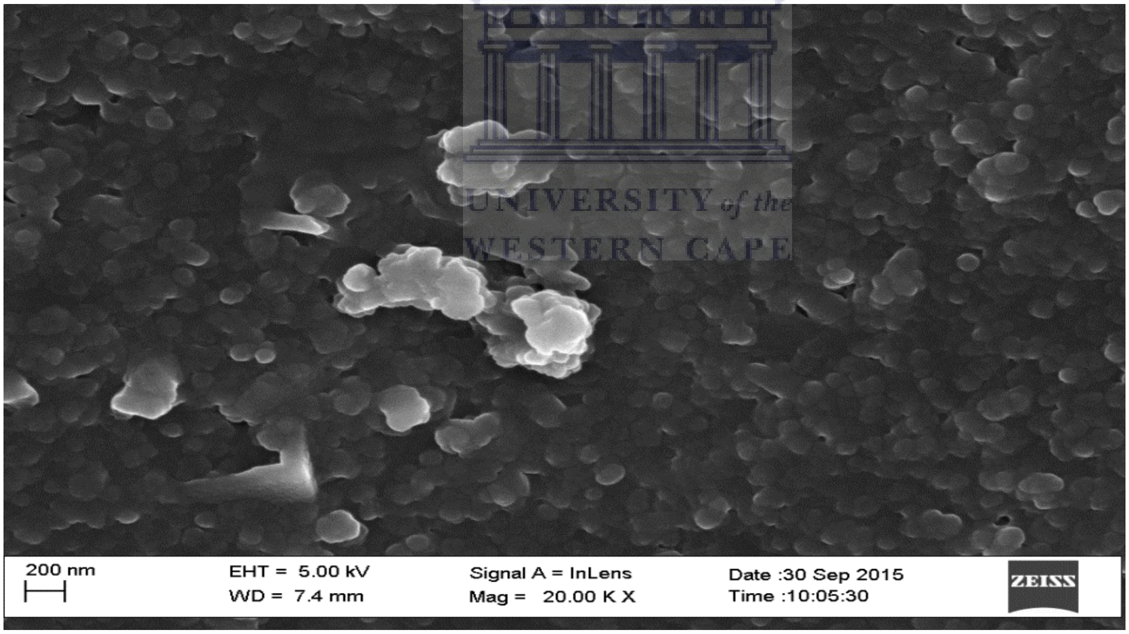


Figure 4.4: SEM image of PAAGO

(Fig.3d) shows the SEM image of polyamic acid –graphene oxide nanocomposite where the PAA is observed to coat the GO completely, resulting in PAA/GO islands, interrupting the laminar structure observed at unmodified graphene oxide.

4.3 Spectroscopic characterization of the transducer materials

4.3.1 Polyamic Acid (PAA)

Both Polyamic Acid (PAA) was recorded as powder (pellet formed using KBr). The FTIR spectra were recorded over the range of 4000 cm^{-1} to 1000 cm^{-1}.

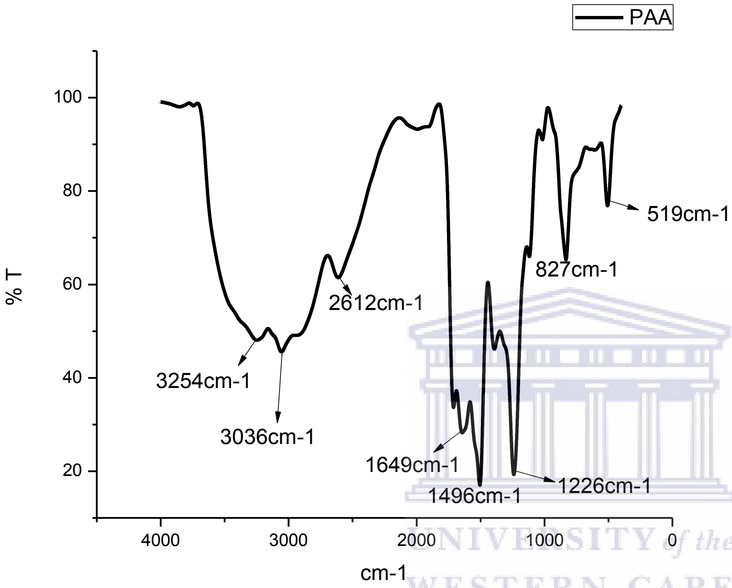


Figure 4.5: FTIR spectrum of Polyamic acid (PAA)

The FTIR spectrum of the Polyamic Acid (PAA) in (Fig.4a) is similar to the spectrum which is in the literature. (Andreescu et al, 2005: Noah et al, 2012). The absorption bands that occur at around 3254 cm^{-1} and 1649 cm^{-1}, and 1392 cm^{-1} indicate the presence of the amide group, whereas the bands occurring at around 2612 cm^{-1} (broad) can be assigned to the vibrational modes of carboxylic acid. A spike appearing at 3049 cm^{-1} shows the existence of NH. The strong peak at around 1239 cm^{-1} indicates the stretching vibration of the ether group.

4.3.2 Graphene Oxide (GO)

Graphene Oxide (GO) was recorded as powder (pellet formed using KBr). The FTIR spectra were recorded over the range of 4000 cm^{-1} to 1000 cm^{-1}.

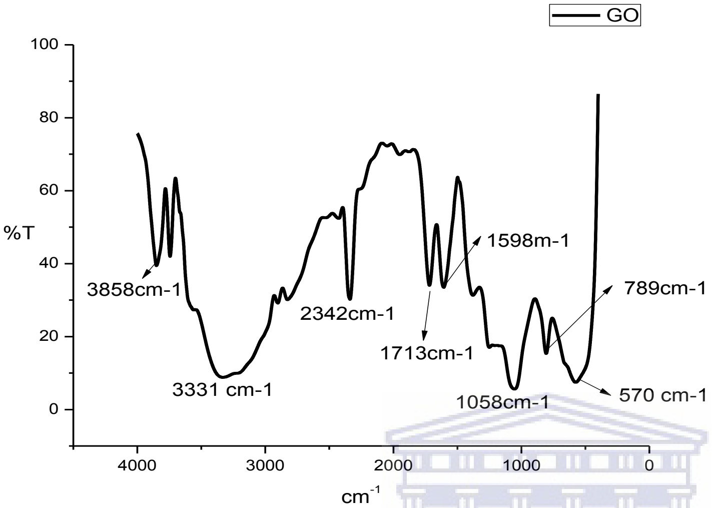


Figure 4.6: FTIR spectrum of Graphene Oxide (GO)

The FTIR spectrum of GO (Fig.4b) shows a weak shoulder at 3858 cm^{-1} indicating the hydroxyl stretching vibrations of the C-OH groups, the O-H stretching vibration at 3331 cm^{-1} .A weak band at 1713 cm^{-1} assigned to the C=O stretching vibrations of the-COOH groups, aromatic C=C stretching vibration at 1598 cm^{-1} a strong band at 1350 cm^{-1} assigned to the O-H deformations of the C-OH groups, and a strong band at 1058 cm^{-1} which is characterize the C-O stretching vibrations. (Pokpas et al 2014: Bourlinos et al, 2013).

4.3.3 Polyamic Acid-Graphene Oxide nanocomposite

Polyamic acid-Graphene oxide (PAA-GO) was dissolved in 0.1 M PBS (0.03 g/mL solution)

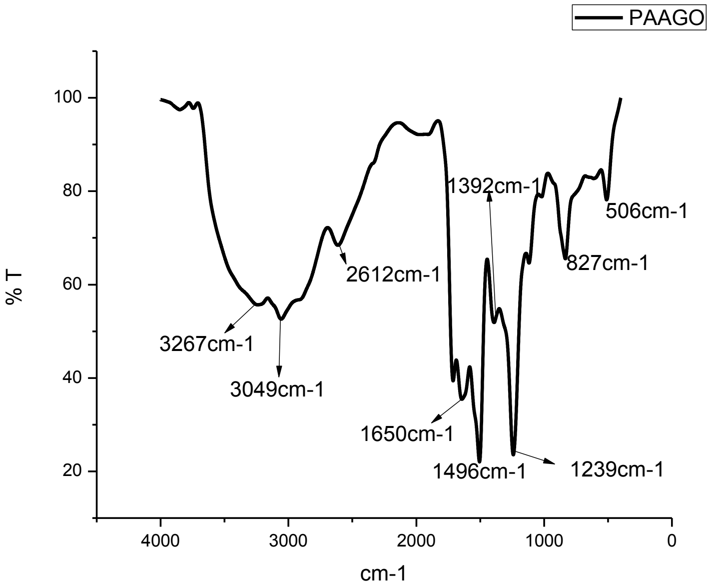


Figure 4.7: FTIR spectrum of PAAGO

The FTIR structure of PAA-GO (Fig.4c) is similar to that of PAA showing the physical dominance of the polymer as a host, interrupting the structure of graphene oxide (GO).

4.4 Electrochemical characterization

Cyclic voltammetry (CV) and Square wave voltammetry (SWV) experiments were carried using PalmSens electrochemical workstation (Bioanalytical Systems, USA.) All experimental solutions were purged with high purity Argon gas and blanketed with Argon atmosphere during measurements. The experiments were carried out at controlled room temperature (25 ºC). The synthesized PAA, GO and PAA-GO were dissolved in 0.1 M PBS pH 7.2 and PAA, GO and PAA-GO were respectively electrochemically deposited onto three different SPCE electrodes using 5 cycles between −1000 mV and 1000 mV at 50 mV/s. The PAA, GO and PAA-GO-modified SPCE electrode were further characterized using CV at different scan rates in 0.1 M PBS with a pH of 7.0.

4.4.1 Effect of scan rate

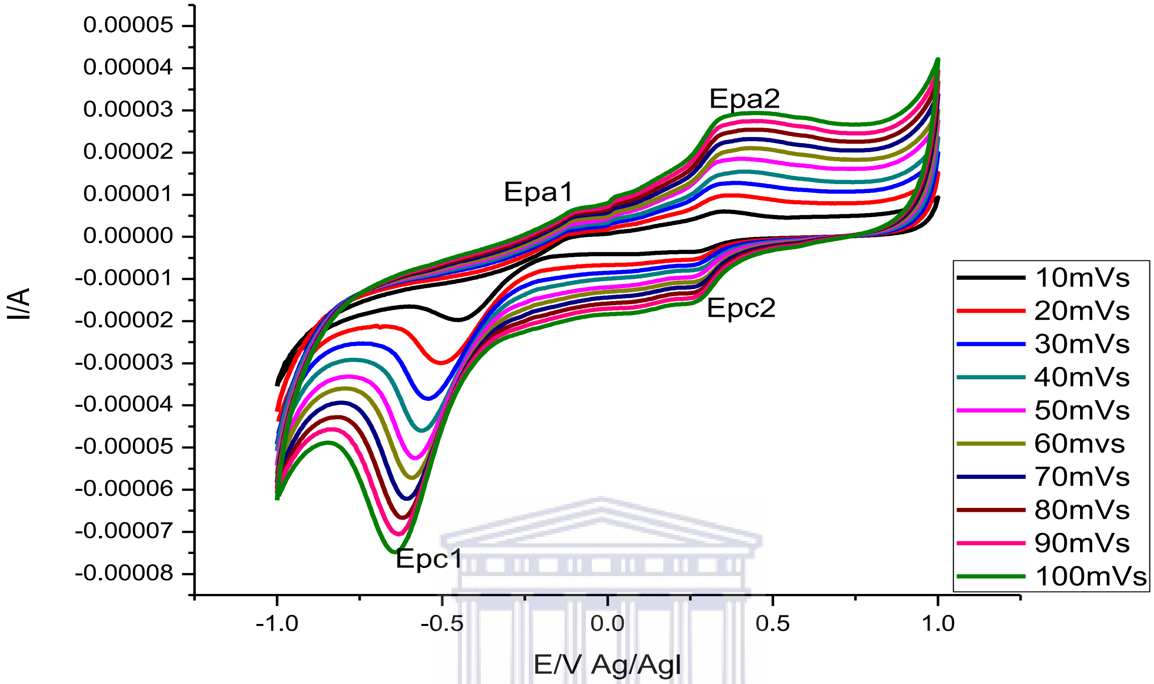


Figure 4.8: Cyclic voltammogram of PAA-SPCE in 0.1 M PBS pH 7.0 at different scan rates (10-100 mVs).

The cyclic voltammetry of polyamic acid showed redox couples which are both reversible. Polyamic acid (PAA) cyclic voltammogram is in agreement with reported studies [Andreescu et al, 2005: Hess et al, 2014) with two anodic peaks which are due to the NH and OH functional groups in the polyamic structure (Epa1 and Epa2) and two cathodic peaks (Epc2 and Epc2). The current peaks of PAA increased with the increasing scan rate.

