Nanostructured polyamic acid electrocatalysts for reliable analytical reporting of sulphonamides as contaminants of emerging concern

By

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Abstract

Polyamic acid (PAA) nanostructured materials were successfully produced by electrochemical deposition and electrospinning using polyvinlypyrrolidone (PVP) as supporting polymer. Polyamic acid thin film and nanofibers were deposited directly at the surface of a screen-printed carbon electrode (SPCE) as electro-catalysts for reliable analytical reporting of sulphonamide as contaminants of emerging concern by electrochemical techniques. Fourier transform infrared (FTIR) spectroscopy was used to confirm the structural integrity of the PAA electrospun nanofibers compared to the chemical synthesized PAA. Brunauer-Emmett-Teller (BET) was used to determine the surface area of the nanofibers. The surface morphology and surface thickness of the polyamic acid (PAA) nanofibers on the screen-printed electrodes was studied using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Cyclic voltammetry (CV) was used to study redox behavior of the nanostructured PAA modified screen-printed carbon electrodes. Electrochemical parameters surface concentration, diffusion coefficient, formal potential and peak separation were determined. Three sulphonamides were selected based on the United States of protection agency (US EPA) and World Health Organization (WHO) list of emerging contaminants and detected sulphonamides in environmental waters in South Africa and other African regions. The selected sulfonamides were evaluated at the unmodified and modified screen-printed carbon electrodes. The sulphonamides were evaluated in three different supporting electrolytes at pH < 7 and >7 to enhance electrochemical signal reporting. Sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfamethazine (SMZ) displayed peaks at 0.80 V vs Ag/AgCl in 0.1 M tris-HCl using square wave voltammetry at the unmodified transducer. At the PAA thin film transducer, SDZ, SMX and SMZ displayed well-defined analytical oxidative peaks at 0.77 V 0.82 V and

0.83 V vs Ag/AgCl respectively. The LOD (n=3) for SDZ was found to be 12.14 µM with a correlation coefficient of 0.9950. The LOD (n=3) for SMX and SMZ was found to 14.59 µM (R^2 =0.9928) and 10.41 µM (R^2 =0.9963). These sulphonamides were also electro-analytical evaluated at the screen-printed carbon PAA nanofiber modified transducer. SDZ, SMX and SMZ produced well-defined analytical signals at 0.79 V, 0.81 V and 0.78 V vs Ag/AgCl respectively. The determined LOD (n=3) for the individual sulphonamides was 8.26 µM, 16.59 µM and 8.81 µM SDZ, SMX and SMZ respectively. The linearity correlation coefficient (R^2) was determined to be 0.9977, 0.9956 and 0.9974 respectively. The efficacy of the proposed nanostructured PAA thin film modified screen-printed carbon sensor was evaluated by performing recovery studies for the selected sulphonamides using square wave voltammetry. Tap water was used to simulate environmental matrix. The recoveries of SDZ with respect to each concentration were 98.84% (RSD 4.98%) to 40.58% (RSD 6.74%). For SMX the recoveries were 154.17% (RSD 11.00%) to 111.03% (RSD 16.80%). The recoveries for SMZ with respect to each concentration were 184% (RSD 8.19%) to 90.26 (RSD 18.26%) indicating the reliability of the analytical results.

Keywords

Antibiotic

Antibacterial resistance

Multi-drug resistant

Contaminants of emerging concern

Sulfonamide

Nanomaterial

Conducting polymer

Polyamic acid

Electrospun nanofiber

Electrochemical sensor

Dihydropteroate synthase

Abbreviations

WHO : World Health Organization

E. U : European union

USEPA : United States of enviornmental protection agency

TB : Tuberculosis

HIV : Human immunodeficiency virus

AIDS : Acquired immunodeficiency syndrome

MDR : Multi-drug resistance

AMR : Antimicrobial resistance

CEC : Contaminant of emerging concern

MRL : Maximum residue limit

WWTW : Wastewater treatment works

SAs : Sulfonamides

DHPS : Dihydropteroate synthase

PABA : Para-amino benzoic acid

SDZ : Sulfadiazine

SMX : Sulfamethoxazole

SMZ : Sulfamethazine

HPLC : High performance liquid chromatography

UV : Ultra-violet

PPy : Polypyrrole

PANI : Polyaniline

PTh : Polythiophene

PEDOT : Poly (3, 4-ethylenedioxythiophene)

CNTs : Carbon nanotubes

SPCE : Screen-printed carbon electrode

PAA : Polyamic acid

PVP : Polyvinlypyrrolidine

FTIR : Fourier transform infrared

HRSEM : High resolution scanning microscopy

AFM : Atomic force microscopy

BET : Brunauer-Emmett-Teller

CV : Cyclic Voltammetry

SWV : Square wave voltammetry

CA : Chronoamperometry

LOD : Limit of detection

LOQ : limit of quantification

RSD : Relative standard deviation

DHPPP : 6-Hydroxymethyl-7,8dihydropterin pyrophosphate

Declaration

I hereby declare that the work of '**Nanostructured polyamic acid electrocatalysts for reliable analytical reporting of sulphonamides as contaminants of emerging concern**` which I now submit for the assessment on the programme of study leading towards the award of a doctoral degree, is entirely my work: quotes and phrases obtained from the work of others have been fully acknowledged and referenced.

Signed: ……

SIYABULELA HAMNCA Date: August 2019

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Publications

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Chapter 1: General background and rationale

This chapter introduces the different classes of antibiotics, hypothesis, research objectives, and thesis outline.

1.1 Background and rationale

Antibiotics are a group of natural or synthetic compounds that kill bacteria or inhibit their growth. These compounds are classified based on their chemical and structural properties. Members of the same class of antibiotics have similar structures, act by similar mechanisms, and are likely to behave similarly in the environment. The important classes of antibiotics in human and animal medicine include aminoglycosides, B-lactams, fluoroquinolones, tetracylines, macrolides and sulphonamides (Huang et al., 2011). Aminoglycosides are derived from antimicrobial substances produced by the bacteria found in the soil such as *Streptomyces* and *Micronospora*. The most common antibiotics in this class are tobramycin, amikacin and gentamicin which are parental agents used for the treatment of gram-negative bacterial infections. Paromomycin and neomycin are not systemically absorbed thus are generally consumed orally for their bowel intra-luminal activity (Avent et al., 2015; Mingeot-Leclercq et al., 1999). Beta-lactam antibiotics are among the most commonly used drugs, grouped together based upon a shared structural feature, the beta-lactam ring. Beta-lactam antibiotics include penicillin, cephalosporins, cephamycins, and carbapenems. The antibiotics which have been used extensively in both veterinary and human medicine practices to prevent the infections of bacteria and fungi with penicillin

G being the most commonly used antibiotic (Svorc et al., 2012). Fluoroquinolones are a class of antibacterial compounds used in both human and veterinary medicine, are commonly used to treat urinary infections (Espinosa-Mansilla et al., 2005: Gober et al., 2012). These agents share common a functional group, the 6-fluoro and 7-piperazino substituent. Substituents at the 1-nitrogen position of the quinolone and the para position of the piperazino group vary from agent to agent. Tetracyclines and analogues with biological effects on bacteria and mammalian targets show a basic chemical structure consisting of a tetracyclic naphthacene carboxamide ring system. Tetracyclines with antibiotic activity have a dimethylamine group at carbon 4 (C4). Removal of the dimethylamino group from C4 reduces its antibiotic properties, but enhances non-antibiotic actions. These antibiotics are mostly effective when used to treat acne. Macrolides are classified into three different groups in causing drug interactions. The first group (e.g. troleandomycin, erythromycins) are those prone to forming nitrosoalkanes and the consequent formation of inactive cytochrome P450-metabolite complexes. The second group (e.g. josamycin, flurithromycin, roxithromycin, clarithromycin, miocamycin and midecamycin) form complexes to a lesser extent and rarely produce drug interactions. The last group (e.g. spiramycin, rokitamycin, dirithromycin and azithromycin) do not inactivate cytochrome P450 and are unable to modify the pharmacokinetics of other compounds. The macrolide antibiotics include natural members, prodrugs and semisynthetic derivatives. These drugs are indicated in a variety of infections and are often combined with other drug therapies, thus creating the potential for pharmacokinetic interaction (Perit et al., 1992). Sulphonamides were the first drugs acting selectively on bacteria, which could be used systemically. Today they are mostly used in combination with other antibiotics, partly due to their widespread resistance. The target of sulphonamides, and

the basis for their selectivity, is the enzyme dihydropteroate synthase (DHPS) in the folic acid pathway. Mammalian cells are not dependent on endogenous synthesis of folic acid and generally lack DHPS (Lindsey et al., 2001; Skold et al., 2000).

The world production and consumption of pharmaceuticals is increasing at a very fast rate (Baran et al., 2011). In the survey study of antimicrobial usage in animals in South Africa with specific reference to food animals conducted by Eagar et al. (2008) (**Figure 1)** which was later published as full paper in 2012 (Eagar et al., 2012), authors reported that macrolides recorded the most sales in the 2002-2004 period followed by tetracyclines, sulphonamides and penicillins. It was reported that a total of 190 400 kg or 12% of the antimicrobials sold from 2002-2004, were indicated for administration through drinking water. Sulphonamides constituted the majority of the antimicrobials used for water medication, which are drugs used to increase water and salt content in the body. It was also reported that a total of 105 4177 kg of the antimicrobials were sold as feed medications for the three years representing up to 68.5% of the grand total (Eagar et al., 2012).

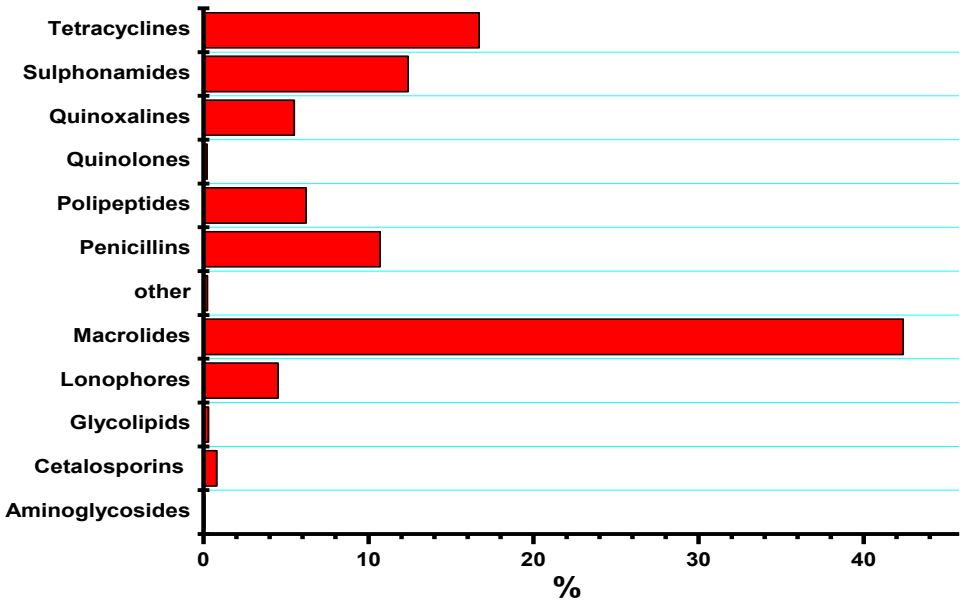


Figure 1. Percentages of volume (kg) for sales of classes of antimicrobials for the period 2002-2004 (Eager et al., 2008).

1.1.1 Public and environmental health assessment of antibiotics

In South Africa tuberculosis (TB) remains the leading cause of death among South Africans in 2013 and among the youth between 2009 and 2014 (Stats SA, 2016). The WHO Global TB Report (2015) estimates the TB incidence rate at 834 cases per 100,000 population. Followed by Human immunodeficiency virus (HIV), statistics South Africa estimates the total number of people living with HIV in South Africa increased from an estimated 4.25 million in 2002 to 7.52 million by 2018. For 2018, an estimated 13.1% of the total population is HIV positive. Approximately one-fifth of South African women in their reproductive ages (15–49 years) are HIV positive. HIV prevalence among the youth aged 15–24 has declined over time from 6.7% in 2002 to 5.5% in 2018 (Stats SA, 2018).

In 2014, there were 306 000 known cases of all types of TB. The treatment success rate for drug susceptible TB is 78% for new and relapse cases registered in 2013, which is below the global target of > 85%. In 2014, South Africa accounted for 15% of the global burden of multidrug-resistant TB (MDR-TB) and about 73% of the burden in the African Region. Furthermore, only 62% of confirmed MDR-TB cases were reported to have been initiated to treatment in 2014, with a 49% treatment success rate. According to statistics SA around 1002 (3.4%) people have died due to multi-drug resistant tuberculosis and a total of 114 (0.4%) deaths were caused by extensively drug-resistant tuberculosis. About 61% of TB cases are co-infected with HIV. About 79% of TB patients co-infected with HIV were on antiretroviral drugs (ARVs) in 2014 (Stats SA, 2016). The uncontrollable misuse and overuse of antibiotics in human medicine and agriculture sector promotes the development of antibiotic resistant bacteria, transmitted to humans through the food chain. Due to public health concerns antibiotic resistance due the overuse of sulfonamides, developed countries have established maximum residue limits (MRL) for sulfonamides. Based on the European Union regulations, the MRL for sulfonamides in foods from animal origin is 100 ng g^{-1} (EU, 2009).

Once these antibiotics are excreted, processes such sorption, abiotic and biotic transformation directly influence the fate and transport of these compounds in the environment as well as their biological activities. Residual amounts of these antibiotics can reach surface waters, groundwater or sediments. Sulfonamides are most likely found in municipal waste water effluents and also in agricultural runoff effluents **(Figure 2)**. The presence of these antibiotics in these water systems is due to the incomplete metabolism, ineffective treatment or disposal of large quantities of these

antibiotics annually in human therapy and in agriculture. Unauthorised use of these drugs in animal breeding can lead to unwanted residues in food (Haasnoot et al., 2005).