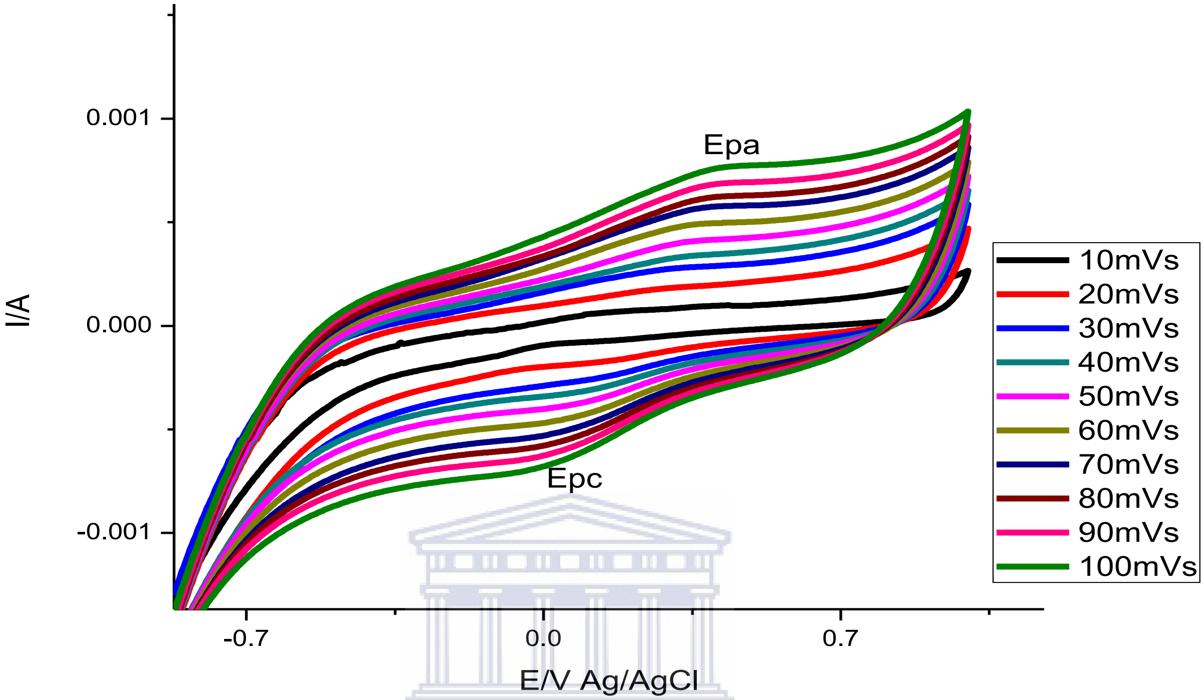


Figure 4.9: Cyclic voltammogram of GO-SPCE in 0.1 M PBS pH 7.0 at different scan rates (10-100 mVs).

Graphene Oxide (GO) Showed one reversible redox couple .The one anodic peak (Epa) and one cathodic peak (Epc) in graphene oxide voltammogram are due to the oxygenated functional groups in the graphene oxide structure such as OH, COOH and epoxides.

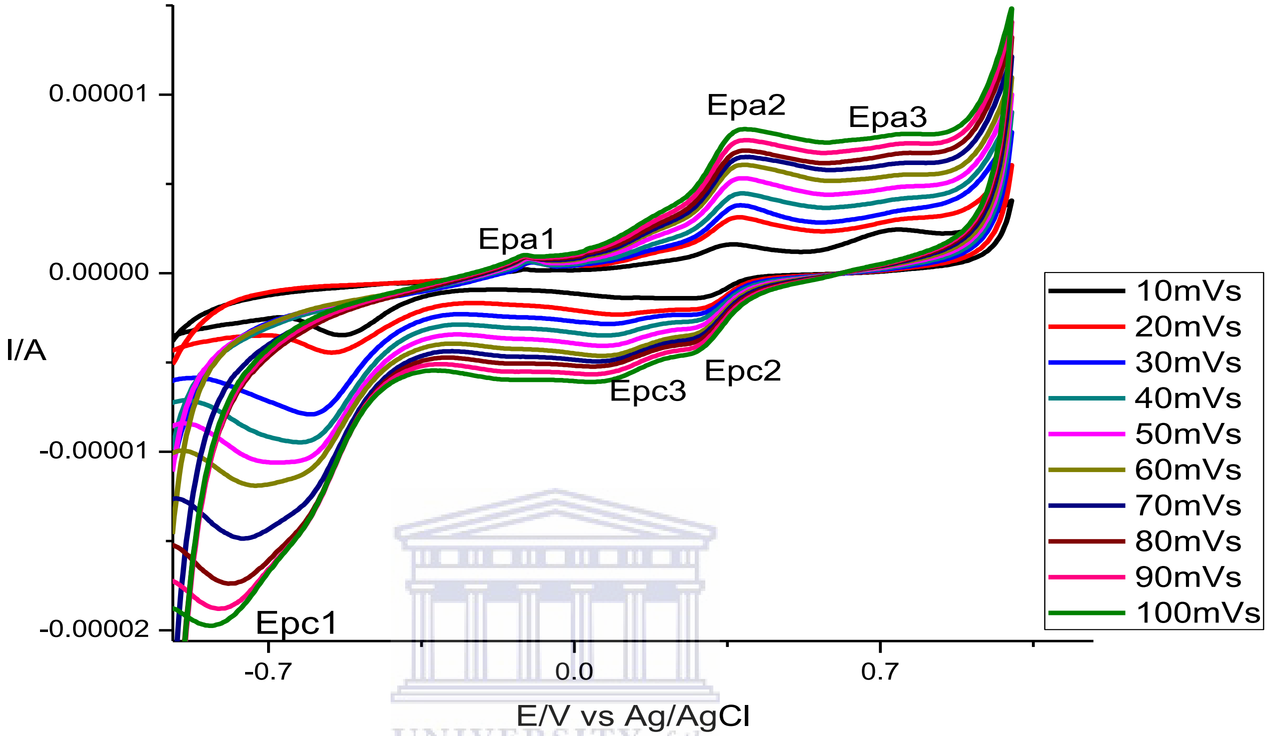


Figure 4.10: Cyclic voltammogram of polyamic acid-graphene oxide (PAAGO-SPCE) in 0.1 M PBS pH 7.0 at different scan rates (10-100 mVs).

The PAAGO nanocomposite displayed three anodic peaks (Epa1, Epa2 and Epa3) and three cathodic peaks (Epc1, Epc2 and Epc3) which confirm the incorporation of the graphene oxide onto the polyamic acid forming a PAA/GO nanocomposite. The redox couple denoted by Epa1/Epc1 and Epa2/Epc2 may be attributed to polyamic acid whilst Epa3/Epa3 is due to graphene oxide incorporation.

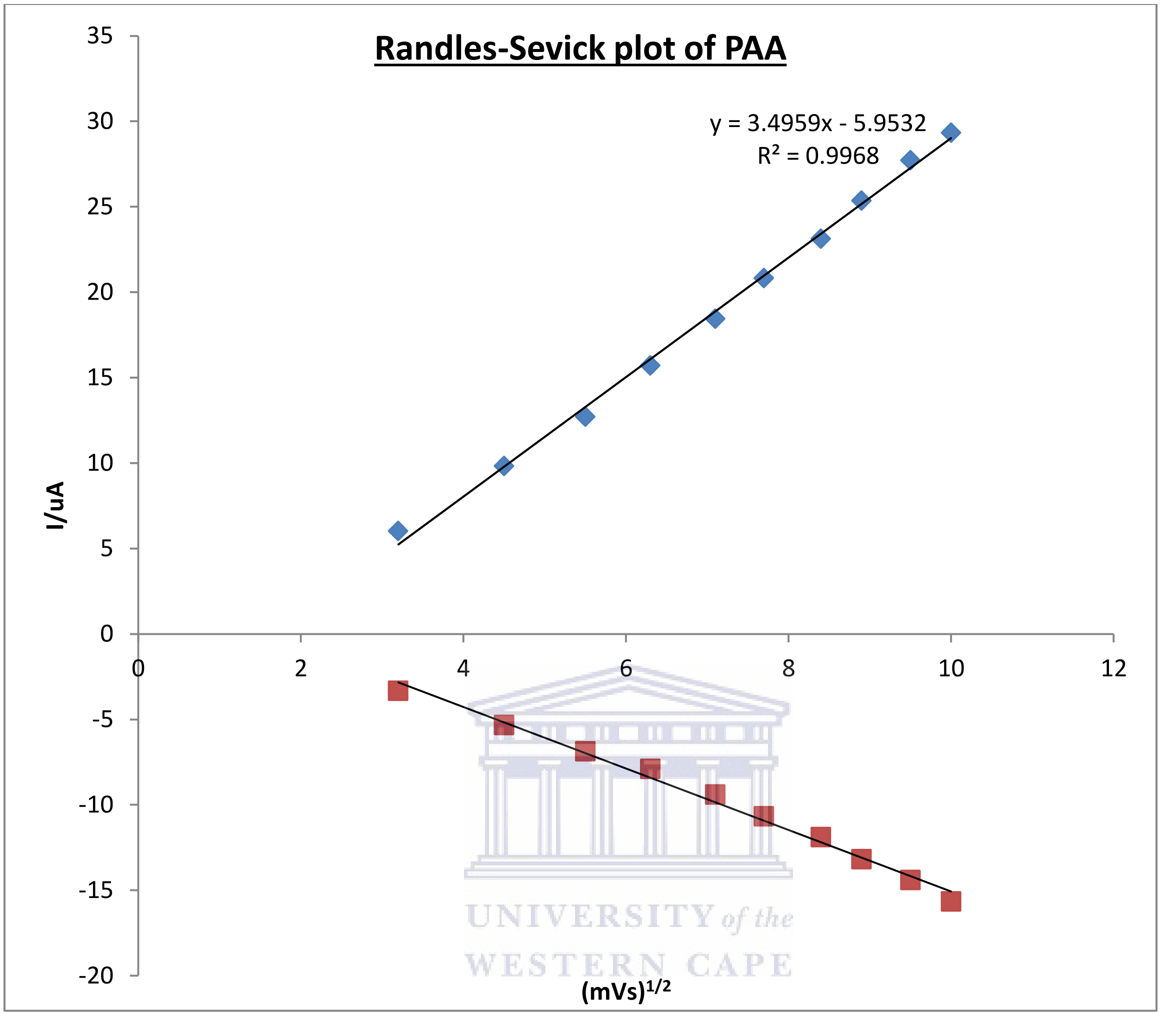


Figure 4.12: Randles-sevick plot of PAA

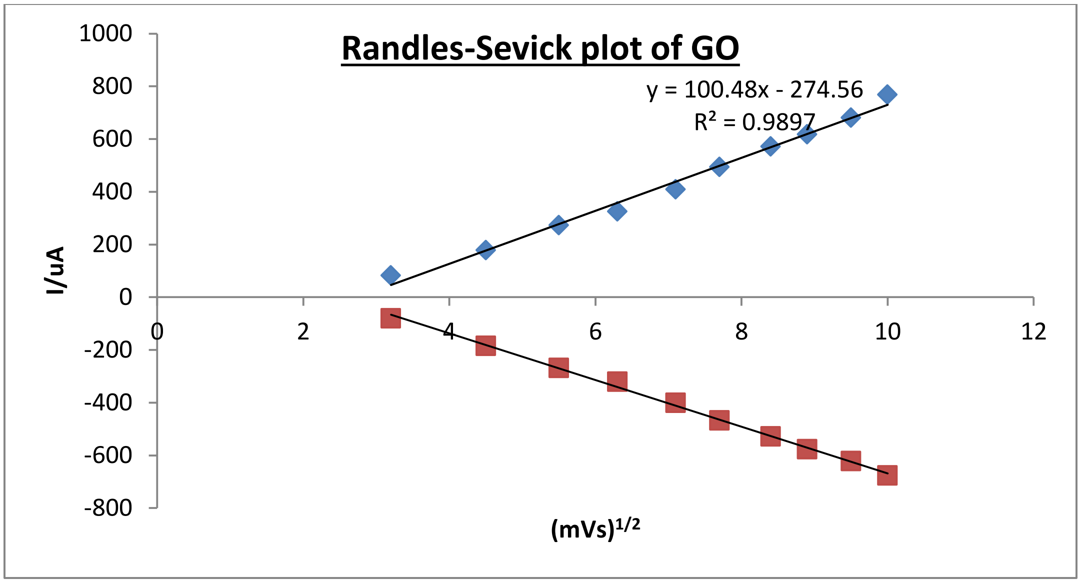


Figure 4.12: Randles-sevick plot Graphene oxide (GO)

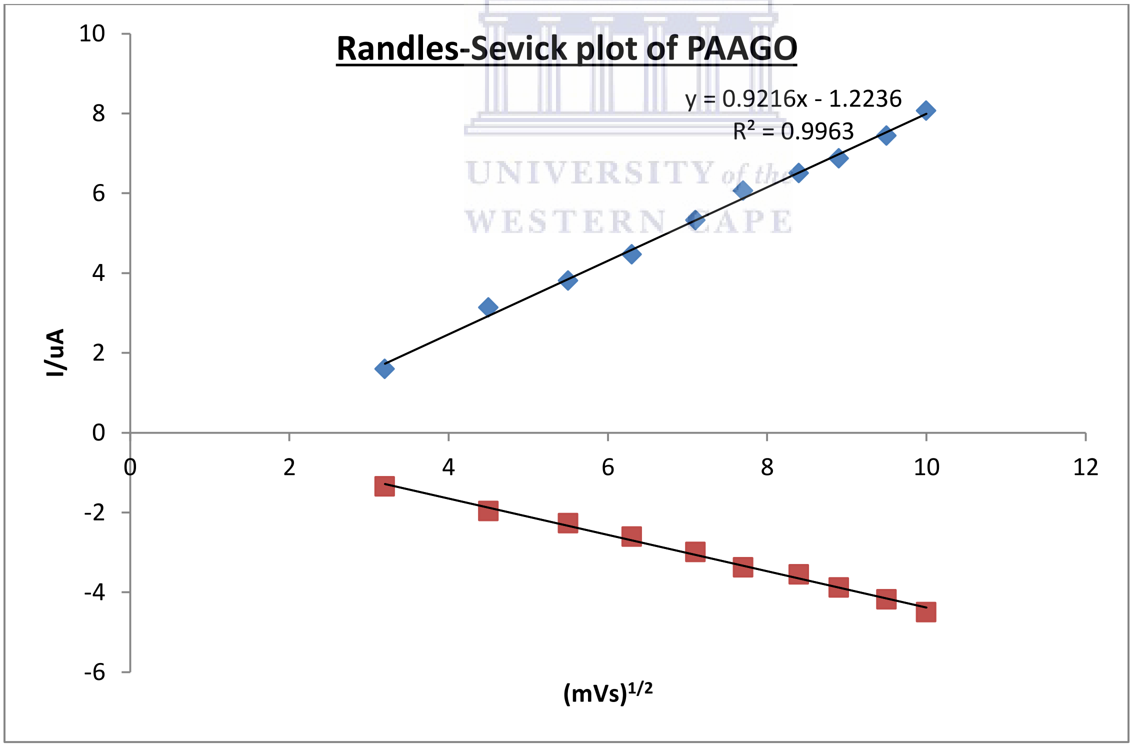


Figure 4.13: Randles-Sevick plot PAAGO

The current peaks of PAA .GO and PAAGO increased with the increasing scan rate. To understand the kinetics of the electron transfer process, the effects of varying the scan rate (m) on the peak currents and peak potentials of all the electrodes were investigated. The diffusion coefficient of anodic response of each electrode was calculated using the Randles– Sevcik equation.

$I_p = (2.69 \times 10^5) A n^{3/2} C D^{1/2} v1/2$

Electrode	Epa1 [V]	Epc1 [V]	Epa2 [V]	Epc2 [V]	Epa3 [V]	Epc3 [V]	ΔP [V]	Eº [V]	D [cm^2s^{-1}]
PAA/SPCE	-0.102	-0.64	-0.40	0.26			ΔP1=0.54 ΔP2=-0.66	E1º =0.37 E2º =0.33	(Ep1) 3.2×10^{-5}
GO/SPCE	0.35	0.024					0.33	0.19	(Ep1) 1.7×10^{-6}
PAA/GO/SPCE	-0.11	-0.74	0.38	0.30	0.72	0.07	ΔP=0.63 ΔP2=0.08 ΔP3=0.65	E1º =0.43 E2º=0.34 E3º=0.40	(Ep3) 1.7×10^{-7}

Table 4.1: Electrochemical parameters of PAA, GO and PAAGO obtained from CV and SWV.

In the above Table 4.1 the obtained electrochemical kinetics of polyamic Acid (PAA), Graphene oxide (GO) and Polyamic acid-graphene nanocomposite respectively are shown. Polyamic acid-graphene oxide was found to have a larger peak separation (Δp3= 0.0.65 V) compared to graphene oxide (GO) which had a peak separation of (Δp3=0.33 V) indicating a faster electron transfer in the PAA/GO platform. The diffusion coefficient of GO and PAA/GO at peak 3 (Ep3) were comparable proving that that peak is due graphene oxide. The calculated diffusion coefficient of the PAA in this study is lower compared to the previously reported diffusion coefficients of PAA (Andreescu et al, 2005, Hess et a, 2014) .This could be due to the different electrode used with Gold electrode (Au) and Glassy carbon electrode (GCE) having a small effective surface area (A) compared to screen printed electrode (SPCE).

The calculated diffusion coefficient of GO/SPCE was lower compared to that of GO/GCE which is reported the literature (Li et al, 2012). GO modified SPCE had a formal potential of 0.19 V. The lower formal potential (Eº) of GO compared to PAA and PAAGO indicated the superior electrical conductivity ability of GO when incorporated appropriately, these atomically thin carbon sheets of GO can significantly improve physical properties of host polymers at extremely small loading (Kim et al, 2010).

4.4.2 Square wave voltammetry (SWV) analysis of the transducer materials

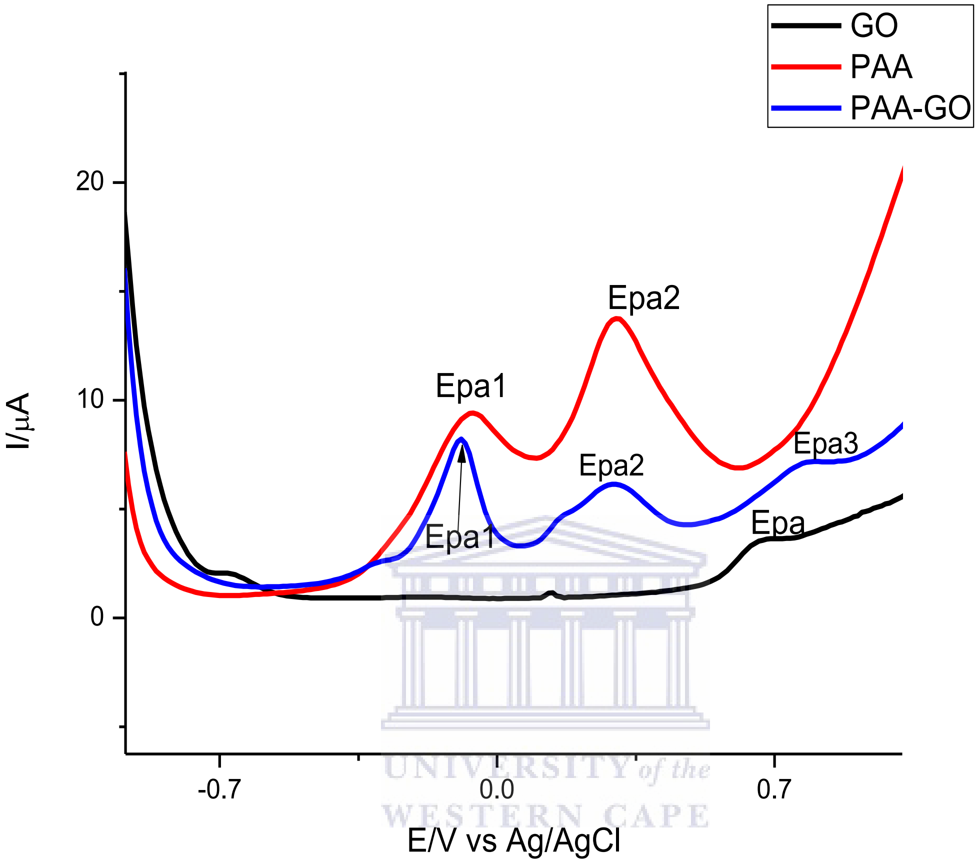


Figure 4.14: oxidative square wave voltammograms of GO, PAA and PAA/GO in 0.1 M PBS pH=7 at a scan rate of 50 mVs

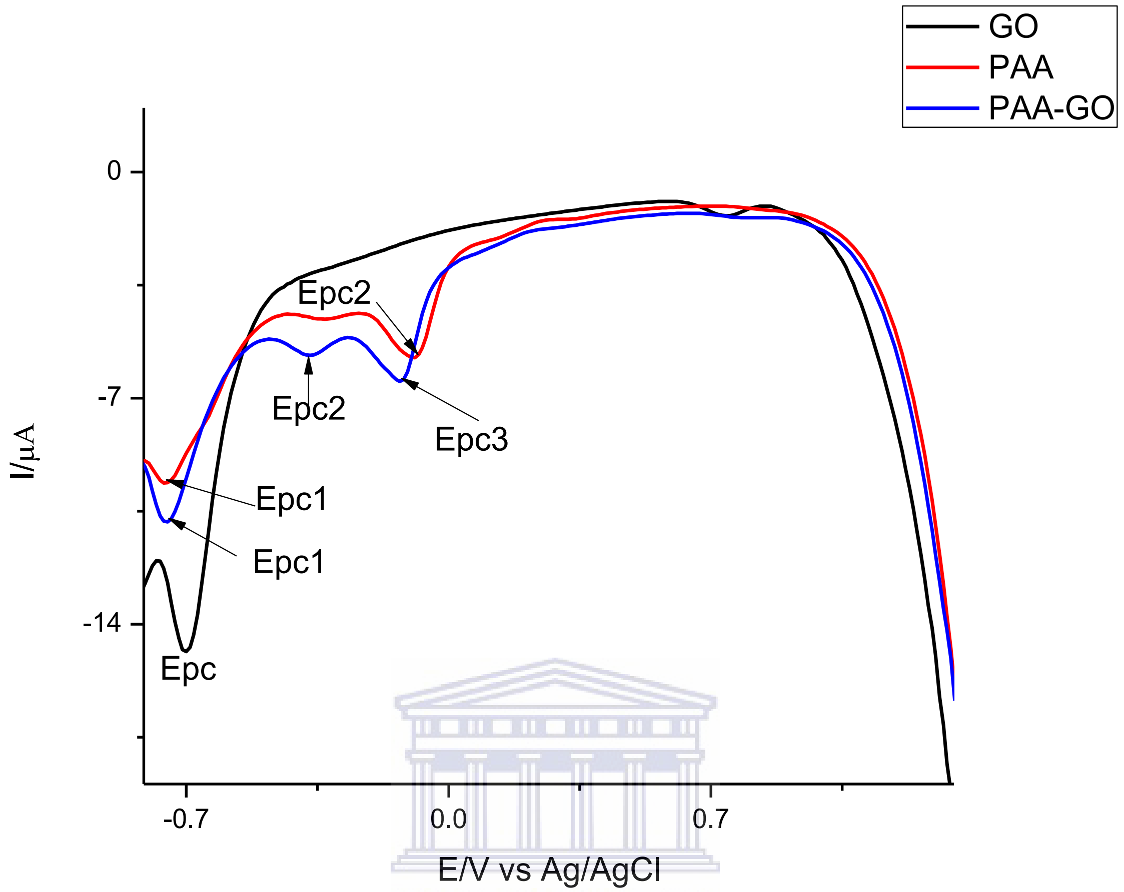


Figure 4.15: Reductive square wave voltammograms of GO, PAA and PAA/GO in 0.1 M PBS pH=7 at a scan rate of 50 mVs

Square wave voltammetry (SWV) confirmed the oxidation and reduction peaks observed in the polyamic acid (PAA), graphene oxide (GO) and polyamic acid -graphene oxide(PAA/GO) the formal potentials for the redox couples identified in the cyclic voltammetry (CV) were calculated for SWV (see table 4.1).

Chapter 5

In this chapter we present the Uv-vis analysis and electroanalytical response of norfloxacin and neomycin at the novel electrode.

5.1 Uv-Vis analysis of Norfloxacin

5.1.1 Sample preparation

A standard solution of 0.1 M of norfloxacin was prepared by dissolving 0.319 g of norfloxacin in 10 mL of 0.1 M HCl and further diluted to different concentrations for analysis on a NICOLET evolution 100 Uv-vis instrument

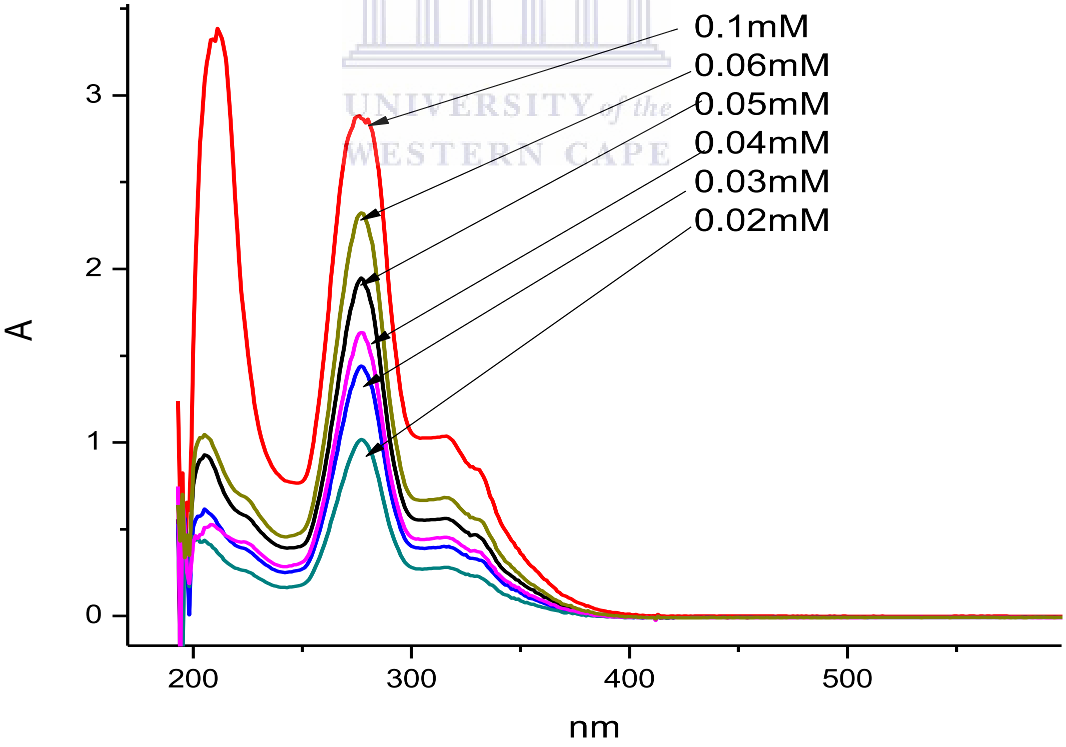


Figure 5.1: Ultraviolet-visible absorption spectra of norfloxacin at λmax. = 277 nm

The absorption spectrum of norfloxacin in 0.1 M HCl solution is shown in Figure 5.1. The λmax was found to be at 277 nm. This wavelength was used for all measurements.