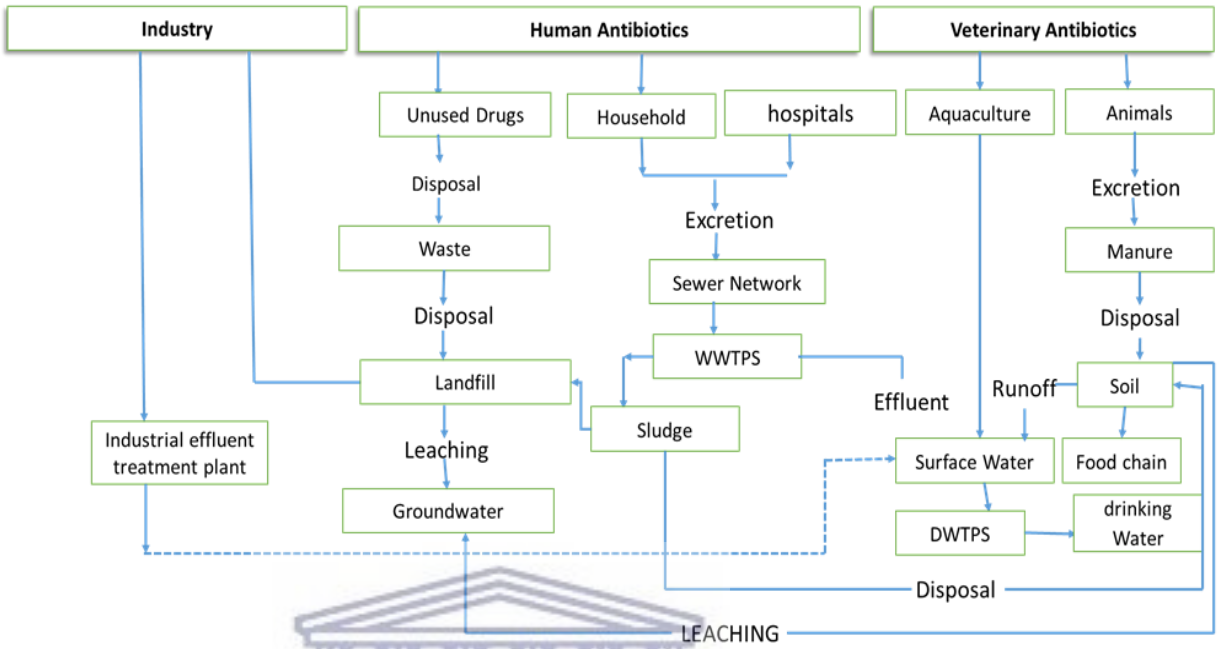


Figure 2. Fate of pharmaceuticals into the environment (adapted from Frade et al., 2014).

The is a growing concern regarding the presence of antibiotics in the environment due to the potential spread of antimicrobial resistance in different bacterial communities (Garcia-Galan et al., 2009). Numerous studies have focused on the understanding bacterial diseases and their antibiotic resistant status in around the world. South eastern Asia and Africa were identified by World Health Organization (WHO) 2014 as of the 40% regions in the world without established antibacterial resistance medium surveillance systems (WHO, 2014). It reported that out of the 41 WHO African member states only 15% of them conduct surveillance for bacterial antimicrobial resistance with external quality assurance laboratories.

Multi-drug resistant (MDR) diseases are defined as resistance to at least three classes of antimicrobials and have been reported in South Africa and other African regions as

presented in **Table 1**. Multi-drug resistant pneumonia caused by the bacteria *Klebsiella pneumoniae* exhibited high resistance to penicillin type and sulfonamides combination antimicrobials in South Africa. MDR pneumonia caused *Streptococcus penumoniae* also showed high resistance to Ampicillin and cotrimoxazole (sulfonamide combination antimicrobials) and trimethoprim showed the least resistance percentage to penicillin combination antimicrobials. MDR shigellosis reported in all nine provinces in South Africa, has high resistance to penicillin type, sulfamethoxazole, streptomycin and trimethoprim. The MDR shigellosis diseases reported in Southern Mozambique showed resistance to chloroamphenicol, ampicillin, tetracycline, trimethoprim and sulfamethoxazole. The majority of the MDR combinations involved *Klebsiella pneumoniae*. The documented extreme case of multi-drug resistance *Klebsiella pneumonia* infection, which was resistant to all available antibiotics.

Table 1. Diseases and reported antibiotic resistance current status in South Africa and neighboring regions (Faleye et al., 2018).

Diseases	***Bacteria***	***Resistance***	***Target drug***	**Location**	**Reference**
Shigellosis	*Shigella*	MDR	Ciprofloxacin, Ceftriaxone, Tetracycline, Ampicillin	Gauteng, South Africa	Agunbiade and Moodley, 2014
Pneumonia	*Klebsiella pneumoniae*	MDR	Ampicillin, Cefuroxime, cefotaxime,	Johannesburg, Pretoria,	Brink et al., 2007

			Cefepime, Piperacillin-tazobactam, Ciprofloxacin, levofloxacin.	Cape Town and Bloemfontein all in South Africa	
Shigellosis	*Shigella*	MDR	Ampicillin, Chloroamphenicol, Streptomycin ,sulfamethoxazole, Trimethoprim, tetracycline, Nalidic Acid, Ciprofloxacin ,b-lactamase based antibiotics.	All nine provinces in south Africa	Keddy et al., 2012

Pneumonia	*Klebsiella pneumoniae*	MDR	Amoxicillin Amox/clav, cefuroxime and cotrimoxazole	Gauteng, Western Cape, Eastern Cape, Kwazulu-Natal, Mpumalanga and Orange Free state provinces in south Africa.	Liebowitz et al., 2013
Pneumonia	*Streptococcus pneumoniae*	MDR	Penicillin, Amoxillin, amox/clav, Cefuroxime, Azithromycin, Clarithromycin and Cotrimoxazole		
Pneumonia	*Streptococcus pneumoniae*	MDR	Chloroamphenicol, Clindamycin, Erythromycin, Gentamicin, oxacillin, Tetracycline, Trimethroprim	Lilongwe district Malawi	Makoka et al., 2012

Shigellosis	*Shigella*	MDR	Chloroamphenicol, Ampicillin, Tetracycline, Trimethroprim, Sulfamethoxazole	Southern Mozambique, Mozambique	Mandomando et al., 2009
Pneumonia	*Streptococcus pneumoniae*	MDR	Ampicillin, Chloroamphenicol, Cotrimoxazole, Gentamicin, Ampicillin plus Gentamicin, Chloroamphenicol plus Ampicillin	Maputo provinces, Southern Mozambique	Mandomando et al., 2010
Pneumonia	*Klebsiella pneumoniae*	MDR	Cefotaxime, Carbepenens, Piperacillin/ tozabactam Ceftazidime,	Gauteng, Kwazulu-Natal, Limpopo and	Perovic et al., 2014

			Cefepime, Ciprofloxacin	Western Cape provinces, South Africa.	

In a separate study by Tadesse et al. (2017) titled "Antimicrobial resistance in Africa: systemic review "the reported medium resistance of trimethoprim-sulfamethoxazole combination was high as 75%. South Africa is one of the few countries in Africa which has taken the initiative to develop measures against antimicrobial resistance. The South African department of health produced a report on the antimicrobial resistance (AMR). The document focused on the antimicrobial resistance (AMR) situation in the country and current efforts to address AMR. AMR research needs to incorporate the data generated from environmental pharmaceutical chemical analyses, provide an opportunity to understand whether low concentrations of antibiotics detected in environmental waters might help induce further antibiotic resistance in pathogenic bacteria (Atcher et al., 2017), thus a need to develop analytical tools that can detect and quantify antimicrobials and resistance bacterial strains in natural environments.

1.1.2 Detection of pharmaceuticals in South Africa and other African countries in the aquatic environment

Pharmaceuticals are identified as contaminants of emerging concern (CECs), defined as those chemical substances that are not regulated and are suspected to affect the environment or with unknown effects (Chang et al., 2018). United Sates Environmental Protection Agency (USEPA) uses the term "contaminants of emerging concern" for the identification of a variety of chemical compounds with no regulatory

standards that have been recently discovered in the natural environment due to the improved analytical chemistry detection levels. These chemical compounds may pose a potential threat to aquatic life at environmentally relevant concentrations (USEPA, 2008). Several classes of pharmaceuticals have been detected in the South African surface water and treated sewage effluents. Fluoroquinolones (0.12 μgL^{-1}) from the quinolones class have been detected in treated sewage (Hendrick and Pool, 2012). Ciprofloxacin and nalidixic acid have been found in surface waters with maximum concentrations of 15 μgL^{-1} and 23.6 μgL^{-1} detected respectively (Agunbiade and Moodley, 2014). Macrolides have also been detected in surface water with a maximum concentration of 20 μgL^{-1} (Mantongo et al., 2015).

Sulfamethoxazole one of the most commonly used sulfonamide drugs and listed as one of the pharmaceuticals considered as contaminants of emerging concern. Sulfamethoxazole has previously been detected (0.1-0.20 µg/L) in raw and treated water by Enzyme Linked Immunosorbent Assays (ELISAs). This study by Hendricks and Pool (2009) focused on the efficiency of sewage treatment plants in some of the South Africa regions. Mantongo et al. (2015) also detected this sulfonamide drug in wastewater treatment works (WWTW) (34.5 µg/L) and in surface water in the Kwazulu-Natal province with concentrations ranging from 1.2 µg/L to 5.3 µg/L. Another sulfonamide (sulfamethazine) was also detected in the same study with maxim concentration of 4.6 μgL^{-1}. Most recently Archer et al. (2017) used the ultra performance liquid chromatography (UPLC) system coupled quadrupole mass spectrometer (UPLC /TQD-MS) to detect sulfamethoxazole in WWTW effluent (0.6-2.6 μgL^{-1}) and surface water (0.6-1.4 μgL^{-1}). This sulfonamide has also been found in sea waters using HPLC in the Western Cape (Petrik et al., 2017). Trimethoprim an antibiotic generally used in combination with sulfamethoxazole has also been detected

in the South African surface waters, the measured maximum concentrations are 0.80 μgL^{-1} (Mantongo et al., 2015), 1.20 μgL^{-1} (Archer et al., 2017) and 3.70 μgL^{-1} (Mantongo et al., 2015).

1.1.3 Hypothesis

Analytical reporting of these pharmaceuticals will play an important role in the creation of regulations and policies for pharmaceuticals in water systems in South Africa and other developing countries, thus a need to develop cost-effective analytical chemistry techniques that can sense and monitor the concentration levels of these type of antibiotic classes in the natural environment. In support of the proposed antimicrobial surveillance and monitoring programmes, incorporation of the data generated from environmental and chemical analysis of pharmaceuticals will create a platform to understand whether low concentrations of antibiotics detected in environmental waters might help induce further antibiotic resistance in pathogenic bacteria. This will require portable, easy to use and cost-effective analytical tools. Electrochemical sensors are set to provide fast, low cost, portable and reliable tools for early detection and quantification of these pharmaceuticals.

1.2 AIMS AND OBJECTIVES

The overall goal of this project was to develop electrocatalysts for analytical reporting of sulphonamides based on nanostructured carbon based polyamic acid (PAA). The nanostructured PAA thin films will be fabricated using two parallel techniques. PAA nanostructures will firstly be fabricated by *situ* electrochemical deposition on commercial screen-printed carbon electrodes (SPCEs). The second technique will involve the deposition of nanostructured PAA fibers on the commercial screen-printed carbon electrodes (SPCEs) by electrospinning of polyamic acid.

1.2.1 Aim 1

To synthesize polyamic acid and prepare transducer materials (prepared by electrochemical deposition and electrospining.

1.2.1.1 Objective 1

Synthesis of polyamic acid was conducted in organic medium using 4.4 oxydianiline (ODA) and 1,2,4,5 –benzenetetra carboxylic acid (Pyromellic dianhydride, PMDA) precursors. The chemically synthesized PAA powder used to prepare the nanostructured thin films by electrochemical deposition onto a commercial screen-printed carbon electrodes (SPCEs).

1.2.1.1 Objective 2

Polyamic acid was dissolved in an appropriated organic solvent required for electrospinning. Polyamic acid solution will be subjected to electrospinning under high voltage. In this study the produced polymer fibers will be deposited directly onto a commercial graphenated screen-printed electrodes. Electrospinning will be explored as a synthesis method to produce high surface polyamic acid fibres.

1.2.2 Aim 2

To characterize the chemically synthesized PAA and modify with nanostructured PAA electrodes for further characterization by a variety of techniques.

1.2.2.1 Objective 1

Spectroscopic techniques will be employed to study the optical properties of the synthesized polyamic acid powder and the free-standing polyamic acid nanofibers. Physical characterization techniques to study the surface area of the nanofibers. Microscopic techniques to study surface morphology of the polyamic acid. Electrochemical techniques to study the redox chemistry of commercial screen-printed

carbon electrodes modified with the nanostructured and also employed for electrochemical detection of the selected sulphonamides.

1.2.3 Aim 3

To apply the prepared electrodes (unmodified and modified) as sensor platforms in the electro-catalytical determination of the selected sulphonamides using square wave voltammetry and chronoamperometry.

1.2.3.1 Objective 1

Electroanalytical detection of the selected sulphonamide on commercial screen-printed carbon electrodes (SPCEs), at PAA thin film and nanofiber modified SPCEs. Recovery studies of the sulphonamides from tap water. Inhibition studies of PABA and sulphonamides on dydropteroate synthase (DHPS).

1.3 Thesis outline

Chapter 1: *Introduces the different types of antibiotics, problem statement research objectives and thesis outline***.**

Chapter 2: *This chapter discusses selected sulfonamide chemistry, conventional analytical techniques and electrochemical sensors.*

Chapter 3: *Study design and methodology.*

Chapter 4: *In this chapter electrochemical evaluation of the selected sulphonamides of different supporting electrolytes at the surface of screen-printed carbon electrolytes and analytical evaluation at the unmodified electrodes is discussed.*

Chapter 5: *Characterization of polyamic acid thin films and polyamic acid nanofibers by spectroscopic, microscopic and electrochemical techniques.*

Chapter 6: *Analytical reporting of the selected sulphonamides on commercial screen-printed carbon electrodes modified with PAA nanostructures. Recovery studies of the selected sulphonamides at the modified electrodes.*

Chapter 7: *Future perspectives (Enzyme based biosensor for sulphonamides)*

Chapter 8*: Conclusions and future recommendations.*

Chapter 2: Literature review

This chapter discusses selected sulfonamide chemistry, mode of action, extraction and analytical techniques and electrochemical sensors.

2.1 Introduction to sulphonamides

Prontosil a prodrug discovered in the 1920s is the first commercially available antibacterial against gram-positive cocci. Prontosil is an azo-dye with a sulfonamide structure. In a human body, with a use of cellular enzymes prontosil is metabolized into sulfanilamide (Touts et al., 1957; Tačić et al., 2017). Sulfanilamide also known as the original sulfonamides are the first agent to exhibit activity against systemic bacterial diseases broad-spectrum (Greenwood, 2011). Sulfonamide containing drugs are further divided into antibiotics and non-antibiotics. Sulfonamides antibiotics compounds contain two structural characteristics that are not found in the non-antibiotic sulfonamides. The arylamine group at the N4 position of the sulfonamides is the basis to differentiate between sulfonamides antibiotics and non-antibiotics. In this N4 arylamine group, amino group is attached *para* to the benzene ring. (Bracket et al., 2004). Sulfonamides become inactive when the p-amino group (NH_2) is acylated (1), benzene ring substituted (2) and sulfonamide group is not directly attached to the benzene ring (3) (Kolaczek et al., 2014). In the sulfanilamide the nitrogen atom of -SO_2NH_2 is numbered as 1 (N1) and the -NH_2 group is 4 (N4). The antibiotic sulfonamides groups are further distinguished from non-antibiotic sulphonamides by the presence of 5-6 membered ring to the N1 nitrogen of the sulfonamide group. These structural characteristics N4 and N1 substituents are also reported as powerful predictions to immunological response (Bracket et al., 2004).