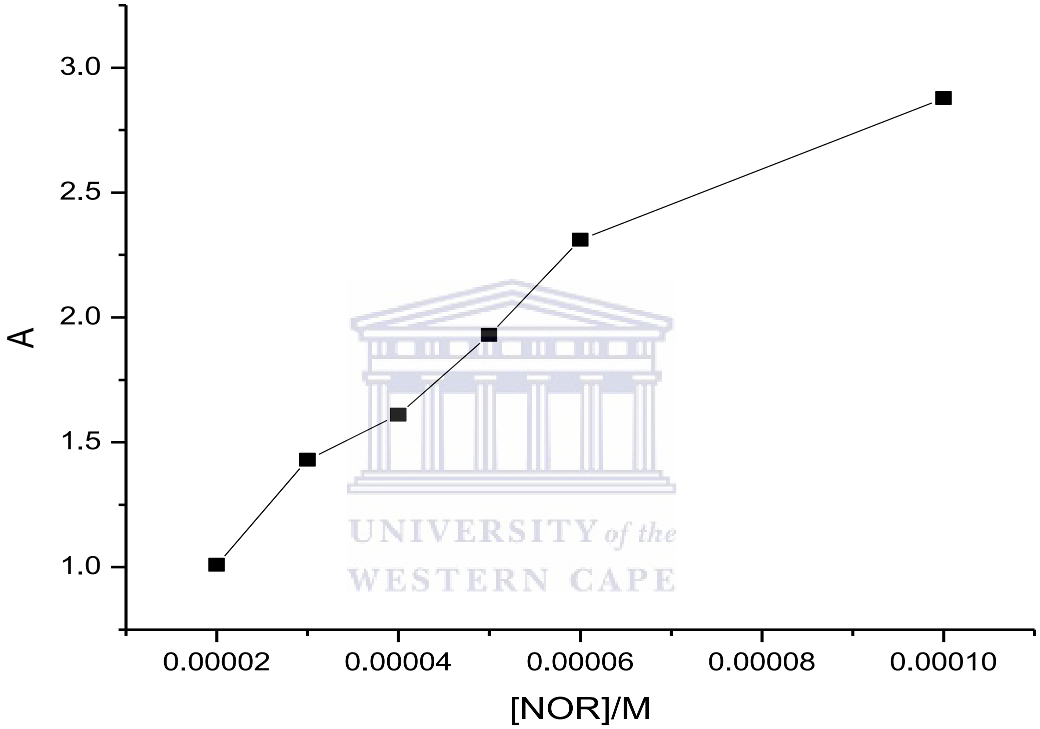


Figure 5.2: Full calibration curve of the UV-vis spectra of norfloxacin at λmax = 277 nm.

The absorption vs concentration response followed a linear relationship at low concentration and reached a plateau at higher concentrations.

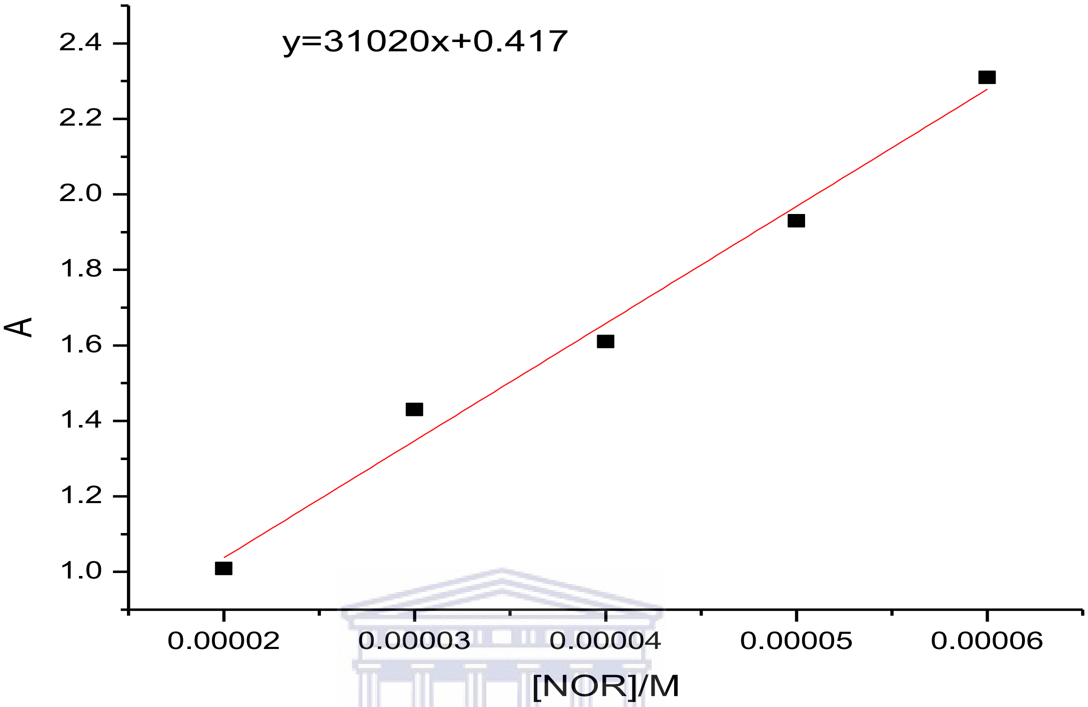


Figure 5.3: Linear range of calibration curve (0.02 mM-0.06 mM) for norfloxacin at λmax = 277 nm

The calibration curve was constructed in the range of expected concentrations (0.02 mM-0.06 mM). Beer's law is obeyed in this concentration range. The value of the determination coefficient was calculated (r^2 = 0.983, y = 31020 x + 0.417), where, x is concentration and y is the absorbance value). From the linear range the LOD was found to be 1.61x10^{-5} M and the LOQ was 4.83 x10^{-5} M (Figure 5.3). The sensitivity of the UV/vis method for detection of norfloxacin in solution using standard solutions was found to be 31020 Au/M.

5.2 Electrochemical detection of Norfloxacin (NOR)

5.2.1 Novel electrode preparation

The novel PAAGO/SPCE electrode was prepared by electrodepositing PAAGO nanocomposite onto a screen printed carbon electrode (SPCE).

5.2.2 Experimental

Experiments on the electroanalytical response of norfloxacin were studied using Square Wave voltammetry (SWV) with the modified electrode in 0.1 M PBS at pH 7.01.

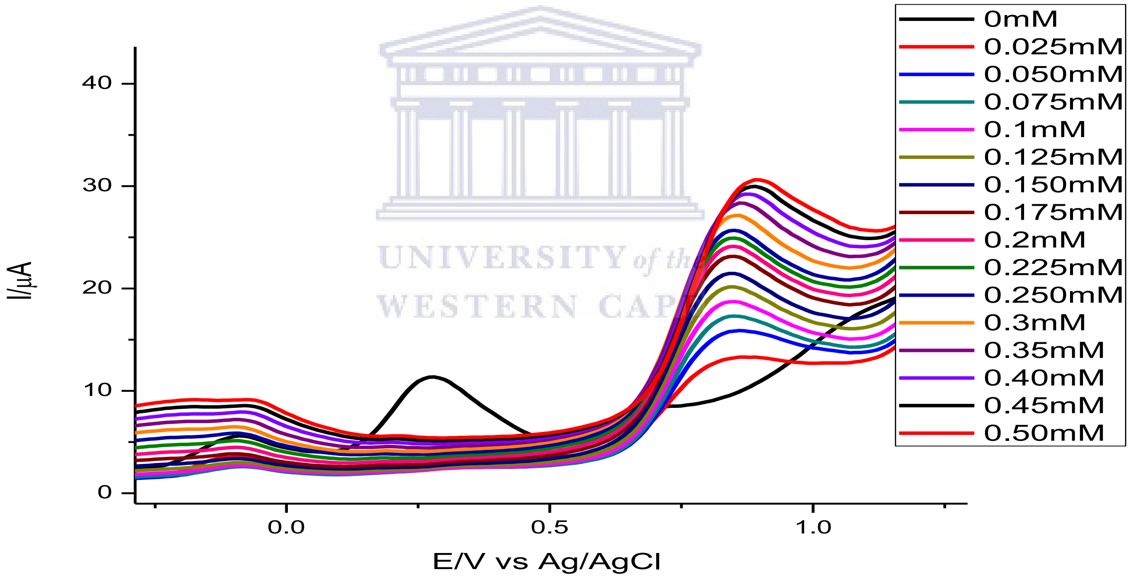


Figure 5.4: Current response measured by SWV at PAA/GO as a function of increasing concentration of norfloxacin, in 0.1 M PBS at pH = 7, vs Ag/AgCl, scan rate 50 mV/s.

In this Figure 5.4 the current response of norfloxacin at different concentrations measured by SWV at PAA/GO in 0.1 M PBS pH=7 vs Ag/AgCl scan rate 50 mVs is shown .The 0 mM represents the bare PAAGO electrode, which is the background response at 50 mVs scan rate, the peak at 0.85 V increased with an increase in concentration of norfloxacin. It is widely reported that the potential peak at 0.85 V represents the electrochemical oxidation of NH group. In the literature it is reported that the oxidation of NH group in the norfloxacin occurs by the transfer of the same number of electrons and protons, thus two electrons and two proton transfer was involved in the reaction at PAAGO/SPCE electrode (Goyal et al, 2012).

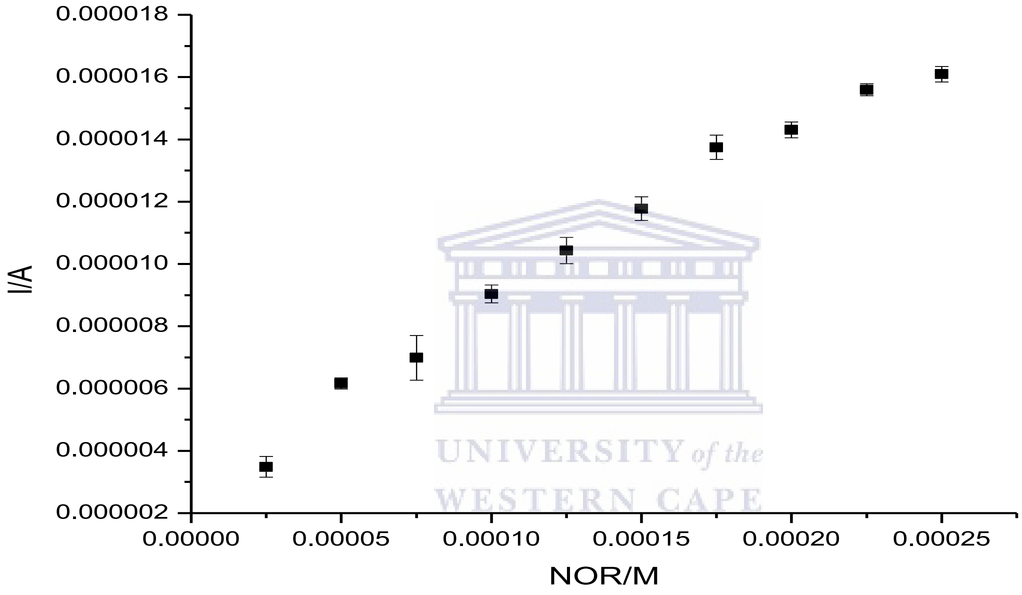


Figure 5.5: Full calibration curve for norfloxacin at PAA/GO electrode, n =3 measurements.

The full calibration curve evaluated form the lowest concentration 0.025 mM to the highest concentration 0.5 m. The linear response region was fitted to linear regression line to evaluate analytical parameters.

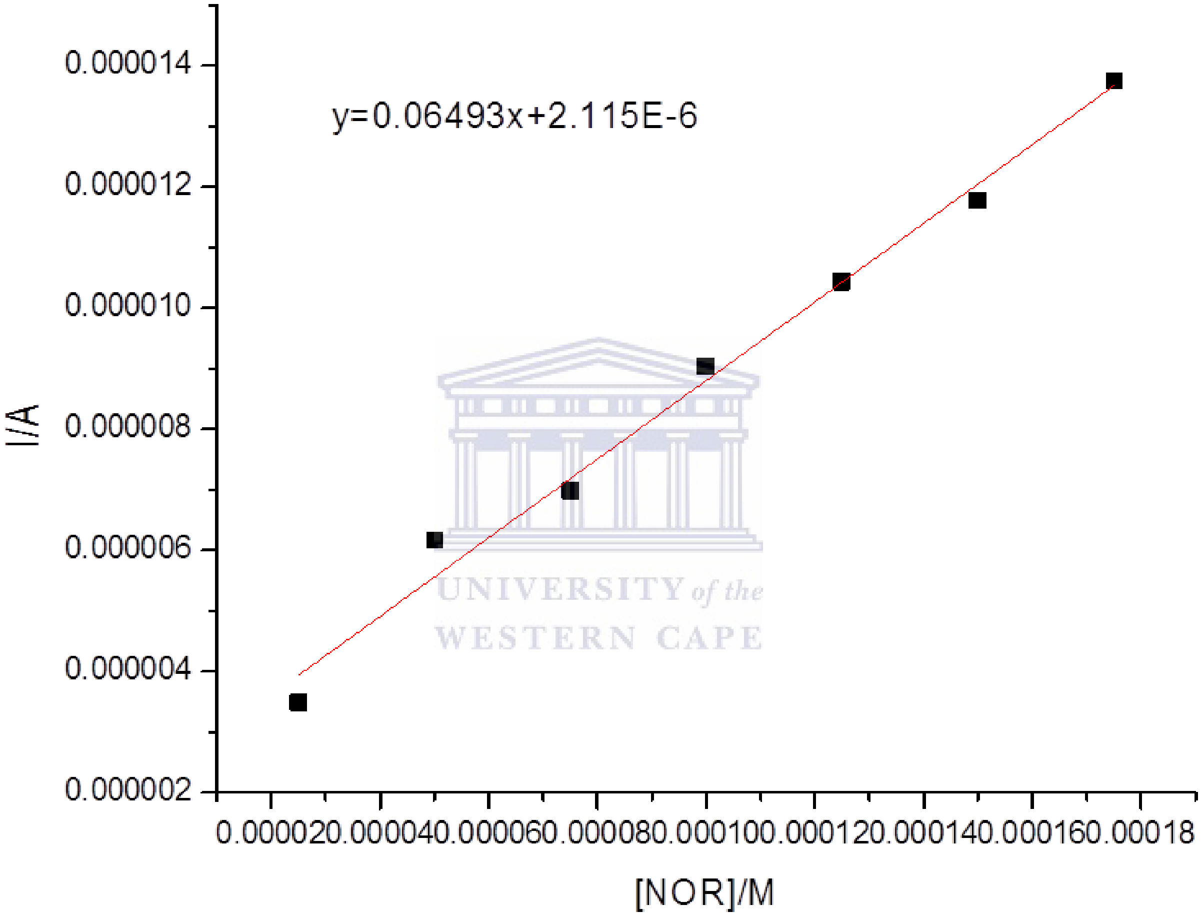


Figure 5.6: Linear plot of the current response of norfloxacin in the range 0.025-0.04mM

The experiment was repeated n=3 times therefore 3 LODs were calculated. The LODs for the 3 experiments were 3.28x10^{-7} M, 2.82x10^{-7}M and 4.02x10^{-7}M with a Standard deviation (STD) value of 6.07799^{-6} .The average LOD| was found to be 3.37x10^{-5} M s/n =3 with a sensitivity of 0.06493 A/M. The analytical performance of the PAA/GO was comparable to that of a widely used analytical method Uv-vis spectroscopy (chapter 5.1) in the determination of norfloxacin and other antibiotic residues.