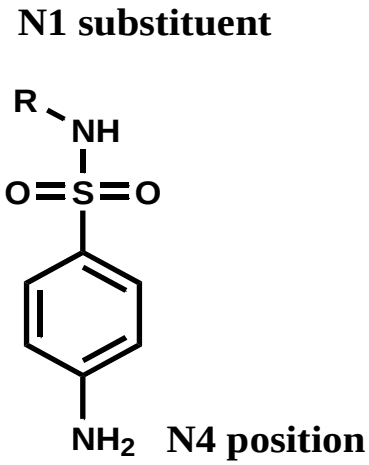
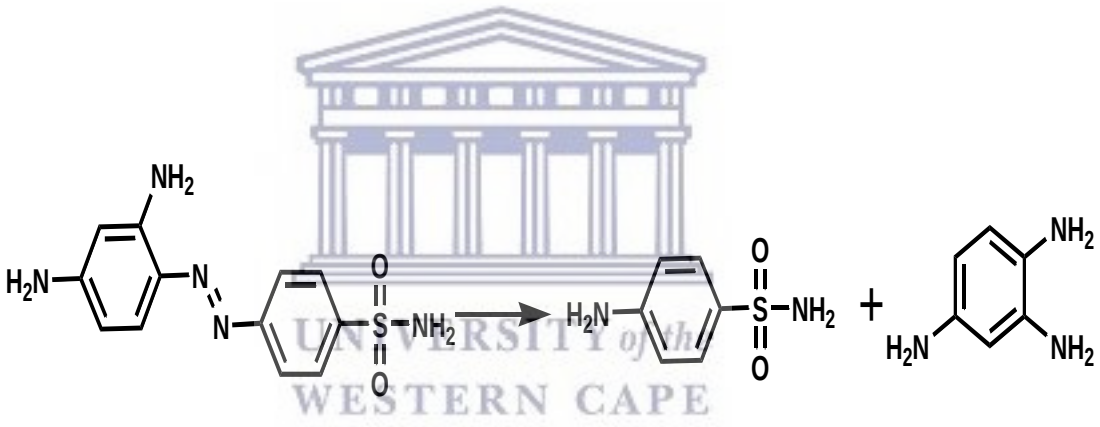


Figure 3. The general chemical structure of sulfonamides (R=H is sulfanilamide).



Scheme 1. The metabolisation of prontosil into sulfanilamide.

The most active antibiotic sulfonamide contains heterocyclic radicals. Most sulfonamides are based on pyrimidine, pyridiazine and other heterocyclic substituents. Sulfonamides are described as class of broad spectrum synthetic bacteriostatic antibiotics which inhibit the multiplication of bacteria by acting as a competitive inhibitor of the enzyme (dihydropteroate synthase) which uses para-aminobenzoic acid (PABA) in the folic acid metabolism cycle. This due to the structural resemblance of sulfonamides with PABA (**Figure 4**) (Lavanya, 2017).

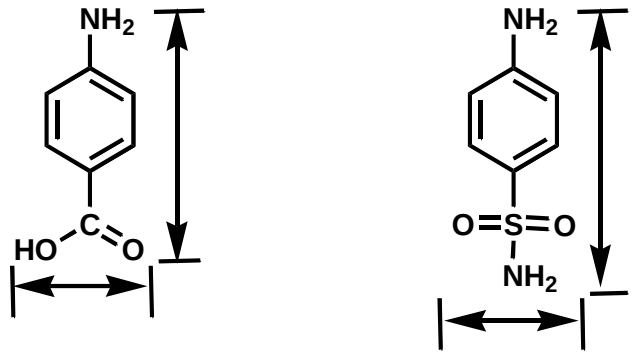


Figure 4. Chemical structures of para-aminobenzoic acid (PABA) and sulfanilamide.

2.2 Antibacterial activity of sulfonamides

As antimicrobials sulfonamides are used to inhibit gram negative and gram-positive bacteria, Nocardia, *Chlamydia trachomatis* and some protozoa. Enteric bacteria such as *Escherichia coli*, Klebsiella, Salmonella, Shigella and Enterobacter are also inhibited by sulfonamides (Lavanya, 2017). Sulfonamides at first were grouped based on the half-time of elimination. The recent various modifications to the parent sulfonamide which has yielded a great number of sulfonamides with relatively similar antibacterial spectrum with different number of pharmacokinetics and toxicological properties. Nowadays we can distinguish long-acting (half-life greater 24 hours) sulfonamides from middle-acting (half-life between 10-24 hours) sulfonamides. Typical long acting sulfonamides are sulfalene and sulfadoxine, and middle-acting sulfonamides are sulfadiazine and sulfamethoxazole. Sulfonamides can also be classed based on their pharmacokinetics properties, sulfonamides such as sulfasalazine, sulfaguanidine are poorly absorbed and systemic sulfonamides such as sulfamethoxazole, salfadiazine, sulfadimidine are rapidly absorbed and excreted

(Mondal, 2017). Long-acting sulfonamides are typically absorbed quickly however are excreted slowly (Fuchs and Elsner, 2003).

Sulfonamides are useful in therapeutic and prophylactic applications in human medicine (Hasnoot et al, 2003; Mcgrath et al., 2005). Sulfamethoxazole (SMX) is mainly used to treat urinary infections, pneumocystic pneumonia, chronic bronchitis, meningococcal meningitis and toxamoplosmosis, SMX drug can be easily excreted by human organisms due to its slow adsorption (Souza et al., 2008). SMX and trimethoprim combination is commercially used to treat simple urinary tract infection caused by *E. coli* (Ronald, 2003). Sulfathiazole (in co-trimoxazole) and dapsone (4.4-diamino-diphenyl sulfone) have been employed in the prophylaxis of *Pneumocystis carinii*. This life-long prophylaxis is recommended for HIV-positive patients (Skold, 2000). Sulfadiazine (4-amino –N-(pyrimidinyl) benzene sulfonamide) is also used in the prophylaxis and treatment of infections caused by *Pneumocystis carinii* in patients with acquired immunodeficiency syndrome (AIDS) and other patients with compromised immune systems (Vree et al., 1995). The combination of sulfadiazine and pyrimethamine is generally used in the treatment of ocular toxoplasmosis and sulfamethoxazole/trimethoprim combination provides an alternative (Soheilian et al., 2005). Sulfonamides are also administered in animal husbandry as preventive and therapeutic agents for bacterial infections (Sun et al., 2009). Sulfonamides are used in animals for the treatment of conditions such as bacterial pneumonia, bacterial scours, coccidiosis, foot rot, calf diphtheria, acute mastitis and metritis. Sulfamethoxine, sulfamethazine chlorotetracycline bisulfate, sulfaethoxypyridazine are amongst a variety of sulfonamides which are used in animals (Faries and Fajt, 2008). Sulphonamides play a part in the livestock production industry by increasing feed efficiency (mostly incorporated in food) (Crooks et al., 1998; Long et al., 1990).

2.3 Pharmacokinetics of sulphonamides

Most orally administered sulphonamides are well absorbed and therefore can reach a peak concentration in the blood 50-100 mg/l 2-4 h a dose of 2 g (Greenwood, 2011), therefore traceable amounts of sulfamethoxazole/ trimethoprim are present in in the blood 24 hours after drug has been administered (Kremers et al., 1974). Sulfamethoxazole and trimethoprim are excreted by the kidneys via both glomerular filtration and tubular secretion. The concentration of sulfamethoxazole and trimethoprim are reported to be higher in urine than compared to blood. The average percentage of the drug dose recovered in urine from 0 to 72 hours after a single dose of sulfamethoxazole /trimethoprim was reported to be 84.5%. In the excreted amount of the total sulphonamide 30% is excreted as free sulphonamide and the rest as N_4-acetylated metabolite (Kaplan et al., 1973). Sulfonamides (and their metabolites) enter the agricultural cycle and interact with the soil and wash off into the different types of environmental waters (Lamshoft et al. 2007; Rong et al. 2014).

2.4 Extraction and analytical techniques for sulphonamide detection

Sulphonamides (SAs) have become one of the antibiotic families most frequently found in all kind of environmental water, surface water, groundwater and systems wastewater as they are water soluble and demonstrate little chelating abilities (Lindsey et al., 2001). Therefore, sulfonamides can be easily quantified in any water source using a variety of extraction methods and analytical tools. Rapid resolution liquid chromatography –tandem mass spectrometry (Yang et al., 2010), High performance liquid chromatography (HPLC) (Premarathane et al., 2015) have been previously used for the detection of sulfonamides. High performance liquid chromatography can be also be coupled with extraction, analytical techniques and detectors to improve analytical performance. Sun et al. (2009) established a method for the determination

of sulfonamides, in this study the authors used a HPLC technique coupled with a ultra-violet (UV) detector (wavelength detector). Tolika et al. (2011) determined ten sulfonamides using HPLC coupled with diode-array detector. These techniques are satisfactory analytical performance however they are expensive, time-consuming and required skilled experts. Hence the development of real-time techniques for the detection of sulfonamides and other pharmaceuticals in the environment remains a target. Electroanalytical means have emerged as sensitivity, selectivity and fast methods which can be used for the real-time analysis as early detection methods for sulfonamides and other pharmaceuticals.

2.5 Nanomaterials in sensor applications

Nanotechnology is a multi-disciplinary field defined as the design, fabrication and application of nanostructured materials. Nanotechnology deals with the fundamental understanding of the relationships between physical properties and materials diameters. Nanostructured materials are in the scale ranging from sub-nanometers to several hundred nanometers (Cao, 2004). Nanomaterials exhibit high surface-to-volume ratio and small dimensions. These properties are beneficial in sensor applications. The high surface accelerates the interactions between the surface of the material and the analytes leading to a high sensitivity whilst small dimensions provide fast adsorption/desorption kinetics for analytes thus enables fast response time (Yoon, 2013).

2.5.1 Polymeric electrochemical sensors

Conducting polymers are generally used in sensor applications due to their electrical conductivity, these polymers can grow electrochemically without the use of oxidising agents thus forming nanostructured thin films on the surface of the electrode. The following conducing polymers are commonly used in sensor applications, polypyrrole

(PPy) (Tan et al., 2016), polyaniline (PANI) (Huang et al., 2014), polythiophene (PTh) and poly (3, 4-ethylenedioxythiphene (PEDOT) (Xu et al., 2013). Polypyrole modified graphite electrodes have been used in sensor applications for electrochemical determination metal ions (Quintana et al., 2013). Polymeric nanocomposites based on the conducting polymers has been studied. In these polymeric nanocomposites, nanosized particles or nanostructures are added to a polymer matrix for performance improvement (Oliviera and Machado, 2013). Inorganic nanomaterials such as metals, metal oxides, carbon and silica-based nanomaterials are the most popular nanomaterials used in the synthesis of polymeric nanocomposites in the sensor applications (Ciu et al., 2015). A variety of nanomaterials have been used to fabricate polypyrrole nanocomposites as sensor platforms. Polypyrole/ quantum dots-based sensors have used for electrochemical detection bisphenol A (Tan et al., 2016) and L-cyteine (Wang et al., 2016). Over-oxidized polyprrole/graphene modified electrodes have been used for electrochemical detection of dopamine in the presence of ascorbic acid (Zhuang et al., 2011). Polyaniline (PANI) a strong conducting with excellent electrochemical properties is often combined with other functional nanomaterials such as nanoparticles in sensor applications. Chen and co-authors developed a thin-layered molybdenum disulfide/polyaniline (MoS_2/PANI) nanocomposite for highly sensitive electrochemical detection of chroramphenicol, a broad-spectrum antibiotic (Chen et al., 2016). MoS_2/PANI nanocomposite with gold nanoparticles-based sensor has been developed for electrochemical determination of dopamine (Huang et al., 2014). PEDOT based-nanocomposites are widely used in electrochemical sensor applications. PEDOT nanomposites have been used detect a variety of analytes such as cysteine (Hsiao et al., 2011), nitrites (Zhang et al., 2013), dopamine (Xu et al., 2013; Xu et al., 2018) and mercury (II) (Zuo et al., 2016).

2.5.2 Carbon nanomaterials as electrochemical sensors

Carbon based nanomaterials are attracting theoretical interest because of their unique structures and properties. The ease of functionalization/modification expands their use and improves their electrocatalytic properties. Compared to other electrode materials, carbon nanomaterials offer advantages such as high surface area, a wide working potential window, in both aqueous and non-aqueous media, high electrocatalytic activity and chemical inertness (Fernandes et al., 2016). Carbon nanomaterials such as carbon nanotubes, graphene and diamond are polymorphs which have been explored as electrochemical sensing platforms. Carbon nanotubes (CNTs) are one of the most studied nanostructures in electroanalysis. CNTs based electrochemical sensors have been explored in the detection of organic species and biomolecules such as antibiotics (Sgobbi et al., 2016), neurotransmitters, dopamine (Uge et al., 2018) and as gas sensor sensors for ammonia detection (Sharma et al., 2014; Abdulla et al., 2015). Due to the uniqueness of graphene properties, graphene based-sensors has been employed for electrochemical detection heavy metal ions (Ruecha et al., 2015; Yang et al., 2015; Lee et al., 2016). It is evident that carbon-based nanomaterials are employed in a wide variety of electro-analytical sensor applications due to the excellent electrocatalytic properties.

2.5.3 Sensors based on electro-spun nanofibers

The electrospinning technique provides many researchers in this field with an opportunity to produce very thin layer of fibers in the nanometer range with large surface areas and ease of functionalization for applications such as electrochemical sensors and biosensors (Agarwal et al., 2008). Huang et al., (2006) prepared palladium nanoparticle-loaded carbon nanofibers by electrospinning technique and modified the carbon paste electrode with the nanofibers for the simultaneous electrochemical

determination of dopamine, uric acid and ascorbic acid. The prepared electrochemical sensing platform was reported to have shown excellent electrochemical catalytic activities towards the selected analytes (Huang et al., 2008).

Carbon nanomaterials are very popular in the electroanalytical field due to the chemical inertness, high sensitivity and low background current noise (Liu et al., 2010). Tang et al. (2010) synthesized carbon nanofibers by electrospinning and subjected the nanofibers to a thermal treatment. A carbon paste electrode was then modified with the synthesized carbon nanofibers to develop an electrochemical sensor for the determination of three amino acids (L-tryptophan (Try), L-tyrosine (Tyr) and L-cysteine (Cys). The carbon nanofiber modified electrode is reported to have demonstrated high catalytic activity and high analytical performance towards the detection of the selected amino acids (Tang et al., 2010). Guo et al. (2012) also modified a carbon paste electrode with carbon nanofibers to fabricate an electrochemical sensor for determination of dihydroxy-benzene isomers (catechol (CC) and hydroquinone (HQ)). The authors reported that compared to the bare carbon paste electrode, the carbon nanofiber modified electrode exhibited high electrocatalytic activity towards the detection of the dihydroxy benzene isomers (Guo et al., 2012).