Method	LOD	LOQ	Peak Potential	Sensitivity	Reference
CuO/MWCNT/GCE	3.21×10^{-7} M	9.63×10^{-7} M	+0.91 V	0.028 A/µM	Devaraj et al, 2013
Edge plane pyrolytic graphite	2.8×10^{-7} M	8.4×10^{-7} M	+0.95 V	0.0733 µA/µM	Goyal et al, 2012
Poly (methyl red) film modified GCE	1.0×10^{-7} M	3.0×10^{-7} M	+1.12 V	Not reported	Huang et al, 2008
MWCNT/nafion/GCE	5×10^{-7} M	1.5×10^{-7} M	+1.1 V	0.2410 µA/M	Huang et al, 2008
PAA/GO/SPCE	3.37×10^{-7} M	1.01×10^{-7} M	0.89 V	0.065 A/M	This study

Table 5.1: Comparison of analytical performance of the novel PAA/GO chemical sensor to the published data for norfloxacin detection.

In comparing the analytical performance of the PAA/GO chemical sensor to the published work, the PAA/GO chemical sensor was found to have a comparable LOD and peak potential which proves that the PAA/GO electrochemical sensor is a stable, low cost, environmentally friendly and easy alternative method to detect norfloxacin in the aqueous medium. The analytical

performance of the PAA/GO sensor was second only to CuO/MWCNT/GCE sensor reported by Devaraj et al, 2013; however the PAA/GO fabrication is simpler, in situ and highly reproducible.

The oxidation of norfloxacin may result in other pharmaceutical products which can cause the same antibiotic resistance as norfloxacin in the water systems.

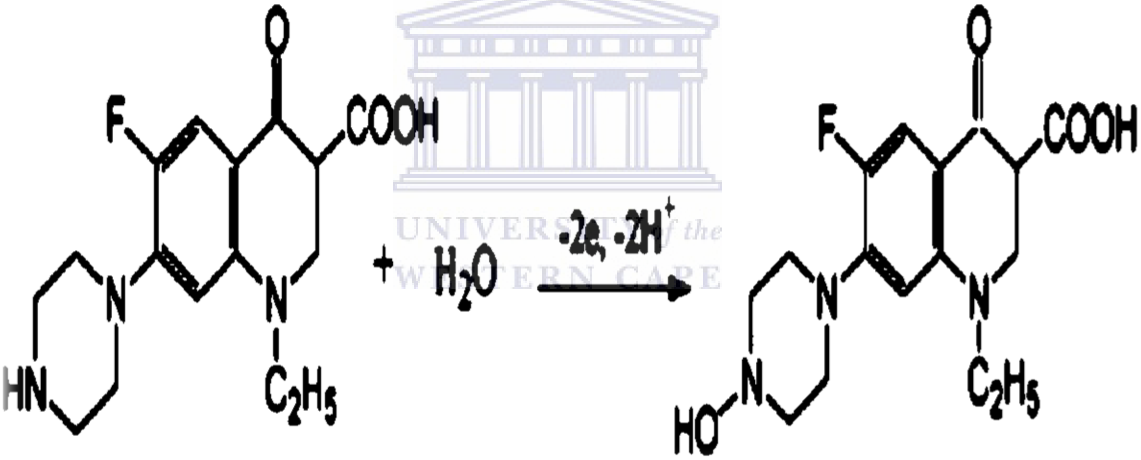


Figure 5.7: Oxidation mechanism norfloxacin

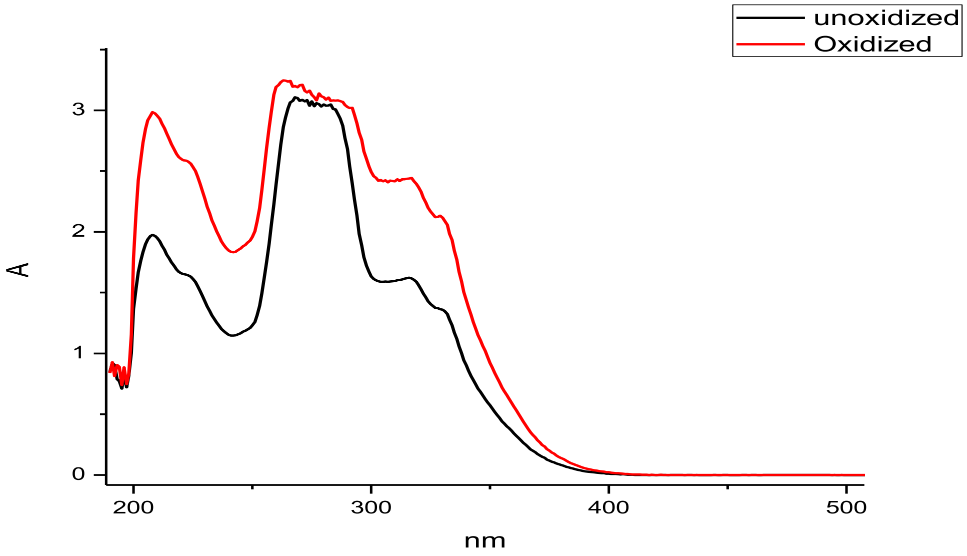


Figure 5.8: UV-vis spectra of 0.1 mM norfloxacin before and after oxidative SWV scan at 50 mVs.

Uv-vis spectra of norfloxacin were recorded before (un-oxidized) and after (oxidized) electrochemical oxidation in the SWV experiment at 50mVs (figure 5.8), showed very similar profiles. This indicates that the analytical peak current measured by SWV was due to a potential induced activation state rather than oxidative degradation of the norfloxacin. This is very important in analytical chemistry, since the SWV detection of norfloxacin is non-destructive and the same sample can either be validated by another method or subjected to repetitive analysis, with easy regeneration of the working electrode.

5.3 Uv-Vis analysis of neomycin

5.3.1 Sample preparation

A standard solution of 1 mM of neomycin was prepared dissolving 0.023 of neomycin sulfate in 25 mL of H_2O and further diluted to different concentrations for analysis on a NICOLET evolution 100 Uv-vis instrument

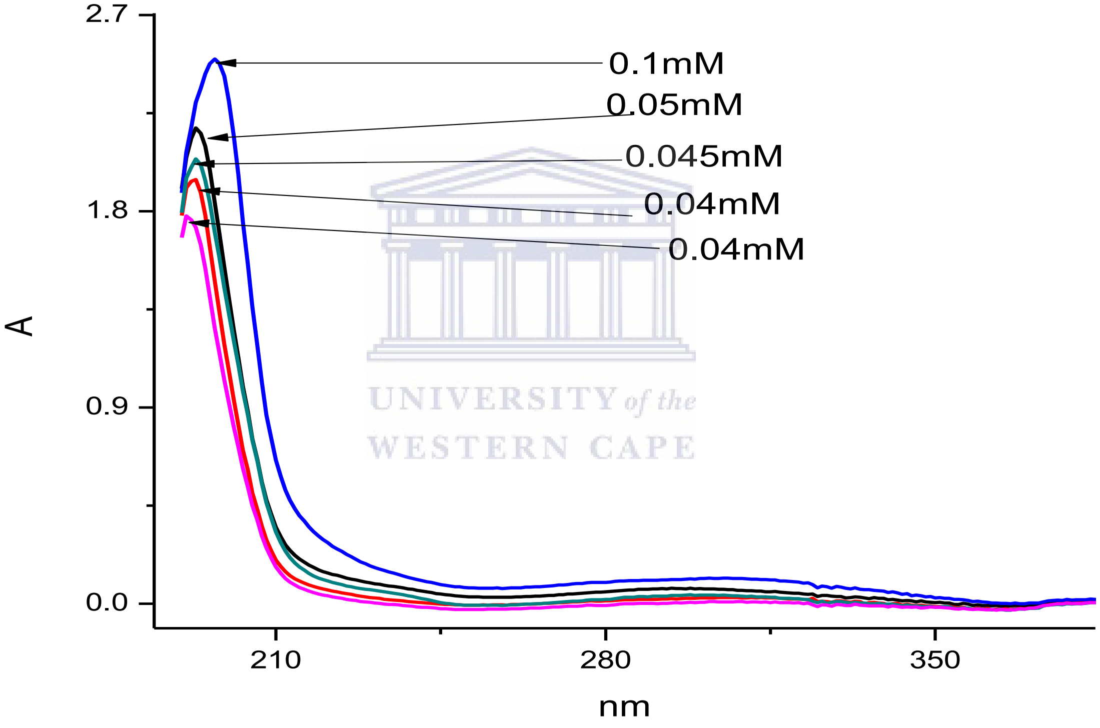


Figure 5.9: Ultraviolet-visible absorption spectra of neomycin at λmax. = 196.7 nm

The absorption spectrum of neomycin in an aqueous solution is shown in Fig. 5.9. The λmax was found to be at 196.7 nm. This wavelength was used for all measurements.

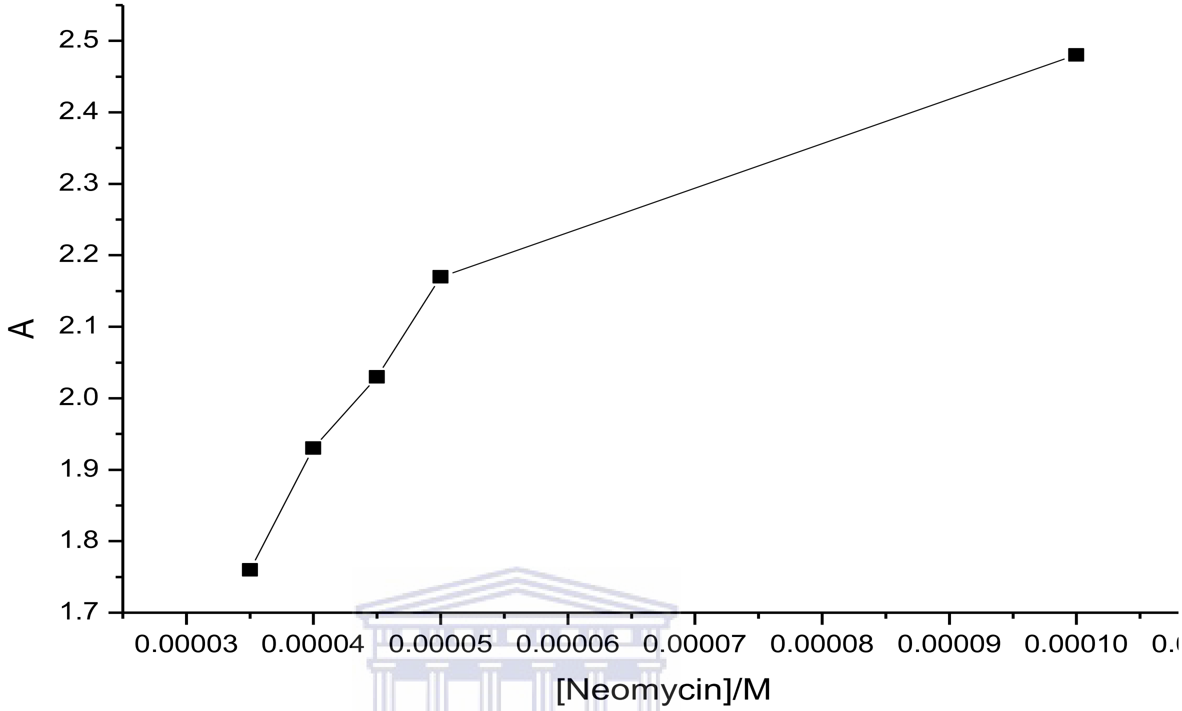


Figure 5.10: Calibration curve of the UV-vis spectra of neomycin at λmax = 196.7 nm

The absorbance values increased with the increasing concentration and started to deplete at higher concentrations indicated