Electrochemical sensors based on electrospun polymer is also reported, authors Mercante et al. (2015) developed an electrochemical sensor based on electropsun polyamide 6/poly (allyamine hydrochloride) (PAH6/PAH) nanofibers functionalized with carbon nanotubes for the determination of neurotransmitter dopamine (DA). Promphet et al. (2015) also used an electrospun polymer nanofiber as an electrochemical sensor platform. The electrospun graphene/polyaniline/polystyrene

nanoporous fiber modified electrode was for a simultaneous determination of lead (Pb) and Cadmium (Cd) (Promphet et al., 2015).

2.6 Electro-catalytic activity of sulfonamides

One of the first studies involving study of the electrochemical behavior of sulphonamides was conducted by Voorhies and Adams (1958). In this study the electroactivity of the sulphonamide amino (N4) group was tested by using aniline and sulfanilamide compounds at the platinum electrode. The study showed that the amino (N4) group is electroactive at the anode while the amide (N1) group is inert (Voorhies and Adams, 1958). The electrode reaction proceeds via a loss of two electron and two protons from the amino (N4) group (Voorhies and Furman, 1958). Whilst the reduction of the sulfonamide occurs at the (-SO_2-) which accounts for the cathodic property of the sulphonamides (Msigati and Ngila, 2002). The well-defined oxidation electrochemistry of sulphonamides at solid electrodes gave rise to the development of electrochemical sensors and biosensors. Solid electrodes surface is more mechanically stable with larger potential range compared to mercury-based electrodes (Okzan et al., 2015). Solid and platinum-based electrodes (Voorhies and Furman, 1958); Carrazón et al., 1987) were very popular in sulfa drugs electro-analytical determination in 1950s to 1980s. Carbon-based solid electrodes gained popularity as surface electrodes for electrochemical oxidation (Goyal et al., 1988; Goyal and Mittal, 1990) and electroanalytical determination (Carrazón et al., 1989: Carrazón et al., 1992) of sulfa drugs in the late 1980s to 2000s. A systemic literature review of the published work relating to electro-analytical of sulfonamides in the last 10 years was conducted.

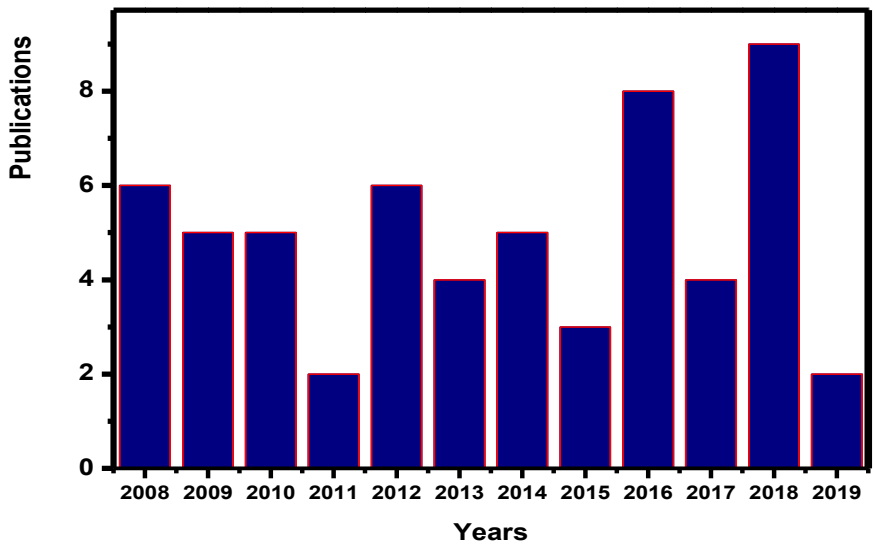


Figure 5. Papers on electro-analytical/catalytical determination of sulfonamides during the 2008-2019 period (published in journals indexed in the Web of Science database).

During the period 2008-2019 more than 50 papers were published in journals indexed in the Web of Science database, are presented in **Figure 5**. In 2008 reviewed articles covered different types of sensors, an enzyme-based electrochemical biosensor (Loaiza et al., 2008), molecular-imprinted sensor (Özkorucuklu et al. 2008) and electrochemical sensing at solid electrodes (boron-diamond electrode) (Andrade et al., 2008). Analytical methods coupled with electrochemical detection were explored for sulphonamide detection (Chue et al., 2009; Andrade et al., 2009) and on boron-diamonds (Issac and Kumar, 2009) in 2009). In 2010 a wide variety of solid electrodes (Braga et al., 2010; Andrade et al., 2010; Joseph and Kumar, 2010; Centi et al., 2010) were used in the electroanalytical detection of sulphonamides.

Polymer modified carbon-based electrodes (Özkorucuklu et al. 2011; Özkorucuklu et al. 2011b; Hong et al., 2012; Sadeghi et al., 2012; Cai et al., 2012) were popular for sulphonamide electrochemical sensing between 2011 and 2012, followed by multi-

walled carbon nanotube modified electrodes (Fotouhi et al., 2012; Hong et al., 2012). The popularity and effectivity of multi-walled carbon nanotubes continued in the following few years (2013-2014 period) as sensor platform for determination of sulphonamide drugs (Ghoreishi et al., 2013; Bueno et al., 2013; Bueno et al., 2014; Sadeghi and Garmroodi, 2014). In the same period scanning electrochemical cell microscopy was introduced as possible transducer for the detection of sulphonamides (Conzuelo et al., 2014). In the year 2018 nine journals indexed by Web of Science database were published, eight of them were based on developing nanocomposite for electrocatalytic determination of different sulphonamides. From 54 published reviewed journals in the 2008 to 2018 period only 5.6% were about enzyme based-electrochemical biosensors and 7.41% covered electrochemical immunosensors (**Figure 6**).

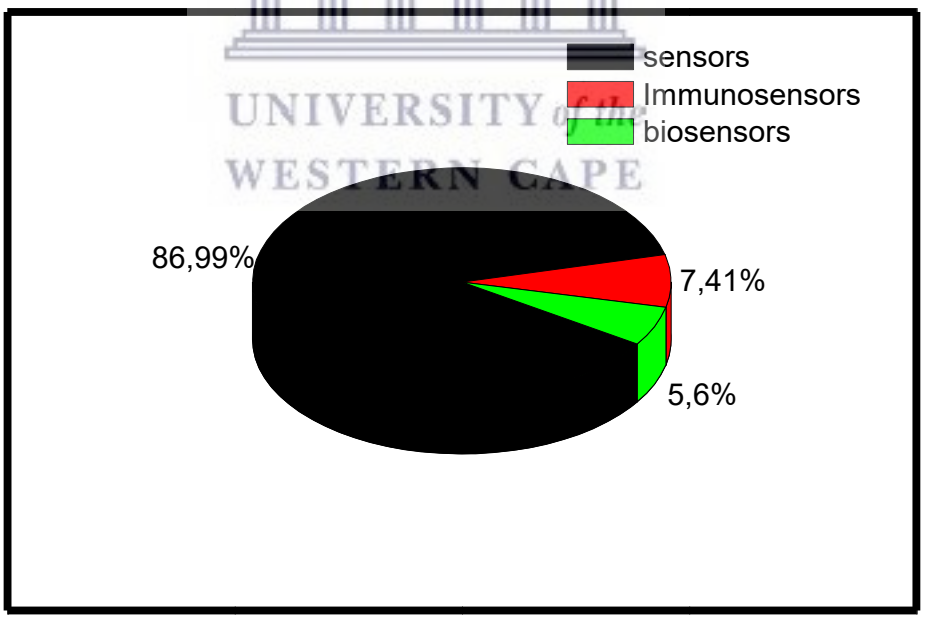
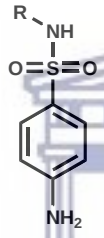
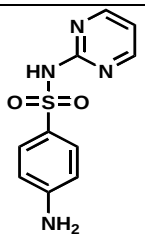
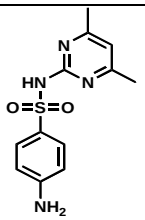
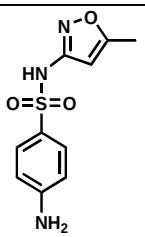


Figure 6. Electrochemical sensors, biosensors and immunosensors towards sulfonamide detection published in the 2008-2019 period.

Recent studies have focused more on developing nanomaterials, nanocomposites nanoparticles and polymeric thin films towards the detection sulphonamides. The following section will review nanomaterial electrodes that have been used for determination of selected sulfonamides for this study. The selected sulfonamides were chosen based on their popularity and persistence in the natural environment, these sulfonamides have been detected in different surface and treated effluents in South Africa and around the world. Sulfadiazine, sulfamethazine and sulfamethoxazole were selected the sulfonamides for the purpose of this work.

Table 2. Chemical structure of sulphonamides analysed in this work.

Sulfonamide			
	Chemical name	Molecular mass	Chemical structure
Sulfadiazine	4-amino –N-(pyrimidinyl) benzene sulfonamide	250.28 g/mol	
Sulfamethazine	4-Amino-N-(4,6-dimethyl-2 pyrimidine) benzene	278.33 g/mol	

Sulfamethoxazole	4-amino –N-(5-methylisoxazol-3-yl)-benzene	253.28 g/mol	

2.6.1 Sulfadiazine (SDZ) electro-catalytic determination

As one of the emerging pollutants in water surfaces it is important to develop tools to detect and quantify sulfadiazine and other sulfonamides in different environmental systems. Electrochemical determination of sulfadiazine is reported in literature both at electrochemical oxidation and reduction. Braga et al, 2016 used a simple bare glassy carbon electrode for electrochemical determination of sulfadiazine by square wave voltammetry at reduction. In an attempt to improve the analytical performance these electrochemical sensors researchers have modified the glassy carbon electrode with a variety of carbon-based nanomaterials. Hong et al. (2011) fabricated a voltammetric sensor for determination of sulfadiazine using N-octyl –pyridinium hexafluorophosphate ($OPPF_6$) ionic liquid as the binder to the conductive multi-walled carbon nanotubes.

Fatouhi et al. (2013) later on also prepared a sensor based on the multi-walled carbon nanotubes modified glassy carbon electrode to detect and study electro-analytical activity of sulfadiazine oxidation (fatouhi et al. 2013). Hong et al. (2012) later aimed to improve the multi-walled carbon nanotubes based electrochemical sensors for sulfadiazine by electrochemical deposition of poly (cobalt) tetraaminophtholocyanine ($polyCo^{11}$) directly on the surface of the multi-walled carbon nanotubes-nafion electrode. This sensing platform was used for determination of sulfadiazine in urine (Hong et al., 2012).

According the studied literature Sadeghi and Mataharian, (2013) are one few researchers that have develop a sensor for sulfadiazine determinations using a molecular imprinted polymer. This sensor was based on carbon paste electrode modified with sulfadiazine molecular imprinted polymer (MIP) as a recognition element (Sadeghi and Mataharian, 2013). Recently Ebrahim et al. (2017) developed a sensor for electrochemical determinatio and behavior of sulfadiazine at the modified carbon paste electrodes (CPEs) platform. These modified electrodes were prepared by reduced graphene oxide (rGO) nanosheets, ceria nanoparticles and reduced graphene oxide (rGO) decorated CeO_2 nanocomposite. This sensor was studied by fast Fourier transform with square wave voltammetry (FFTSWV) technique (Ebrahim et al., 2017). The analytical performances of the electrode surfaces for electrochemical sensing are summarized in the table below.

Table 3. Electrochemical and analytical data for sulfadiazine detection reported in literature.

Reference	Electrode	Method	LOD (µM)	Peak potentials (V)	Sensitivity (µA/µM)
Bara et al., 2010	GCE	SWV	10.9	-0.15	0.027
Hong et al., 2011	GCE-MWCNTs	Amperometry	0.21	0.90	0.016

Hong et al., 2012	GCE-MWCNTs –nafion poly(co[11])	Amperometry	0.71	0.90	0.04
Fatouhi et al., 2013	GCE-MWCNTs	Chrono amperometry	7.10	0.98	0.03
Sadeghi and Motaharian. 2013	CPEs-MIP	DPV	0.14	0.92	4.22
Ebrahim et al., 2017	CPEs-Ceri NPs nanocomposite	FFTSWV	0.17	0.90	0.70
Hong and Ma, 2017	GCE-MWCNT-PSS	Amperometry	0.6	0.95	0.0054

The electrochemical sensors towards the detection of sulfadiazine are mostly based on carbon electrodes (unmodified and modified). Multi-walled carbon nanotubes are popular nanomaterials which have been used in the modification of carbon electrodes

to develop sensors for sulfadiazine detection. Multi-walled carbon nanotubes improve the electron transfer between electrodes surface and electroactive species (Lahcen and Amine, 2017). Molecular imprinted polymer modified carbon paste electrode showed a very sensitivity ($4.22x10^5$ µA L mol^{-1}) towards the detection sulfadiazine. Carbon paste electrodes showed low detection limits compared to the modified glassy carbon electrodes.

2.6.2 Sulfamethoxazole (SMX) electro-catalytic determination

Electrochemical detection of sulfamethoxazole is reported in the literature using a variety of modified electrodes including the pencil graphite electrode modified with the overoxidised polypyrrole (PGE-OPPy) (Özkorucuklu et al., 2008), 5,10,15, tetrakis [3-methoxy-3-hydroxy phenyl porphyrinato] Cu (II) modified carbon paste electrode CPE- TMHPP Cu (II) (Joseph and Kumar, 2010), Iron zinc oxide modified carbon paste electrodes FeZnO/CPE (Meshki et al., 2015) , screen-printed carbon electrode based tyrosinase biosensor SPCE-Au-TRY (Román et al., 2016), screen printed electrodes modified with multiwalled-carbon nanotubes and prussian blue nanocubes SPE-MWCNT/PBnc (Sgobbi et al., 2016), molecularly imprinted polydopamine PDA-MIP (Tacco et al., 2018) and graphitic carbon nitride and oxide modified glassy carbon electrode, GCE-gC3N4/ZnO (Balasubramanian et al., 2018) nanocomposite have been previously reported in literature for electro-analytical oxidation of sulfamethoxazole. The electrochemical determination of sulfamethoxazole is also reported at unmodified electrodes such as boron-doped diamond electrodes BDD (Souza et al., 2008; Andrade et al., 2009) and glassy carbon electrode (GCE) (Calaca et al., 2014). The analytical performances of the different electrodes used as sensor platforms for sulfamethoxazole electrochemical detection are summarized in the table below (**Table 4**).

Table 4. Summarized analytical parameters of sulfamethoxazole at different electrode sensing surfaces in the literature.