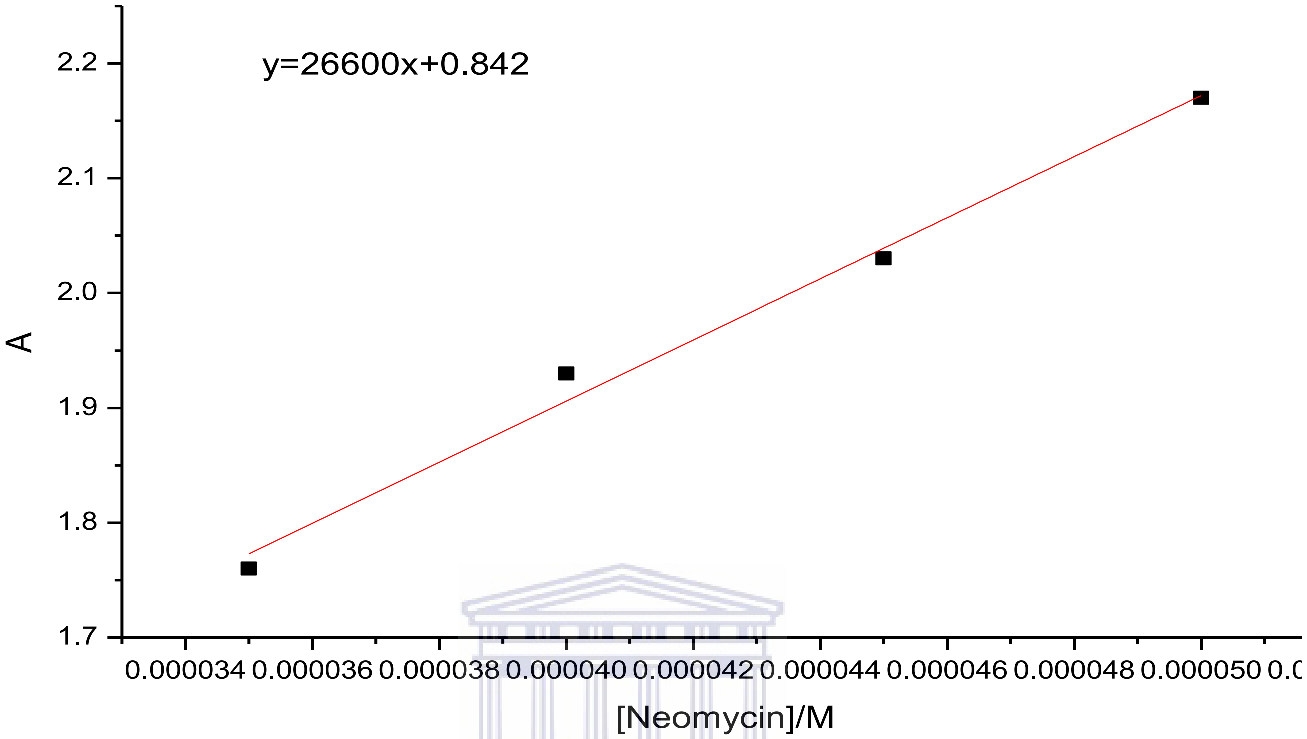


Figure 5.11: linear plot (0.04-0.05 mM) of neomycin at λmax = 196.7 nm

The calibration curve was constructed in the range (0.04 mM-0.05 mM) where Beer’s law was obeyed. The value of the determination coefficient was calculated (r^2 = 0.986, y = 26600 x + 0.842, where, x is concentration and y is the absorbance value). From calibration curves (figure 5.9 and 5.10) the LOD was found to be 1.41x10^{-5} M and LOQ was 4.23x10^{-5} M. The sensitivity of the UV/vis method of detecting neomycin in solution using standard solutions was found to be 26600 Au/M.

5.3 Electrochemical detection of Neomycin

5.3.1 Novel electrode preparation

The novel PAAGO/SPCE electrode was prepared by electrodepositing PAAGO nanocomposite onto a screen printed carbon electrode (SPCE).

5.3.2 Experimental

Experiments on the electroanalytical response of neomycin were studied using Square Wave voltammetry (SWV) with the modified electrode in 0.1 M HCl.

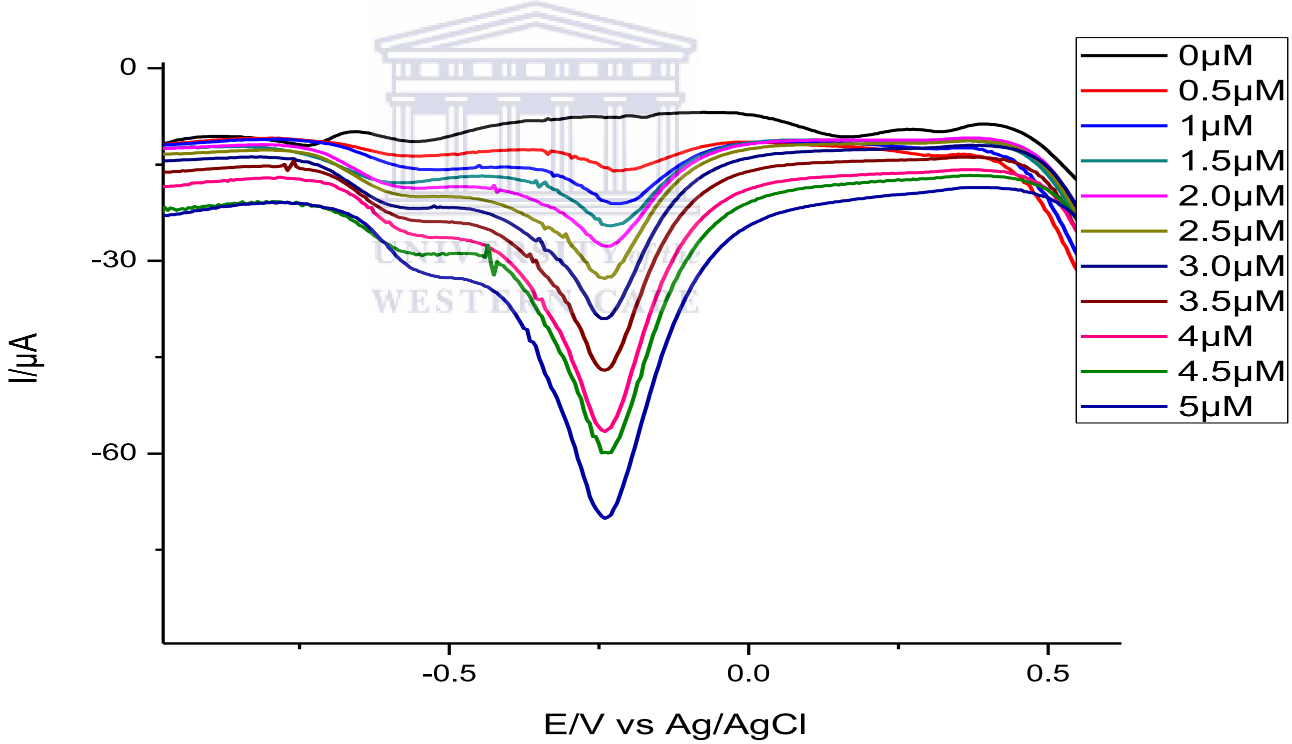


Figure 5.12: Current response measured by SWV at PAA/GO as a function of increasing concentration of neomycin, in 0.1 M PBS at pH = 7, vs Ag/AgCl, scan rate 50 mV/s

The neomycin current response at the PAA/GO is displayed in figure 5.12, showed a well-defined reductive peak with the peak potential of -0.24 V. The reductive peak at -0.24 V increased with the increasing concentration of the neomycin onto the PAA/GO.

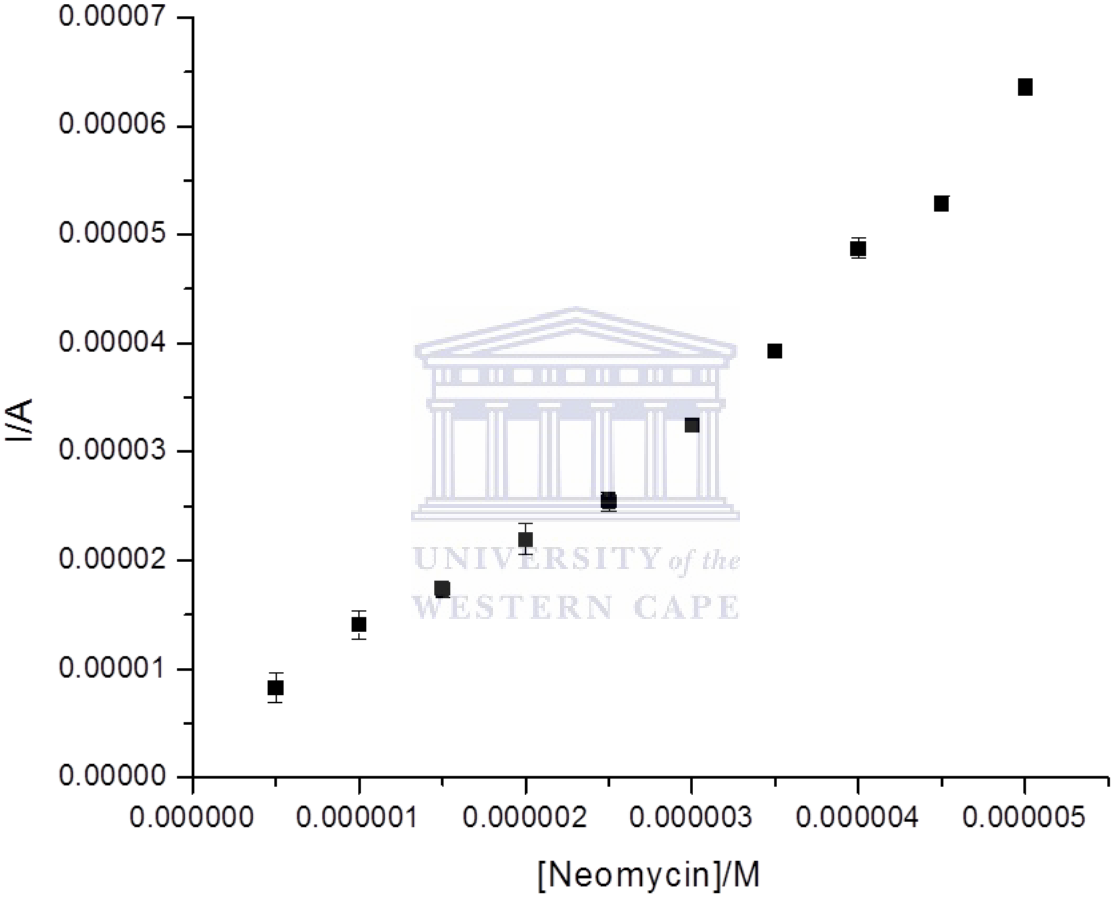


Figure 5.13: Full calibration curve for neomycin at PAA/GO electrode, n =3 measurements

The full calibration curve in figure 5.13 shows the increase of the current response at lower concentration to higher concentration. The linear response (0.5-3 μM) region was then fitted linear regression line to evaluate the analytical performance of the PAA/GO electrochemical sensor towards the detection of neomycin.

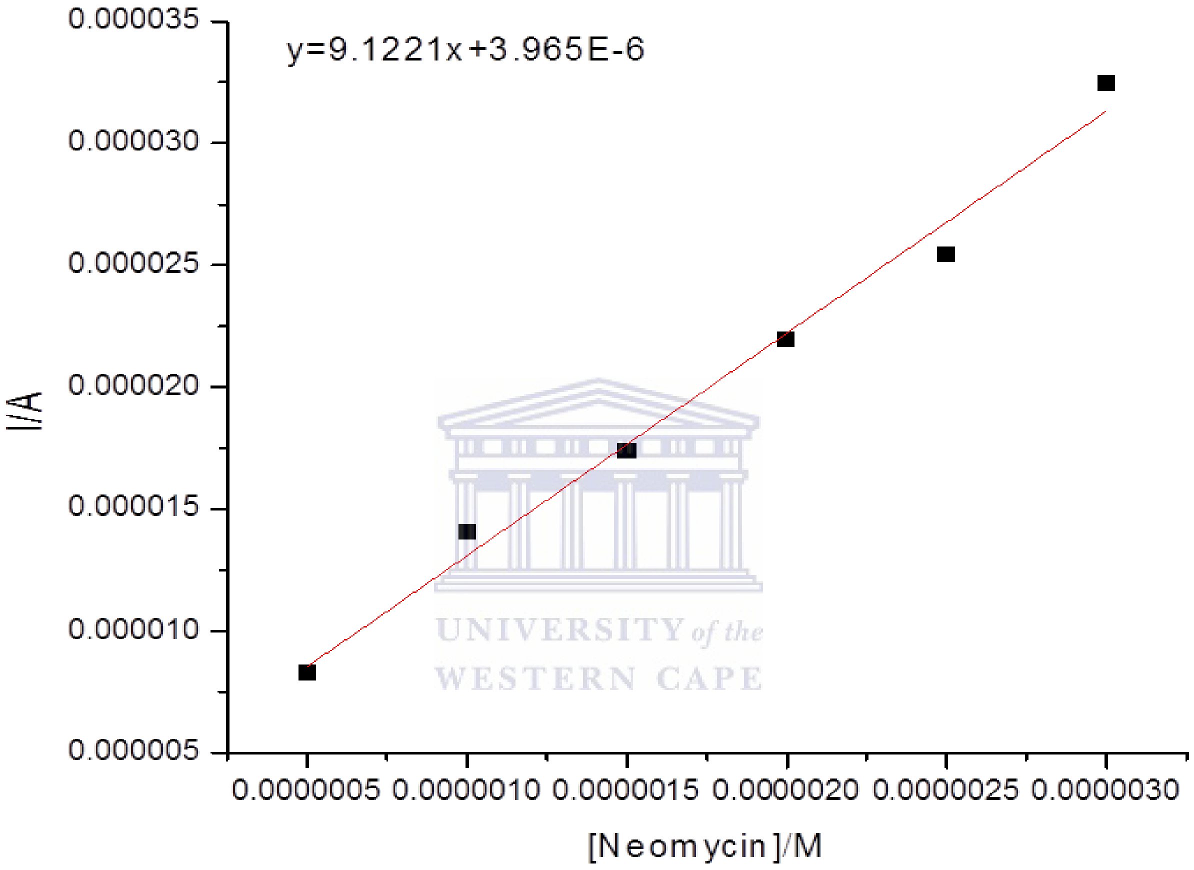


Figure 5.14: linear plot of the current response of neomycin in the range 0.5-3 µM

The linear plot in figure 5.14 was used to study the analytical performance of the PAA/GO towards the detection of neomycin in the aqueous medium. The electrochemical detection of neomycin in the aqueous medium using SWV was performed n=3 times, the 3 experiments had the following LODs 1.79x10^{-6} M, 1.21x10^{-6} and 8.11x10^{-7}. The average LOD s/n=3 for PAA/GO sensor towards the detection of neomycin was found to be 1.067x10^{-6} M and the sensitivity of the sensor was 9.1221 A/M. The analytical performance of the PAA/GO electrochemical sensor was then compared to the reported chemical sensor in the literature.