Reference	*Electrode*	*Method*	*LOD (µM)*	*Potential (V)*	*Sensitivity (µA/ųM)*
Özkorucuklu et al. 2008	PGE-OPPy	DPV	0.359	1.15	0.013
Souza et al,.2008	BBD	SWV	1.150	1.10	0.83
Andrade et al.2009	BBD	DPV	0.014	0.92	nr
Joseph and Kumar,.2010	CPE- TMHPP Cu (II)	DPV	0.005	-0.14	nr
Calaca et al. 2014	GCE	SWV	8.560	0.96	0.034
Román et al. 2016	SPCE-Au-TRY	Amperometry	22.60	0.90	0.120
Sgobbi et al. 2016	SPE-MWCNT/PBnc	DPV	0.038	0.58	0.039
Tacco et al. 2018	PDA-MIP	Amperometry	0.800	1.30	nr
Balasubramanian et al.2018	GCE-gC3N4/ZnO	Amperometry	0.007	0.88	0.0031

	nanocomposit e

nr=not reported

A wide variety of sensor platforms have been developed for electroanalytical determination of sulfamethoxazole. Bare boron-diamond (BDD) electrodes showed an extremely high sensitivity towards the detection of sulfamethoxazole compared to other electrodes. BDD electrodes exhibit superior material properties which include widest solvent window of all electrode materials; low background current and capacitive currents; reduced fouling and the ability to withstand extreme potentials (Macpherson, 2015). Carbon-based bare and modified electrodes are mostly used by researchers in electro-catalytic detection of sulfamethoxazole because of their conductive nature, surface properties and affordability.

2.6.3 Sulfamethazine (SMZ) electro-catalytic determination

Modified carbon-based electrodes such as glassy carbon electrode (GCE), carbon paste electrode and screen-printed carbon electrode have emerged as the most popular sensing platforms for the determination of sulfamethazine using voltammetric techniques. Fatouhi and Zabeti (2014) modified the glassy carbon electrode (GCE) with multi-walled carbon nanotubes (MWCNTs) for electro-catalytic oxidation of sulfamethazine by cyclic voltammetry (CV) (Fatouhi and Zabeti, 2012). Cesarino et al., 2016 studied the oxidation mechanism of sulfamethazine on glassy carbon electrode modified with rGOAuNP using cyclic and differential pulse voltammetry (Ceserarino et al., 2012).

Carbon paste electrodes (both unmodified and modified) are commonly used for the electro-catalytic oxidation of other sulphonamides, however rarely used in the

electrochemical determination of sulfamethazine. Urzua et al. (2018) modified the carbon paste electrodes with the ionic liquid 1-methyl-3-octyl imidazolium hexafluorophosphate. The authors used these modified electrodes to study the electro-catalytic oxidation and voltammetric determination. Screen-printed electrodes are emerging as one of the most popular sensor platforms for environmental analysis of organic pollutants. Su and Cheng (2018) modified the screen-printed carbon electrode with PEDOT/MnO_2 to fabricate an electrochemical sensor for the determination of sulfamethazine in food samples by square wave voltammetry.

Table 5. Electrochemical sensing of sulfamethazine based on the literature

Reference	*Electrode*	*Method*	*LOD (µM)*	*Potentials (V)*	*Sensitivity (µA/µM)*
Fotouhi and Zabeti. 2014	GCE-MWCNT	CV	6.1	0.91	0.057
Cesarino et al. 2016	GCE-rGOAuNPs	CV	nr	0.89	nr
Su and Cheng. 2018	SPCE/PEDOT/MnO2	SWV	0.16	1.02	0.112
Urzula et al., 2018	CPE-IL	DPV	193	0.70	0.042

Carbon based-electrodes and nanomaterials are popular in electroanalytical evaluation of sulfamethazine. The electro-catalytic surface properties of carbon nanomaterial modified electrodes has also attracted a lot of researchers, particularly in the electrocatalytic determination of pharmaceuticals. Conducting polymer based-nanocomposite modified screen-printed carbon electrode showed sensitivity and low detection limits towards the detection of sulfamethazine. Polymer based nanocomposite are applicable as sensor materials due to the properties such excellent electrocatalytic activity and strong adsorptive ability when compared to conventional polymer, however to date carbon-based nanomaterials are still the most utilized electrodes in electroanalytical determination of sulfamethazine.

2.7 Conclusions

Sulphonamides can enter the agricultural cycle and interact with the soil and wash off into the different types of environmental waters. Residual amounts of sulphonamides can reach surface waters, groundwater or sediments which could be hazardous to human and animal health. As class of antibiotics, sulphonamides are expected to have a smilar behavior in the environment as they exhibit the same mode of mechanism. As contaminants of emerging concern, sulfonamides are frequently detected in all kind of environmental water, surface water, ground water and systems wastewater. Hence the development of real-time techniques for the detection of sulphonamides and other pharmaceuticals in the environment remains a target. Sulphonamides are electro-active organic species, electrochemical sensors and biosensors have a significant role in delivering data in simple, portable and cost-effective device. Electrochemical sensors are sensitive, selective and fast methods which can be used for the real-time analysis as early detection methods for sulphonamides and other pharmaceuticals. The high surface-to-volume ratio and small dimensions properties of nanomaterials are

beneficial in sensor applications. Carbon based electrodes and nanomaterials remain the most used electrochemical platform of sulphonamides. Polymer based nanocomposite are also applicable as electrode sensing platforms for electrocatalytic determination of sulphonamides. The reported sensors in the literature can detect at low concentration however with low sensitivity, thus a need to utilize the interesting properties of novel nanomaterials to develop selective and sensitive sensors that can detect sulphonamides and at low concentration. Our study focuses on developing polymeric based nanostructures as electrocatalysts for electrochemical determination and analytical reporting of sulphonamides.

Chapter 3: Study design and methodology

Electrochemical methods will be employed for electro-analytical detection of sulphonamides. Electro-analysis of is one of the simplest yet cost-effective method to quantitatively and qualitatively determine trace levels of electro-active species. Voltammetric techniques will be used for the electro-analytical detection of the selected sulphonamides. Voltammetry is an analytical technique based on measuring the current flowing through an electrode dipped in as solution containing electrode-active compounds while the potential is applied on the system. The advantages of electro-analytical techniques over other techniques include low cost, accuracy and reliability (Power et al., 2018). Efficiency of the electrochemistry methods rely strongly on the transducer from the literature it was evident that the forward anodes (oxidative) for the detection of environmental species at low concentration are based on the carbon-based semi-conductive materials with high surface area and excellent electro-catalytic properties. In our work, commercially screen-printed carbon electrodes (SPCEs) are used to study the electro-oxidation behaviour of three selected sulphonamides in three different supporting electrolytes. In our work unmodified SPCEs were used to investigate the effect of electrolyte on the electrochemical behaviour of the sulphonamide. Using square wave voltammetry, the commercial unmodified screen-printed carbon electrodes will also be employed as sensing platforms for screening and profiling of sulphonamides. Polymeric sensor systems have been explored in the development of electrochemical sensors. Conducting polymer materials in electrochemical sensors may participate in the chemical or

electrochemical reactions which make them both selective agents and transducer materials. This simultaneous participation of conducting polymers in electrochemical sensors may involve properties such as redox activity coupled with sensing reactions; electronic and ionic conductivities; and conformational and structural changes.

Here we report on the use of polyamic acid (PAA) in electrochemical sensors. (PAA) a semi-conductive polymer with amide and carboxylic groups in its backbone (**Figure 7**). Due to its versality, well-defined electrochemistry and solubility in aprotic solvents, PAA has many potential sensor applications.

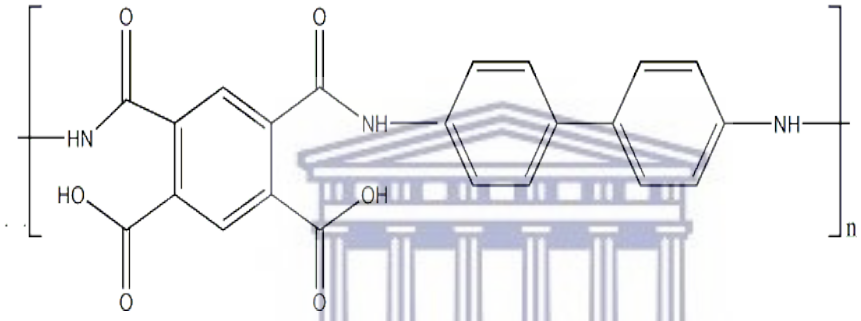


Figure 7. Chemical structure of polyamic acid (PAA).

The nanostructured PAA electrodes prepared using two different methods; electrodeposition of PAA on screen-printed electrodes (SPCEs) (**Figure 8**) and electrospinning directly on SPECs (**Figure 9**). Spectroscopic and morphology investigation will play a vital role in characterizing and understanding the new materials formats. Electrochemical techniques will be employed for the investigation of these new material formats and their interaction with the selected group of analytes. The prepared PAA thin film electrodes prepared by *in situ* electrochemical deposition will be employed as sensing platform for the three selected sulphonamides using voltammetric techniques. The main advantage of deposited thin films is the ability control their formation on solid electrode, this makes them suitable layered systems

for sensing reactions. Nanofibers produced by electrospinning are among a variety of nanomaterial. Properties of electrospun nanofibers provide a large surface area, high porosity and the ability to be easily functionalized. Nanofibers are a new generation of nanomaterials that can enhance the performance of analytical devices such as chemical sensors and biosensors. The polymer nanofibers developed in our work were employed as direct chemical transducers for the electro-catalytic analysis of selected sulphonamides. The two types PAA electrodes (in situ deposited thin films and electrospun fibers) will evaluated in parallel to assess their effect as electro-analytical platforms in the detection of standard samples of the selected analytes and their application in real sample recovery studies.

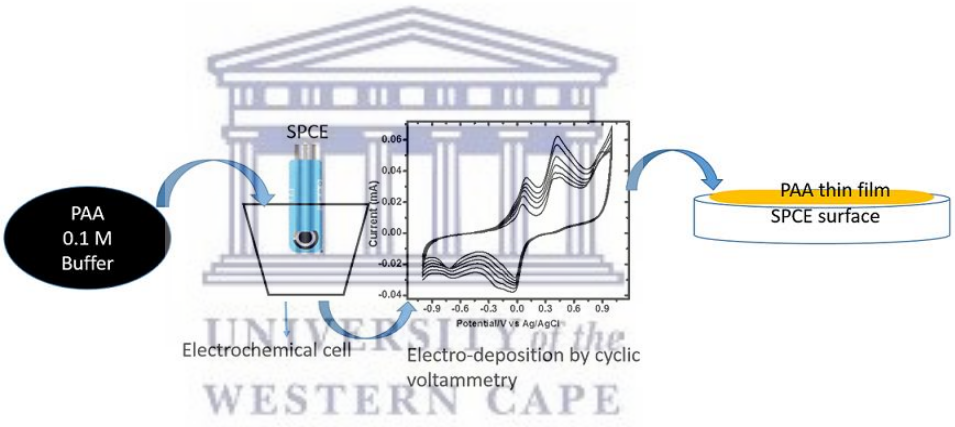


Figure 8. Electrochemical deposition of PAA to form PAA thin film at a screen-printed scarbon electrode (SPCE).

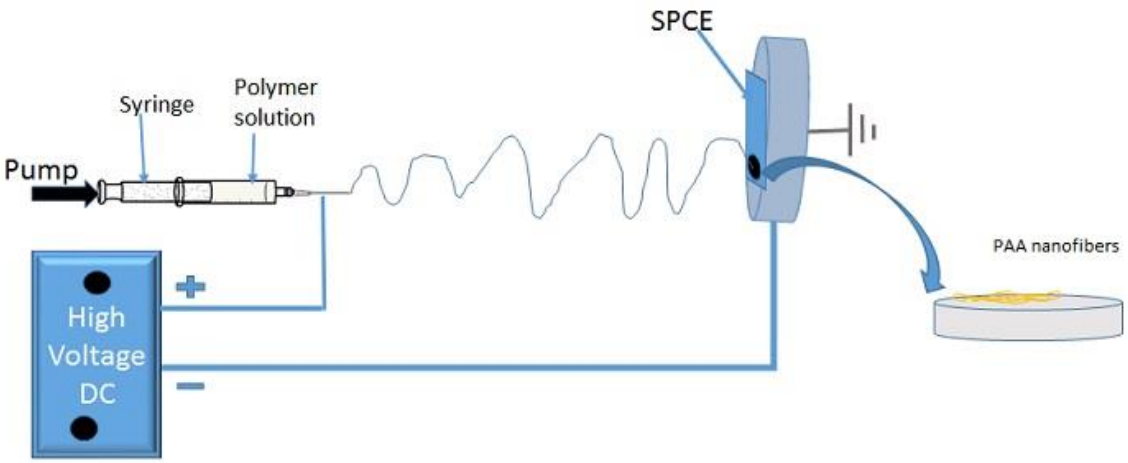


Figure 9. Electrospinning of polymer solution to produce electrospun PAA nanofibers

3.1 Electrospinning

Electrospinning is an efficient and highly scalable method used for the preparation and production of a variety of nanofiber polymer materials. The process of electrospinning applies voltages to produce fibers with diameters ranging from micron to nanometer size fiber. Electrospinning instrument from IME technologies situated in chemical engineering department (HySA) at the University of Cape Town was used to produce the polyamic acid nanofibers.

3.2 Fourier transform-infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy an experimental technique generally used for qualitative and quantitative analysis of organic compounds, provides specific information on the molecular structure, chemical bonding and molecular environment. PerkinElmer Spectrum 100 Fourier transform infrared (FTIR) spectroscopy instrument was used to study the structure and functionalized as well as the integrity of polyamic acid functional groups as nanofibers by comparing them to the chemical synthesized

polyamic acid. The IR spectrometer will provide information about the structural and vibration changes (if any) of newly electrospun PAA nanofibers.

3.3 Microscopy

High resolution scanning microscopy (HRSEM) and atomic force microscopy (AFM) is primarily used for imaging the surface morphology of different type nanomaterials. HRSEM uses an electron gun to produce an electron beam that is focused on the specimen surface. The electrons are then accelerated usually with a voltage between 1kV to 30kV. The information or signals result from the interaction of the electron beam with atoms near the surface of the sample. Scanning electron produces an image by scanning it with a focused beam of electrons in a raster. HRSEM-Zeis (SmartSEM software) (UWC) and MIRA3 TESCAN high performance SEM (UCT) operated at 5kV was used to study the surface morphology the polyamic acid (PAA) thin film and electrospun PAA nanofibers morphology on the screen-printed electrodes.

Atomic Force Microscopy (AFM) images were obtained using a Thermo Microscopic M5 (Nanosurf easy scan controller) using a non-contact mode and scanning over a range of 25μm by 25 μm at a resolution of 256×256 data points. AFM is used to study the correlation between the surface topography of PAA nanostructures and electrochemical behaviour.

3.4 Brunauer-Emmett-Teller (BET) analysis

Nitrogen sorption experiments were performed on a Micromeritics TrisStar II 3020 version 2.0 instrument from the University of Cape, chemical engineering department (HysSA). The Brunauer-Emmet-Teller theory explains the physical adsorption of gas molecules on solid surfaces. This theory assumes that the surfaces of the material are homogeneous and adsorption occurs equally across the whole surfaces without any

specific sorption (Brame and Griggs, 2016). The amount of gas molecule gas (nitrogen gas) physically adsorbed by the pores of the nanofibers provides an indication about of the size and distribution pores. In this study the BET equation was used to determine the surface area of the polyamic acid nanofibers.

3.5 Electrochemistry

Cylic voltammetry (CV), square wave voltammetry (SWV) and chronoamperometry (CA) were used for electrochemical deposition, electrochemical characterization and also to study the analytical performance of transducer platforms. Electrochemical electrodeposition and evaluation were performed using a PalmSensPTrace 4.4 workstation (Bioanalytical Systems, USA) at the University of the Western Cape.

Cyclic voltammetry (CV) is one the electrochemical techniques that allows us to probe the mechanics of redox and transport properties of a system in a solution. In this study CV was used to determine the number electrons transferred in the system, surface concentration, diffusion coefficient (D_e) and formal potentials at nanostructured (thin films and nanofibers) PAA modified commercial screen-printed carbon electrode.

Square wave voltammetry (SWV) is powerful linear sweep technique which is suitable for analytical applications, mechanistic study of electrode redox processes and electro kinetic measurements. SWV is mostly used for analytical purposes due to its high sensitivity making them a very suitable tool for low-level quantifications. SWV in this study was used to detect sulfonamide drugs and develop standard calibration curves.

Chronoamperometry (CA) was used to confirm peak potentials of sulphonamides also for the analytical reporting of the sulphonamides. The principle of amperometric sensing is based on measuring the generated current by redox reaction of an analyte at

the working electrode, where the current is subjected to Faraday's law and dynamic reaction which achieves steady-state conditions in the system.

In summary nanostructured PAA electrodes will be prepared using two different methods; electrodeposition of on screen-printed electrodes (SPCEs) and electrospinning directly on SPECs. Microscopic, spectroscopic, physical and electrochemical instruments will be employed for characterization of the newly developed PAA nanostructures. The two nanostructured PAA formats will employed in parallel to electroanalytical evaluate the selected analytes.

Chapter 4: Electrochemical evaluation of sulphonamides at the bare Screen-Printed Carbon Electrodes

In this chapter redox behaviours of selected sulphonamides at an unmodified screen-printed carbon surface will explored, using square wave voltammetry.

4.1 Introduction

Screen printed technology is widely used for the mass fabrication of disposable electrochemical sensors and biosensors. The notable commercially application of this well-established technology is the development of glucose biosensor, devices which are used to measure the glucose concentration in diabetic patients (Honeychurch and Hart, 2003; Morrin et al., 2003). This technology allows for the microfabrication of thick film electrode by sequential deposition of layers with a variety of conductive and non-conductive inks on chemically inert substrates, mostly ceramic and plastic based materials (Fanjul-Bolado et al., 2007). Compared to other methods of fabricating electrodes, in the screen-printing process the electrode area, electrode thickness and electrode composition can be readily controlled (Fletcher, 2015).

Carbon, platinum and gold are commercially available inks which are commonly used for printing working electrodes and silver-based inks are used as reference electrodes (Wang et al., 1997). The working electrode is the main electrode where electrochemical reactions occur while the reference electrode and the counter (auxiliary) electrode complete the electronic circuit). The electrochemical reactions

occurring at the screen-printed working electrode is converted to into signal which can be detected by a connected transducer element. Electrochemical techniques which can detect the signal include potentiometry and amperometry and conductometry (Hayat and Marty, 2014). Square wave voltammetry (SWV) a potentiometric technique is one of the highly electro-analytical method employed for electrochemical evaluation of electro-active organic and inorganic molecules that are adsorbed on the electrode surface. Carbon based-screen printed electrode have been used more than the metal (gold and platinum) based electrodes. This due to the cost effectiveness, low background current and wide potential window of carbon inks. This chapter will explore the electrochemical behaviour of the selected sulphonamides at the screen-printed carbon electrodes. In this chapter the electroanalytical performance of the screen-printed carbon electrodes will also be evaluated.

4.2 Effect of electrolytes on sulphonamide electrochemical behaviour at the bare screen-printed carbon electrodes

Supporting electrolytes in electrochemistry play an important in potential and current response. The nature of the electrolyte, its concentration and their pH value affect the thermodynamics and kinetics of the electrochemical process as well as mass transfer in the electrochemical systems (Braga et al., 2010). Sulphonamides are amphoteric compounds; their electrochemical behaviour depends on both the supporting electrolytes and their pH values. The effect of pH the electrochemical behaviour of sulphonamides is extensively studied and reported in the literature (Andrade et al.,2009; Joseph and Kumar, 2010; Arvand et al., 2011; Sadeghi et al., 2013; Sgobbi et al., 2016; Ebrahim et al., 2017). From the literature it is evident that the oxidation current peak is pH depended and decreased in acidic pH. The pH affects the position

of the peak potential, there is a shift towards the more negative potential when the pH of the solution is more alkaline.

The type of supporting electrolyte can also be regarded as an important factor in the electrochemical sensor performance towards the detection of the specific analyte. In this study we will investigate the effect of electrolyte in the electrochemical behaviour of the selected analytes. Sulfadiazine, sulfamethoxazole and sulfamethazine were selected to evaluate their electrochemical behaviour in three different supporting electrolytes. The chosen electrolytes include hydrochloric acid (HCl) (pH 1.2), phosphate buffer (pH 7.01) and Trizma hydrochloride (tris-HCl) (pH 8). The electrochemical oxidation behaviour of these sulphonamides in these supporting electrolyte solutions were evaluated using square wave voltammetry using the graphenated commercial screen-printed electrodes with a potential window set between −1000 mV and 1000 mV at a scan rate of 50 mV/s. In this chapter the dependence of electrochemical behaviour of the three selected sulphonamides on the supporting electrolytes is investigated at the bare screen-printed carbon electrodes. Before the analysis the electrodes surface was activated by multiple scans of cyclic voltammetry until stable background was obtained.

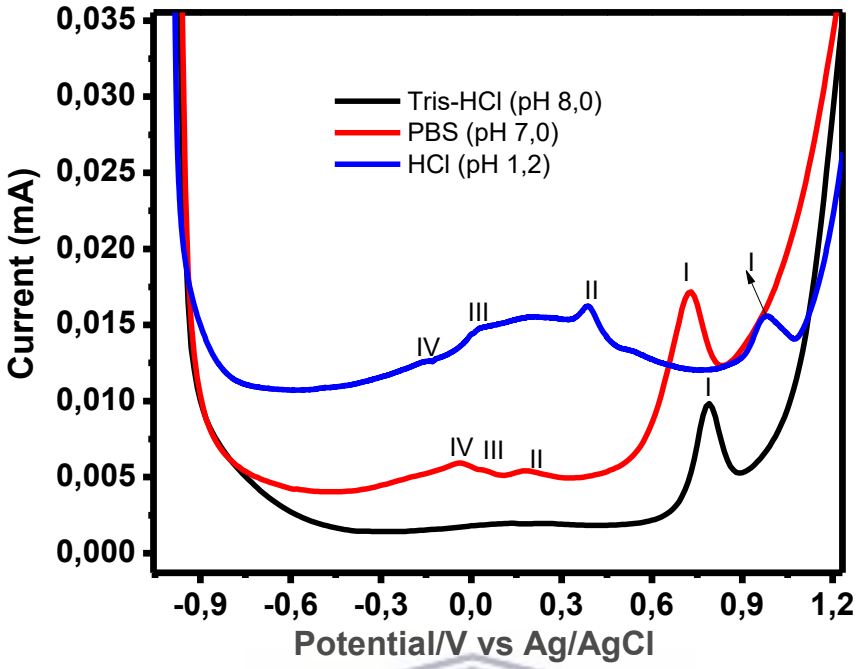


Figure 10. Square wave voltammogram of 40 µM of sulfadiazine at different supporting electrolyte with a step potential and frequency (i.e. 50 mV/s scan rate) at a screen-printed carbon electrode.

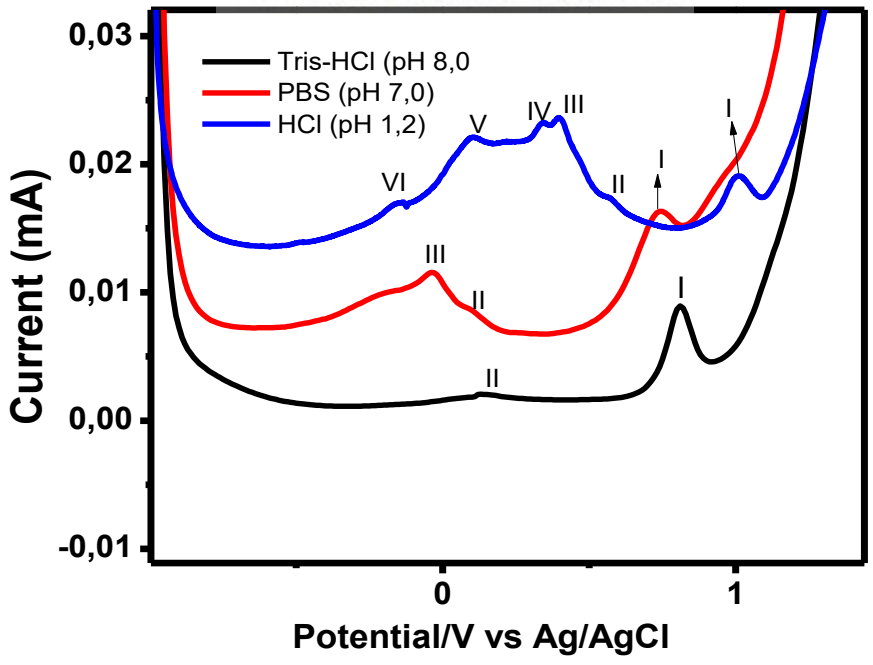


Figure 11. Square wave voltammogram of 40 µM of sulfamethoxazole at different supporting electrolyte with a step potential and frequency (i.e. 50 mV/s scan rate) at a screen-printed carbon electrode.

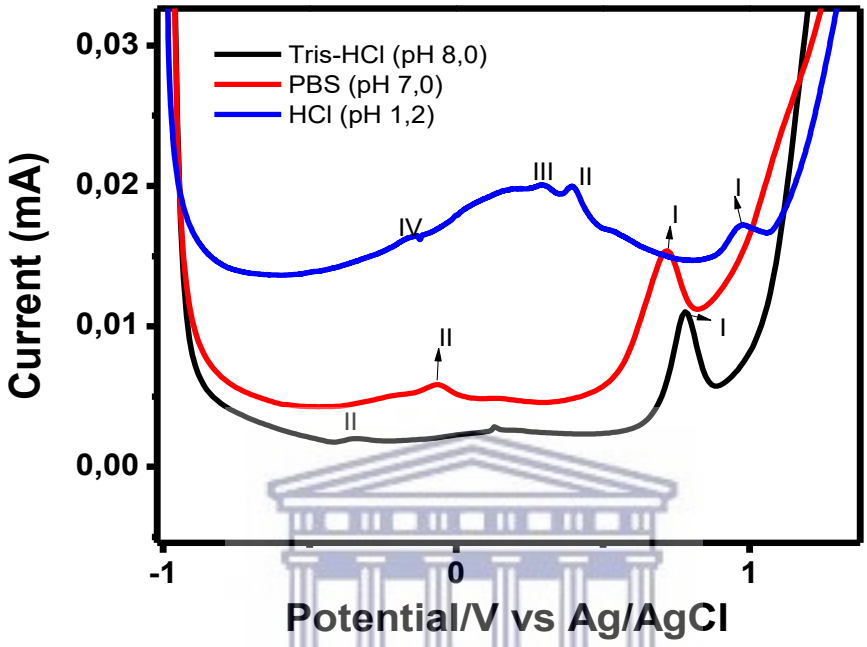
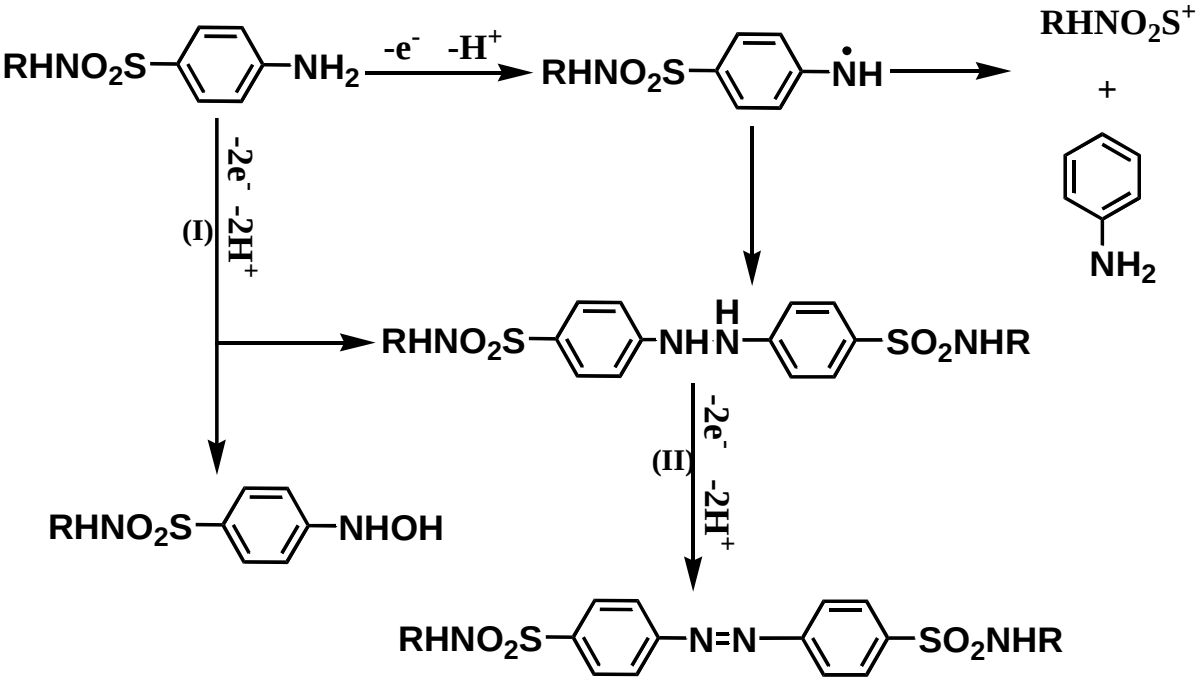


Figure 12. Square wave voltammogram of 40 µM of sulfamethazine at different supporting electrolyte with a step potential and frequency (i.e. 50 mV/s scan rate) at a screen-printed carbon electrode.

The selected sulphonamides were evaluated in 0.1 HCl, pH 7 by square wave voltammetry at scan rate of step potential and frequency (i.e 50 mVs). The peak potential due the oxidation of sulfadiazine was observed at 0.98 V vs Ag/AgCl (**Figure 10**). Three other additional were observed at 0.40 V (peak II), 0.57 V (peak III) and -0.15 V (IV) vs Ag/AgCl. The peak potential for sulfamethoxazole was observed at 1.01 V (peak I) vs Ag/AgCl and other additional peak potentials were observed at 0.11 V (II), 0.33 V (III) 0.40 V (IV), 0.09 V (V) and -0.15 V (VI) vs Ag/AgCl.