Electrode	Linear range	LOD	LOQ	Sensitivity	Author
Chitosan AgNPs/ graphene-MWCNTs (chemically imprinted)	9 nM-7 µM	7.63 nM	22.89 nM	Not reported	Huang et al, 2013
PAA/GO	0.5-3 µM	1.07 µM	9.27 µM	9.12 A/M	This study

Table 5.2: Analytical performance of PAA/GO chemical sensor compared to published data for neomycin detection

The analytical performance of PAA/GO chemical sensor compared to the analytical performance of chemical sensors that are already published is displayed in the above table 5.2. There are fewer studies reporting electrochemical sensors for the detection of neomycin, compared to norfloxacin. The PAA/GO chemical sensor was comparable to the published LOD considering that the PAA/GO is a much easier chemical sensor to make compared to the more complex chemical sensors that have been reported in the literature (Huang et al, 2013).

The reduction peak current of neomycin at -0.24 V (vs Ag/AgCl) was assigned to the reduction of the OH functional groups in the neomycin chemical structure. The mechanism below (figure 5.15) proposed the pathway for a one electron and one proton redox reaction. The reduced form of neomycin may cause the same antibiotic resistance as neomycin.

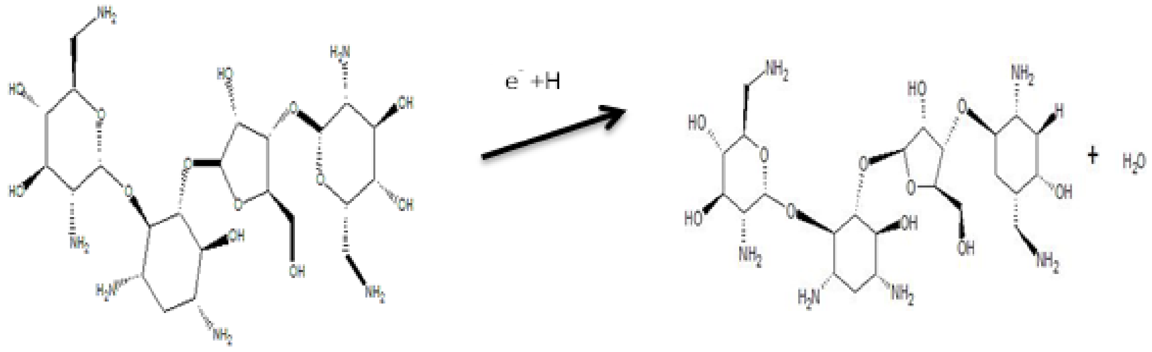


Figure 5.15: Reduction mechanism of neomycin

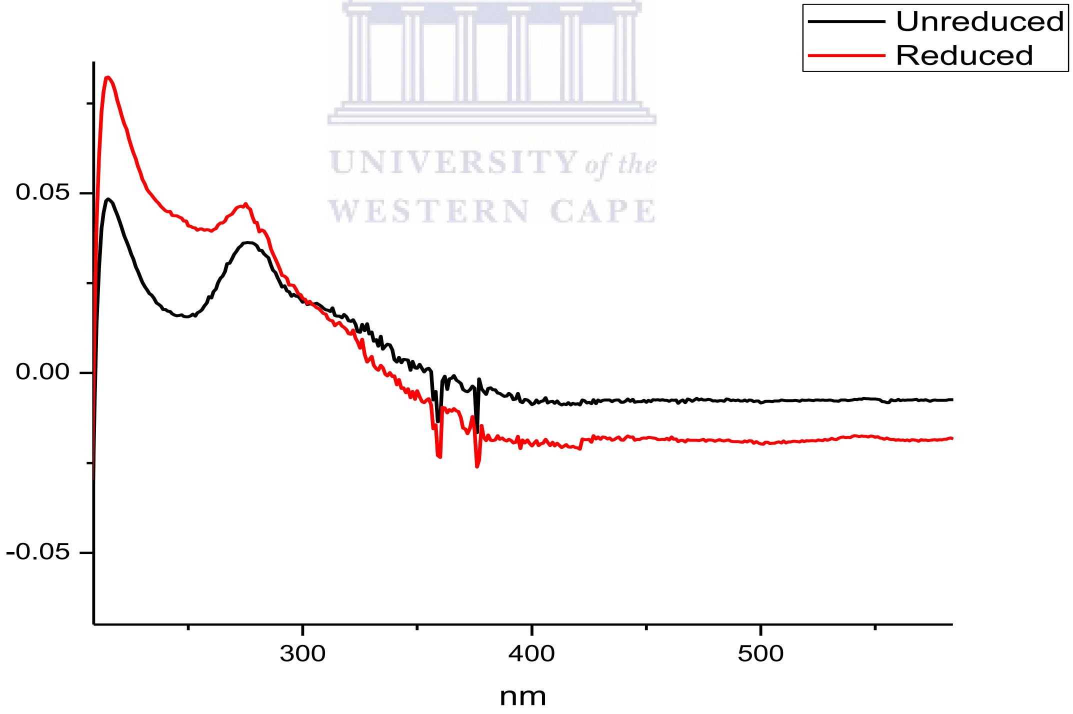


Figure 5.16: a UV-vis spectrum of 2 µM neomycin before and after it was electrochemically scanned in the reductive scan in 0.1 M PBS pH=7 at 50 mVs.

UV-vis spectra of neomycin before (unreduced) and after (reduced) it was electrochemically scanned reductively, using SWV shows that no new product is formed during the reductive scanning of neomycin using the SWV at 50 mVs. The reduction energy results in peripheral electron exchange that leaves the bulk structure intact. The Uv-vis spectra together with the SWV response suggests that neomycin undergoes a temporary electronic state which gives rise to the neomycin current signal, making it a non-destructive technique for neomycin detection and quantification.

Chapter 6:

Conclusions

Polyamic acid and graphene oxide were successfully synthesized, polyamic acid was synthesized in situ polymerization combining ODA and PMDA forming a yellow powder and Graphene oxide synthesized using the Hummer’s method. The polyamic acid-graphene (PAA/GO) nanocomposite was formed by combining polyamic acid and graphene oxide. PAA ,GO and PAA/GO were characterized using Scanning electron microscopy (SEM) ,Fourier Transform infrared spectroscopy (FTIR) ,Cyclic voltammetry (CV), and Square wave Voltammetry (SWV). The SEM was used to study the morphology of the GO PAA and PAA/GO, the SEM image of PAA a typical clustered structure of a polyamic acid which confirmed the successful synthesis of PAA. The SEM of GO showed a laminar wrinkled surface of graphene and sheets stacked together to form a multilayered structured.

The SEM image of PAA/GO confirmed the successful incorporation of GO into the PAA structure ,the image showed PAA which appeared to be coating the GO structure resulting in the PAA/GO islands interrupting the laminar structure observed in the unmodified GO. FTIR spectroscopy confirmed the chemical structures of PAA and GO, also confirmed the functional groups associated with PAA and GO. Cyclic voltammetry (CV) and Square wave voltammetry (SWV) provided evidence on the successful synthesis polyamic acid and graphene oxide by studying their unique electrochemical behavior. CV and SWV confirmed the successful formation of PAA/GO nanocomposite, the electrochemical behavior of PAA/GO showed a combined electrochemical behavior of PAA and GO. Electrochemistry of PAA/GO nanocomposite retained the functional groups of both PAA/GO.

From literature the electrochemical sensors for the detection of norfloxacin which were developed earlier include poly (methyl red) film modified GCE and MWCNT/nafion/GCE were found to have an LOD of $1.0x10^{-7}$ M and $5x10^{-7}$ M respectively. The newly developed electrochemical sensors include CuO nanoleaves MWCNT composite and Edge plane pyrolytic graphite sensor found to have a LOD of $3.21x10^{-7}$M and $2.8x10^{-7}$ M. The electrochemical sensor based on chitosan AgNPs-MWCNTs (chemically imprinted) developed for detecting neomycin had a LOD of 7.63 nM. LC-MS/MS is the advanced method used to detect both norfloxacin and neomycin residues, for which the LOD ranges from <100 ng/L and 1-22 ng/L for neomycin and norfloxacin respectively.

According to WHO for pharmaceuticals in water, these compounds have been reportedly found in places such as hospital wastewaters, wastewater treatment plants and surface waters all over the world with concentrations ranging from approximately 60-120000 ng/, 2-580 ng/L and 5-1300 ng/L respectively. Norfloxacin is mostly found in surface waters in the concentration range of <120 ng/L. The maximum levels for permissible residual fluoroquinolones including norfloxacin in ppm are zero. Neomycin concentrations in water systems have not yet been reported. For consumer protection, the European (EU) established neomycin residual limits (MRL) for animal edible tissues, 500 µg/kg for meat, fat, liver ,eggs and 1500 µg/kg for kidney. The method used to study neomycin in real samples is LC-MS-MS had an LOD of 2-13 µg/L for 9 aminoglycosides including neomycin.

The PAA/GO electrochemical sensors in this work was prepared by simple electrodeposition of PAA/GO onto screen printed carbon electrodes (SPCE). The electrochemical detection of norfloxacin and neomycin were done using the Square wave voltammetry (SWV), a fast reproducible and reliable technique. This was demonstrated by the small standard deviation per sample set (n=3). Well resolved signals for neomycin (-0.24V) and norfloxacin (0.85V) were obtained in phosphate buffer solution (PBS). For comparison norfloxacin and neomycin were also analyzed using a standard analytical method Uv-vis spectroscopy. The LOD determined from Uv-vis spectroscopy calibration curves were $1.61\text{x}10^{-5}$ M and $1.41\text{x}10^{-5}$ M for norfloxacin and neomycin respectively. The PAA/GO electrochemical sensor had a LOD of $3.37\text{x}10^{-7}$ M for norfloxacin and $1.066\text{x}10^{-6}$ M for neomycin. This represents a 100 fold improvement in the LOD using the electrochemical sensor. Even though the information for the regulation of these antibiotics specifically are sparse, the analytical performance of the PAA/GO sensors in terms of LOD, LOQ and sensitivity illustrates that these sensors provide a cost-effective, reproducible and fast analytical tool for screening of water samples for neomycin and norfloxacin (and possibly other) residues.

Future work

We will focus on application of the novel chemical sensors to real sample analysis and develop a prototype for a portable onsite monitoring tool to assess antibiotics and other environmental pollutants. Portable potentiostats that are compatible with the sensors developed e.g. PalmSens electrochemical interface, may be used together with screen printed carbon electrodes modified with PAA/GO nanocomposite to detect the selected antibiotic residues in real sample in the agriculture sector and medical wastewater. This introduces the capability of taking the lab to the point of contamination for the evaluation of multi-element chemical composition using simple sensor technology.

Simulation and recovery studies (spiked real samples) are required to test the technology in a realistic context. Collaboration with colleagues (i.e. Agricultural Research Council) will be necessary for developing real time sampling protocols and evaluating on site analysis efficiency and reliability. The multi-array chemical sensor technology will be evaluated in parallel to current standard protocol for antibiotic residue evaluation.

The efficiency of the multi-array chemical sensor system will be evaluated both from real sample analysis and laboratory trials with a view to develop predictive models for monitoring these pollutants in environmental and industrial waters. Measurements from multi-array chemical sensor technology may then be employed as both predictive signaling and monitoring within the established analytical performance range.

References

Abergel, D., Apalkov, V., Berashevich, J., Ziegler, K., & Chakraborty, T. (2010). Properties of graphene: A theoretical perspective. *Advances in Physics*, 59(4), 261-482.

Adams, E., Schepers, R., Roets, E., & Hoogmartens, J. (1996). Determination of neomycin sulfate by liquid chromatography with pulsed electrochemical detection. *Journal of Chromatography A*, 741(2), 233-240.

Adhikari, R. (2013). Atomic force microscopy of polymer/layered silicate nanocomposites (PLSNs): A brief overview. *Macromolecular Symposia*, 327. (1) pp. 10-19.

Andreescu, D., Wanekaya, A. K., Sadik, O. A., & Wang, J. (2005). Nanostructured polyamic acid membranes as novel electrode materials.*Langmuir*, 21(15), 6891-6899

Badihi-Mossberg, M., Buchner, V., &Rishpon, J. (2007).Electrochemical biosensors for pollutants in the environment.Electroanalysis,19(19-20), 2015-2028.

Balcıoğlu, I. A., & Ötker, M. (2003). Treatment of pharmaceutical wastewater containing antibiotics by O 3 and O 3/H 2 O 2 processes.*Chemosphere*,50(1), 85-95.

Baquero, F., Martínez, J. L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments.*Current opinion in biotechnology*,19(3), 260-265.