Sulfamethazine sulphonamide peak potential was observed at 0.97 V (peak I) and other addition peak potentials at 0.40 V (peak II) 0.56 V (peak III) and -0.22 V (IV) vs Ag/AgCl. The other additional peaks may be due to

Secondly the studied sulphonamides were evaluated using SWV in phosphate buffer solution (pH 7.01) as a supporting electrolyte with step potential and frequency (i.e. 50 mV/s). The sulphonamide peak potential of sulfadiazine was observed at 0.73 V (peak I) vs Ag/AgCl and additional peak potentials at 0.18 V (peak II), 0.05 V (peak III) and -0.04 V (peak IV) vs Ag/AgCl. Sulfamethoxazole had peak potentials at 0.74 V (peak I), 0.09 V (peak II), -0.03 V (peak III) and -0.19 V (peak IV) vs Ag/AgCl. Sulfamethazine peak potentials were observed at 0.72 V (peak) and -0.06 V (peak II) vs Ag/AgCl. Peak (I) in the electrochemical behaviour of sulphonamides represented the oxidation of the $-NH_2$ group to a stable hydroxy product in a $2e^-$ 2H process, Peak (I) oxidation process involves $2e^-$ per molecule (Goyal et al., 1990). The formation of readily free unstable radical species can lead to a combination of other similar species in the solution to form a hydrazo intermediate which can be easily oxidized to give an azo product which can be attributed to peak (II) in the SWV voltammogram. Some of the unstable peaks that are not assigned to any reaction step in the mechanism (**scheme 2**) may be attributed to other transformations and adsorption of species on the carbon electrode surface. The additional peaks (Peak III and VI) in the oxidation of the sulphonamides may be due to the further oxidation of the unstable species generated in peak (I) electrooxidation reaction.



Scheme 2. Proposed mechanism for the electrochemical oxidation sulphonamides.

The peak observed at 0.40 V vs Ag/AgCl common on all the studied sulphonamides was evaluated at fixed by chronoamperometry to observe its analytical behaviour. There was no amperometric response at that potential (0.40 V vs Ag/AgCl) and other additional peak potentials observed in the electrochemical evaluation the selected sulphonamides by SWV. Thus, the additional are oxidation reactions at the carbon surfaces observed can be due to the high concentration of protons from the aqueous medium, promoting the oxidation of other sulphonamide species in the solution.

Trizma hydrochloride (tris-HCl) was also used as supporting electrode in the electrochemical evaluation of some of the selected sulphonamides. The square wave voltammogram was used in the electrochemical evaluation of sulfadiazine, sulfamethoxazole and sulfamethazine. The examined sulphonamides showed well-defined irreversible peak at 0.80 V vs Ag/AgCl (peak I) which involves the oxidation

the amino group to a stable hydroxylamine product. In comparison to the electrolyte with lower pH the peak potentials of the studied sulphonamides shifted towards the negative potential and minimal secondary oxidation reactions at the surface of the screen-printed carbon electrode.

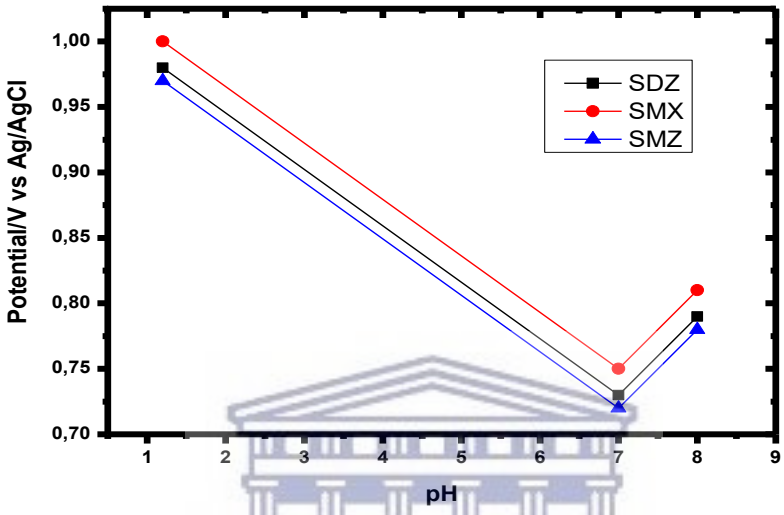


Figure 13. Relationship between the pH values of the supporting electrolytes against the peak potential observed.

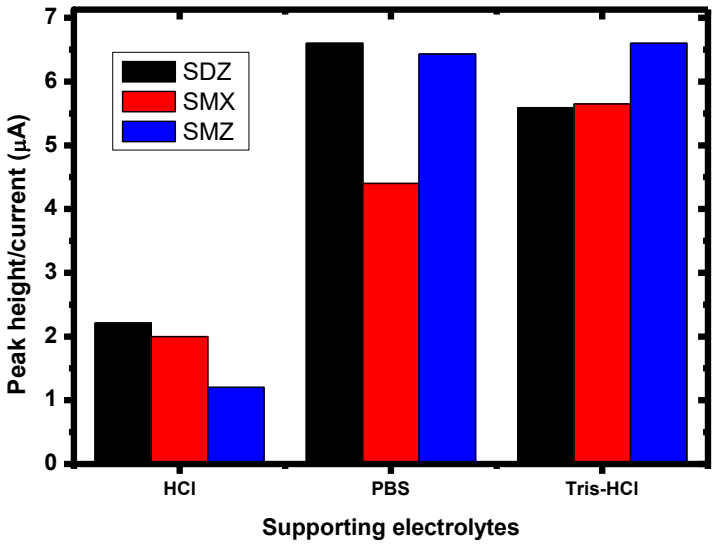


Figure 14. Relationship between the supporting electrolytes against peak heights (current µA).

The potential peak shifted a little towards the positive potential there was no other additional oxidation peak potentials observed (**Figure 13**). Tris-HCl (pH 8) electrolyte presented optimal conditions due to the well-defined peak potential characteristic of sulphonamide with high current peak (**Figure 14**) observed and physiological pH value, thus can be used in biology and medicine.

4.3 Electroanalytical evaluation of the selected at commercial SPCEs surface

Stock solutions (10 mM) of sulfadiazine sulfamethaxazole and sulfamethazine were prepared in 0.1 M HCl. The electrochemical potential window for the oxidative screening of the sulphonamides was set -1000 to 1000 mV. The optimum scan rate used was 50 mVs (i.e SWV set at the step potential of 5 mV vs Ag/AgCl and frequency at 10 Hz). Prior analysis the unmodified screen-printed carbon electrode was conditioned by cyclic voltammetry scans at 50 mV/s scan rate in 0.1 M tris-HCl (pH, 8.0).

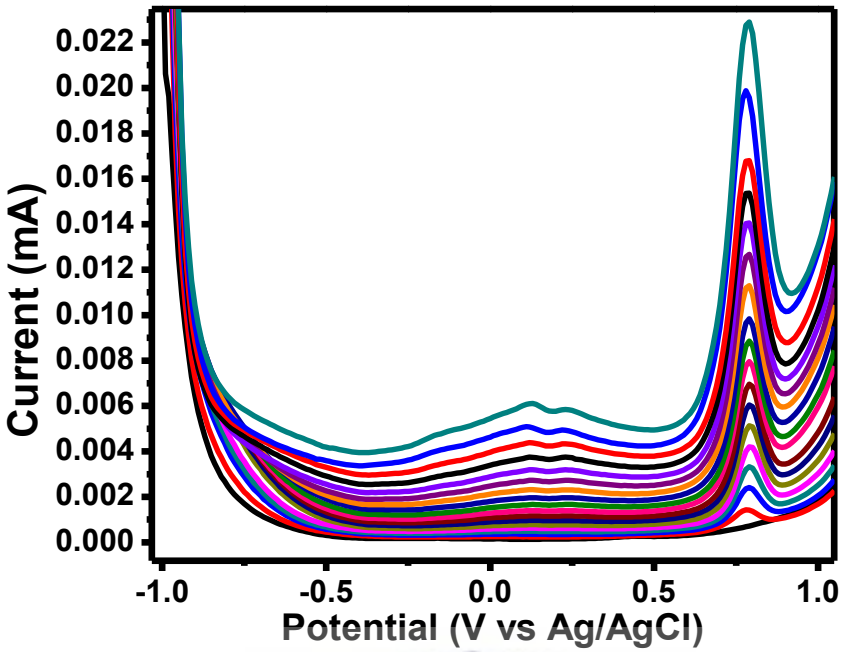


Figure 15. SWV analysis of the sulfadiazine at a screen carbon electrode (SPCE) in 0.1 M Tris-HCl (pH, 8) with concentrations ranging from (25 -300 µM) at 50 mVs.

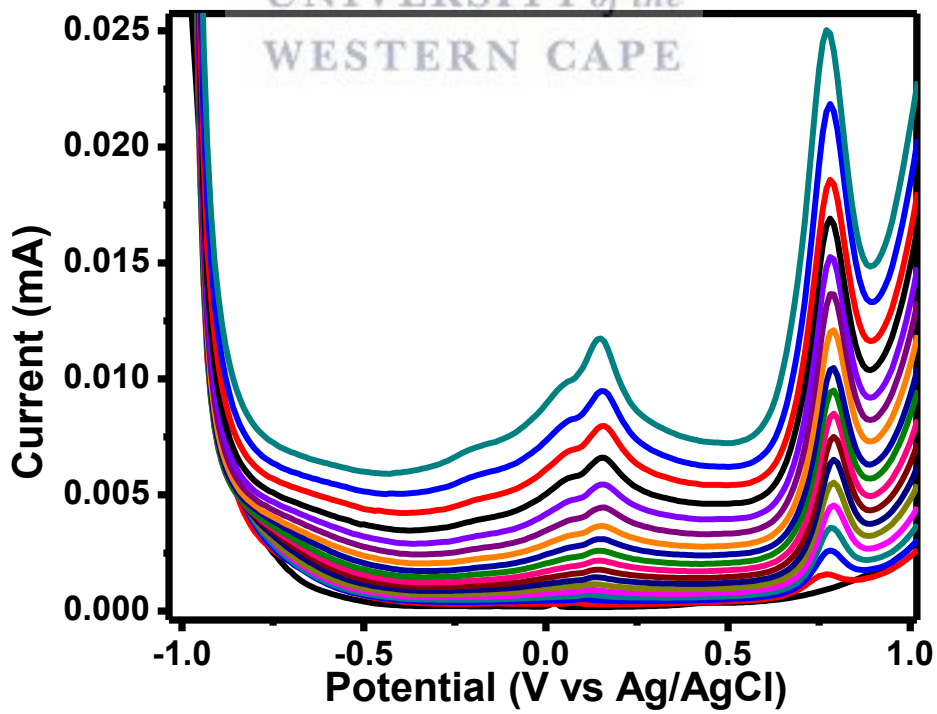


Figure 16. SWV analysis of the sulfamethoxazole at a screen-printed carbon electrode (SPCE) in 0.1 M tris-HCl with concentrations ranging from (25 -300 μM) at 50 mVs.

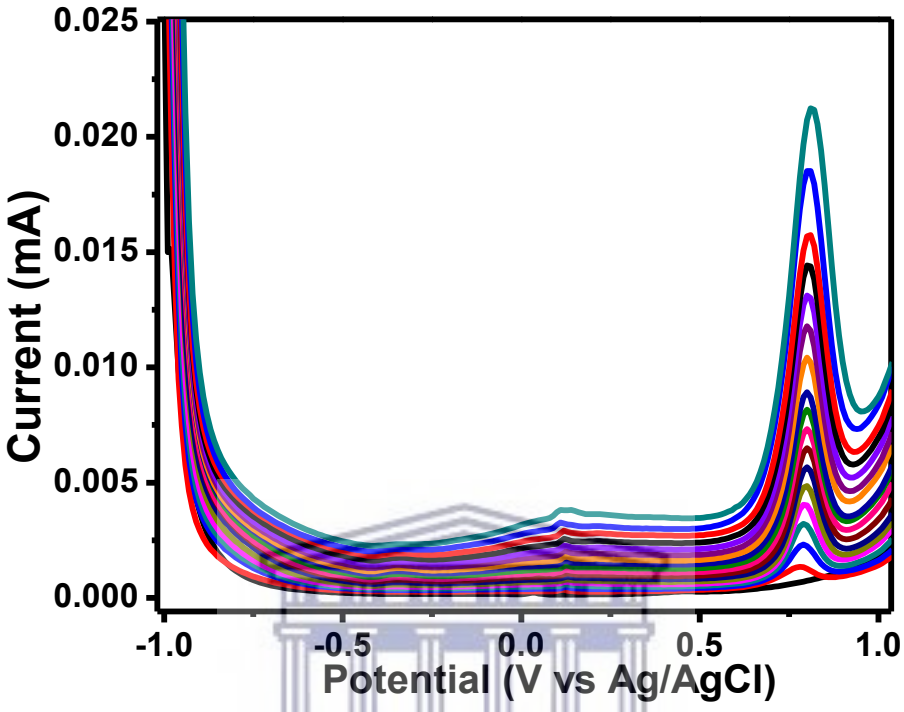
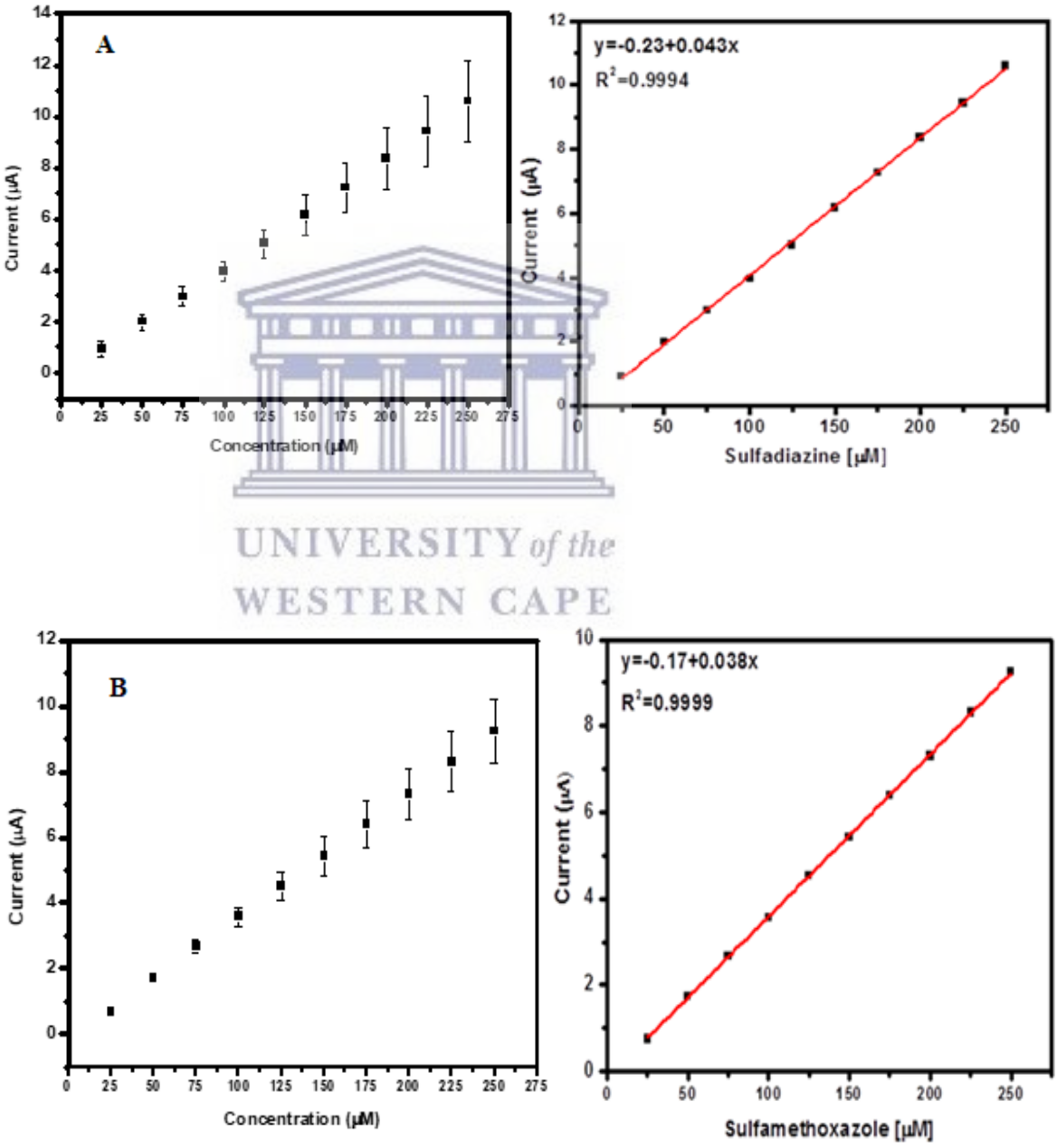


Figure 17. SWV analysis of the sulfamethazine at a commercial screen-printed carbon electrode (SPCE) in 0.1 M tris-HCl with concentrations ranging from (25 -300 μM) at 50 mV/s (step potential vs frequency).