Bbosa, G. S., Mwebaza, N., Odda, J., Kyegombe, D. B., & Ntale, M. (2014). Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance.*Health*, 6(05), 410.

Bourlinos, A. B., Gournis, D., Petridis, D., Szabó, T., Szeri, A., & Dékány, I. (2003). Graphite oxide: chemical reduction to graphite and surface modification with primary aliphatic amines and amino acids. *Langmuir*,*19*(15), 6050-6055.

Collignon, P. J. (2002). 11: Antibiotic resistance. *Medical journal of Australia*,*177*(6), 325-331

Cha, J. M., Yang, S., & Carlson, K. H. (2006). Trace determination of β-lactam antibiotics in surface water and urban wastewater using liquid chromatography combined with electrospray tandem mass spectrometry.*Journal of Chromatography* A,1115(1), 46-57.

Chen, D., Feng, H., & Li, J. (2012). Graphene oxide: Preparation, functionalization, and electrochemical applications. *Chemical Reviews*, 112(11), 6027-6053.

Chen, L., Tang, Y., Wang, K., Liu, C., & Luo, S. (2011). Direct electrodeposition of reduced graphene oxide on glassy carbon electrode and its electrochemical application. *Electrochemistry Communications*, 13(2), 133-137.

Christian, T., Schneider, R. J., Färber, H. A., Skutlarek, D., Meyer, M. T., &Goldbach, H. E. (2003).Determination of antibiotic residues in manure, soil, and surface waters.*ActaHydrochimicaEtHydrobiologica,* 31(1), 36-44.

Das, T. K., &Prusty, S. (2013). Graphene-based polymer composites and their applications.*Polymer-Plastics Technology and Engineering*,52(4), 319-331.

Devaraj, M., Deivasigamani, R. K., & Jeyadevan, S. (2013). Enhancement of the electrochemical behavior of CuO nanoleaves on MWCNTs/GC composite film modified electrode for determination of norfloxacin. *Colloids and Surfaces B: Biointerfaces*,*102*, 554-561.

Dikin, D., Kohlhaas, K., Dommett, G., Stankovich, S., &Ruoff, R. (2006). Scanning electron microscopy methods for analysis of polymer nanocomposites. *Microscopy and Microanalysis,* 12(S02), 674-675.

Dimiev, A. M., & Tour, J. M. (2014).Mechanism of graphene oxide formation. *ACS Nano,* 8(3), 3060-3068.

Du, N., Wong, C., Feurstein, M., Sadik, O. A., Umbach, C., & Sammakia, B. (2010). Flexible poly (amic acid) conducting polymers: effect of chemical composition on structural, electrochemical, and mechanical properties. *Langmuir*, 26(17), 14194-14202.

Espinosa-Mansilla, A., de la Pena, A. M., Gómez, D. G., & Salinas, F. (2005). HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning: application to urine and serum. *Journal of Chromatography* B,822(1), 185-193.

Fernández, J., Navasa, M., Gómez, J., Colmenero, J., Vila, J., Arroyo, V., & Rodés, J. (2002). Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology*,35(1), 140-148.

Gaber M, Khedr .A, El-Kady.A (2012) Spectrophotometric determination of norfloxacin in pure and dosage forms by complexation with Fe3+ and Cu2+ ions . *Int. Res. J. Pharm. And Pharm.*, p. 97

García-Gutiérrez, M. C., Nogales Ruiz, A., Hernández, J. J., Rueda, D. R., &EzquerraSanz, T. A. (2007). X-ray scattering applied to the analysis of carbon nanotubes, polymers and nanocomposites.*OpticaPura Y Aplicada*, 40(2), 195-205.

Ghoneim, M. M., Radi, A., & Beltagi, A. M. (2001). Determination of Norfloxacin by square-wave adsorptive voltammetry on a glassy carbon electrode. *Journal of pharmaceutical and biomedical analysis*, *25*(2), 205-210.

Goyal, R. N., Rana, A. R. S., & Chasta, H. (2012). Electrochemical sensor for the sensitive determination of norfloxacin in human urine and pharmaceuticals.*Bioelectrochemistry*, *83*, 46-51.

Guan, B., & Yuan, D. X. (2007). Determination of neomycin in water samples by high performance anion chromatography with pulsed amperometric detection. *Chinese Chemical Letters*,18(2), 201-204.

Haque, A. M. J., Park, H., Sung, D., Jon, S., Choi, S. Y., & Kim, K. (2012).An electrochemically reduced graphene oxide-based electrochemical immunosensing platform for ultrasensitive antigen detection. *Analytical chemistry*,84(4), 1871-1878.

Hess, E. H., Waryo, T., Sadik, O. A., Iwuoha, E. I., & Baker, P. G. (2014). Constitution of novel polyamic acid/polypyrrole composite films by in-situ electropolymerization. *Electrochimica Acta*, 128, 439-447.

Hernández, F., Sancho, J. V., Ibáñez, M., & Guerrero, C. (2007).Antibiotic residue determination in environmental waters by LC-MS.*TrAC Trends in Analytical Chemistry*, 26(6), 466-485.

Hirsch, R., Ternes, T., Haberer, K., &Kratz, K. (1999). Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment*, 225(1), 109-118.

Hossain, M. M., Hahn, J. R., & Ku, B. C. (2014).Synthesis of Highly Dispersed and Conductive Graphene Sheets by Exfoliation of Preheated Graphite in a Sealed Bath and its Applications to Polyimide Nanocomposites. *BULLETIN OF THE KOREAN CHEMICAL SOCIETY*,35 (7), 2049-2056.

Hua, M. Y., Chen, H. C., Chuang, C. K., Tsai, R. Y., Jeng, J. L., Yang, H. W., & Chern, Y. T. (2011). The intrinsic redox reactions of polyamic acid derivatives and their application in hydrogen peroxide sensor. *Biomaterials*,32(21), 4885-4895.

Huang, K. J., Xu, C. X., & Xie, W. Z. (2008). Electrochemical behavior of norfloxacin and its determination at poly (methyl red) film coated glassy carbon electrode. *Bulletin of the Korean Chemical Society*, *29*(5), 988-992.

Jin, Y., Jang, J. W., Lee, M. H., & Han, C. H. (2006). Development of ELISA and immunochromatographic assay for the detection of neomycin.*Clinica Chimica Acta*,364(1), 260-266.

Kim ,H,Abdala ,A.A, and Macosko, C.(2010).Graphene/polymernanocomposites.Macromolecules .43(16),6515-6530

Kümmerer, K. (2009).Antibiotics in the aquatic environment–a review–part I.*Chemosphere*, 75(4), 417-434.

Le, T. X., & Munekage, Y. (2004). Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. *Marine Pollution Bulletin*,49(11), 922-929

Le-Minh, N., Khan, S. J., Drewes, J. E., & Stuetz, R. M. (2010). Fate of antibiotics during municipal water recycling treatment processes.*Water research*,44(15), 4295-4323.

Lian, W., Huang, J., Yu, J., Zhang, X., Lin, Q., He, X. (2012). A molecularly imprinted sensor based on β-cyclodextrin incorporated multiwalled carbon nanotube and gold nanoparticles-polyamide amine dendrimernanocomposites combining with water-soluble chitosan derivative for the detection of chlortetracycline. *Food Control,* 26(2), 620-627.

Li, D., Yang, M., Hu, J., Zhang, Y., Chang, H., & Jin, F. (2008). Determination of penicillin G and its degradation products in a penicillin production wastewater treatment plant and the receiving river.*Water Research*,42(1), 307-317.

Li, J., Kuang, D., Feng, Y., Zhang, F., Xu, Z., & Liu, M. (2012). A graphene oxide-based electrochemical sensor for sensitive determination of 4-nitrophenol. *Journal of hazardous materials*, *201*, 250-259.

Li, X., Chen, Y., & Huang, X. (2007). Electrochemical behavior of neomycin at DNA-modified gold electrodes. *Journal of inorganic biochemistry*,101(6), 918-924.

Loh, K. P., Bao, Q., Eda, G., &Chhowalla, M. (2010).Graphene oxide as a chemically tunable platform for optical applications. Nature Chemistry, 2(12), 1015-1024.

Marcano, D. C., Kosynkin, D. V., Berlin, J. M., Sinitskii, A., Sun, Z., Slesarev, A (2010) Improved synthesis of graphene oxide. *ACS Nano*, 4(8), 4806-4814.

Morgan, A. B., & Gilman, J. W. (2003). Characterization of polymer-layered silicate (clay) nanocomposites by transmission electron microscopy and X-ray diffraction: A comparative study. *Journal of Applied Polymer Science*, 87(8), 1329-1338.

Navarro, P. G., Blázquez, I. H., Osso, B. Q., de las Parras, P. J. M., Puentedura, M. I. M., &1`García, A. A. M. (2003). Penicillin degradation catalysed by Zn (II) ions in methanol. *International journal of biological macromolecules*,33(4), 159-166.

Noah, N. M., Omole, M., Stern, S., Zhang, S., Sadik, O. A., Hess, E. H., ... & Iwuoha, E. I. (2012). Conducting polyamic acid membranes for sensing and site-directed immobilization of proteins. *Analytical biochemistry*,428(1), 54-63.

Oertel, R., Renner, U., & Kirch, W. (2004). Determination of neomycin by LC–tandem mass spectrometry using hydrophilic interaction chromatography. *Journal of pharmaceutical and biomedical analysis*,35(3), 633-638.

Padavan, D. T., & Wan, W. K. (2010). Synthesis and characterization of a novel versatile poly (amic acid) derived from ethylenediaminetetraacetic dianhydride. *Materials Chemistry and Physics*, 124(1), 427-433.

Pellegrini, G. E., Carpico, G., &Coni, E. (2004).Electrochemical sensor for the detection and presumptive identification of quinolone and tetracycline residues in milk. *AnalyticaChimicaActa*, 520(1), 13-18.

Pena, A., Chmielova, D., Lino, C. M., & Solich, P. (2007). Determination of fluoroquinolone antibiotics in surface waters from Mondego River by high performance liquid chromatography using a monolithic column. *Journal of separation science*,30(17), 2924-2928.

Pokpas, K., Zbeda, S., Jahed, N., Mohamed, N., Baker, P. G., & Iwuoha, E. I. (2014). Electrochemically Reduced Graphene Oxide Pencil-Graphite in situ Plated Bismuth-film Electrode for the Determination of Trace Metals by Anodic Stripping Voltammetry. *Int. J. Electrochem. Sci,9*, 736-759

Sadik, O. A., Mwilu, S. K., & Aluoch, A. (2010). Smart electrochemical biosensors: From advanced materials to ultrasensitive devices. *Electrochimica Acta*, *55*(14), 4287-4295.

Selampinar, F., Akbulut, U., &Toppare, L. (1997).Conducting polymer composites of polypyrrole and polyimide. *Synthetic Metals*, 84(1), 185-186.

Sola, F., Xia, Z., Lebrón-Colón, M., & Meador, M. A. (2012). Transmission electron microscopy of single wall carbon nanotube/polymer nanocomposites: A first-principles study. *Physica Status Solidi (RRL)-Rapid Research Letters*, 6(8), 349-351.

Suzuki, M. A., Kakimoto, M. A., Konishi, T., Imai, Y., Iwamoto, M., & Hino, T. (1986). Preparation of monolayer films of aromatic polyamic acid alkylamine salts at air–water interface. *Chemistry Letters*, 15(3), 395-398.

The European Agency for the evaluation of Medical Products ,Veterinary Medicines and Inspections (2002).

Tovide, O., Jaheed, N., Mohamed, N., Nxusani, E., Sunday, C. E., Tsegaye, A. (2014).Graphenated polyaniline-doped tungsten oxide nanocomposite sensor for real time determination of phenanthrene.*ElectrochimicaActa*, 128, 138-148.

Wang, H., Hao, Q., Yang, X., Lu, L., & Wang, X. (2009). Graphene oxide doped polyaniline for supercapacitors. *Electrochemistry Communications*, 11(6), 1158-1161.

Wang, X., You, H., Liu, F., Li, M., Wan, L., Li, S., ...& Cheng, J. (2009). Large-Scale Synthesis of Few-Layered Graphene using CVD.*Chemical Vapor Deposition*,15(1-3), 53-56.

World Health Organization. (2011).Pharmaceuticals in drinking water.

Winey, K. I., &Vaia, R. A. (2007).Polymer nanocomposites. *MRS Bulletin*, 32(04), 314-322.

Ye. Z., Weinberg, H. S., & Meyer, M. T. (2007). Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry.*Analytical Chemistry*,79(3), 1135-1144.

Zacco, E., Adrián, J., Galve, R., Marco, M., Alegret, S., &Pividori, M. I. (2007).Electrochemical magneto immunosensing of antibiotic residues in milk.*Biosensors and Bioelectronics*, 22(9), 2184-2191.

Zawilla, N. H., Diana, J., Hoogmartens, J., & Adams, E. (2006). Analysis of neomycin using an improved liquid chromatographic method combined with pulsed electrochemical detection. *Journal of Chromatography B*,833(2), 191-198.

Zhang, J., Zhaojun, L. I., Gaofei, G. E., Wanchun, S. U. N., Liang, Y., & Laosheng, W. U. (2009). Impacts of soil organic matter, pH and exogenous copper on sorption behavior of norfloxacin in three soils.Journal of Environmental Sciences,21(5), 632-640.

Zhou, M., Zhai, Y., & Dong, S. (2009). Electrochemical sensing and biosensing platform based on chemically reduced graphene oxide. Analytical Chemistry, 81(14), 5603-5613.

Zhu, Y., Murali, S., Cai, W., Li, X., Suk, J. W., Potts, J. R. (2010). Graphene and graphene oxide: Synthesis, properties, and applications. Advanced Materials, 22(35), 3906-3924