Square wave voltammetry was used to examine the electro-analytical behaviour of sulphonamides at the commercial screen-printed carbon electrode in 0.1 M tris-HCl. Based on the voltammetric behaviour the sulphonamides shown in **Figures 15, 16 and 17.** The obtained peak potential for the three selected sulphonamides was observed at 0.80 V vs Ag/AgCl. The analytical peak represented by the voltammograms in **Figure 15-16** is attributed to the respective steps in the sulphonamide oxidation mechanism. The oxidation mechanism in the SWV voltammogram is irreversible. The influence of concentration of the sulphonamides on current was studied at optimum scan rate of 50

mV/s (step potential and frequency) and tris-HCl (pH 8) was used as a supporting electrolyte. An increase in peak current in the oxidative scan with each addition of respective sulphonamide was observed. The current response of the screen-printed carbon electrode was plotted against the concentration of the sulphonamides.



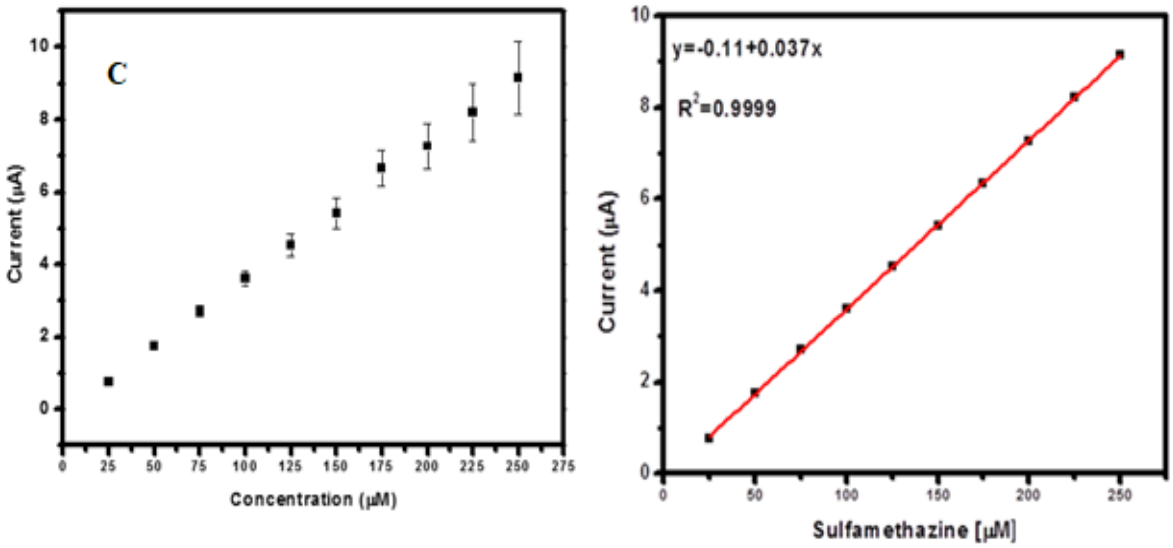


Figure 18. Linear (25 to 300 µM) range of sulfadiazine (a), sulfamethoxazole (b) and sulfamethazine (c) at the commercial graphenated screen-printed electrode (n=3).

The above figure (**Figure 18**) represents calibration curves of sulfadiazine, sulfamethoxazole and sulfamethazine. The error bars represented the discrepancy between three sets of data at the carbon surface. At the carbon electrodes the data (n=3) of the selected sulphonamides showed low variability and similar behaviour of the sulphonamides was observed at the carbon surface. The electrochemical parameters and analytical data obtained from the linear plots of current and against the concentration of the analytes are tabulated below (**Table 6**).

Table 6. Peak potential and analytical performance summary of the selected sulphonamides at the screen-printed carbon electrodes.

Analytes	Peak potential	LOD	LOQ	Sensitivity (Aµ µM^{-1})
Sulfadiazine	0.80 V	4.07 µM	12.34 µM	0.043

Sulfamethoxazole	0.80 V	1.6 µM	4.86 µM	0.038
Sulfamethazine	0.80 V	1.94 µM	5.98 µM	0.037

The anodic current was linearly proportional to the concentration of the sulphonamides with linear regression equations (n=3) of y = 0.23+0.43x (R^2= 0.9998), y=-0.17+0.038x (R^2= 0.9999) and y=-0.11+0.037x (R^2=0.9999) for sulfadiazine, sulfamethoxazole and sulfamethazine respectively. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the LOD =3.3 x standard error (SE)/slope and LOQ 10 x standard /slope equations (Cojocaru et al., 2012). LOD can be defined as the lowest concentration detected by the system and LOQ is the lowest concentration that can be measured with an acceptable uncertainty. The LOD and LOQ values for sulfadiazine were 4.07 µM and 12.34 µM respectively. Sulfadiazine detection at a bare glassy carbon electrode is reported in the literature with a LOD value 10.90 µM (Bara et al., 2010), higher than the LOD value reported in our study at a screen-printed carbon electrode. Sulfamethoxazole had a LOD value of 1.60 µM and an LOQ value of 4.86 µM. Sulfamethoxazole electrochemical determination has been reported at a bare glassy carbon electrode with a LOD value of 8.56 (Calaca et al., 2014). At bare BBD electrode the LOD value as reported in the literature 1.15 µM (Souza et al., 2008). The LOD and LOQ values for sulfamethazine were 1.94 µM and 5.98 µM respectively. The reported LOD value of sulfamethazine at polymer modified screen-printed electrode in the literature is 0.16 µM (Su and Chang, 2018). This value lower than the unmodified LOD reported in our study. The sensitivity of the sensor system was determined from the slope of the calibration curve. The sensitivity of the

bare screen-printed carbon electrodes towards the detection of the selected sulphonamides was higher than other bare electrodes reported in the literature.

The type of supporting electrolyte and its pH value are regarded as important factors in the electrochemical sensor performance towards the detection of the specific analyte. The electrochemical behaviour of the selected sulphonamides was evaluated in three different electrolytes. Use of electrolytes rich in protons promotes further unstable oxidation and adsorptive behaviour of the generated species. Members of the same class of antibiotics such as sulphonamides have similar structures and functional groups may have similar electrochemical properties and may likely to behave similarly in the environment. Similar electrochemical behaviour of the selected sulphonamides was observed in the SWV voltammograms at different electrolytes. Tris-HCl (pH 8) electrolyte presented optimal conditions due to the well-defined peak potential and was the preferred electrolyte in the electroanalytical evaluation of sulphonamides at the bare screen-printed carbon electrodes. The carbon platform as reported in the literature was very sensitive towards the detection of the selected sulphonamides.

Chapter 5: Spectroscopic, microscopic and physical characterization of nanostructured polyamic acids (PAA)

This chapter will focus on the synthesis, preparation and characterization of the polyamic acid (PAA) nanostructures.

5.1 Introduction to polyamic acid (PAA)

Polyamic acid (PAA) is semi-conducting polymer with carboxylic and amide groups. PAA is well-known precursor of the polyimides. Polyimides are synthesized via the imidization of polyamic acids. The synthesis of polyamic acid via diamines and dianhydrides proceeds through the nucleophilic substitution on the carbonyl atom with the amine acting as a nucleophile (Pyun et al., 1989). PAA is easy to synthesize and process due to its high solubility in aprotic solvents. In this study two approaches ((electrochemical electrodeposition and elecrospinning) of processing nanostructured polyamic acid were explored. PAA polymer thin films may easily be produced in situ by electrochemical deposition at Au and glassy carbon electrode surfaces and functionalized in various ways to prepare very efficient electrochemical sensors (Noah et al., 2012; Hess et al., 2014; Hamnca et al., 2016). Nanofibers can also be produced by electrospinning polymer directly onto screen printed carbon electrodes.

Electrospinning is an efficient and highly scalable method used for preparation and production of a variety of nanostructured polymer materials. The process of electrospinning requires the application of controlled voltages to produce fibers from

charged polymers solutions with diameters ranging from micron to nanometer size fiber (Frenot and Chronakis, 2003: Valizadeh and Farkhani, 2013). These polymer nanofibers exhibit unique properties such as high surface area to volume ratio, with good structural mechanical properties, extreme flexibility, low basic weight, and cost effectiveness (Machotova et al., 2016).

Electrospinning works well for polymers with high molecular weight, with good solubility to yield high concentration solutions. Additionally, polymers should also have good conductivity and viscosity. However, finding the correct mix of polymer solution properties for efficient spinning remains a challenging objective. Researchers have adopted different approaches to improve the spin- ability of polymers and other non-polymer materials, such as the use of carrier polymers or additives such as surfactants.

5.2 Chemical synthesis of polyamic acid

Polyamic acid was synthesized from organic solvent acetonitrile (ACN) using 4, 4-oxydianiline (ODA) and benzene 1,2,4,5 –tetracarboxylic anhydride (PMDA) precursors, as previously described in literature (Andreescu et al., 2005: Noah et al., 2012, Hamnca et al., 2016: Hamnca et al., 2017). Briefly 2.003 g of ODA was dissolved in 157 mL of ACN then 50 mL of CAN containing 2.171 g of PMDA was added drop-wise for than an hour and the solution mixture was stirred for 24 hours at room temperature. The resulting precipitates were filtered through a membrane water under suction and finally dried at room temperature.

5.3 Preparation of polyamic acid thin films

The nanostructured polyamic acid thin film modified screen printed electrodes were prepared by electrochemical deposition of polyamic acid in aqueous solution. Prior the

deposition the screen-printed carbon electrode surface was activated by 5 cyclic voltammetry scans in 0.1 M phosphate buffer (pH, 7) at 50 mV/s scan rate, the potential window ranged from -1000 mV to +1000 mV. The screen-printed electrode was dipped in a solution of phosphate buffer (pH, 7) containing 2400 µg/ml of polyamic acid (PAA), using cyclic voltammetry the electrochemical deposition of the PAA was conducted by applying a potential window of -1000 to +1000 mV at 50 mV/s scan rate, the maximum number of scans was found to be 5 which resulted in a uniform deposited layer of PAA thin film on the electrode. The resulted thin films were characterized by cyclic voltammetry (CV), square wave voltammmetry (SWV), scanning electron microscope (SEM) and atomic force microscope (AFM).

5.4 Polyamic acid nanofiber preparation by electrospinning

The solubility of PAA was tested in acetonitrile (ACN), tetrahydrofuran (THF), methanol, chloroform, diethyl ether, dimethylformamide (DMF), dimethylacetamide (DMac) and tetrahydrofuran/methanol (1:1 ratio) at concentrations 1%, 5%, 10% and 20% wt. PAA. However, polyamic acid was only found to be completely soluble in DMF and DMAc, which are high boiling point aprotic solvents (153°C and 165.1 °C respectively). Chemically synthesized PAA on its own did not provide spinnable solutions in DMF and DMAc for spinning on its own. The molecular weight of the polymer plays a role in electrical properties of the polymer or polymer composite solution such as viscosity, surface tension, and conductivity (Machotova et al., 2016). The presence of a network of topological entanglements is required for electrospinning of continuous fibers thus limiting spinnability of low molecular polymers (Wang et al., 2016), therefore cross-linkers, additives and carrying polymers are used to improve the spinnability of low molecular polymers such as PAA. The carrier polymer method for producing electrospun nanocomposite fibers from PEGylated PAMAM

dendrimers, blended with a small amount of high-molecular-weight polyethylene oxide (PEO) has been effectively explored (Aduba et al., 2015). It is also possible to produce high quality spun fibres of alginate by blending it with high molecular weight polyethylene oxide (PEO) (Saquing et al., 2013).

Polyvinylpyrrolidone (PVP) has been widely reported as efficient carrier polymer in spinning of a wide range of materials, due to its high solubility in aprotic solvent such as dimethylformamide (DMF) and N, N-dimethylacetamide (DMAc). Lubasova et al. (2015) used PVP and PVP/poly (acrylic acid) blend to produce hydrogel nanofibers simply by heat treatment of the electrospun nanofibers without the inclusion of any toxic agent for cross-linking as reported by the authors (Lubasova et al., 2015). PVP has also been used as a carrying polymer in the fabrication of titania nanofibers (Chandrasekar et al., 2009). In our work, the semiconducting polymer, polyamic acid (PAA) was used to produce nanofibers by electrospinning PAA from a homogeneous blended polymer solution, using the minimal amount of a high molecular polyvinylpyrrolidone (PVP) for efficient nanofiber production, with good processability and yield.

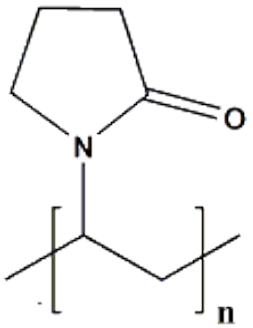


Figure 19. Chemical structure of polyvinylpyrrolidone (PVP).

Low concentrations of PVP (1%, 2%, 3% and 4% and 5% by wt.) were added to 12% by wt. PAA solutions using DMF and DMAc, respectively. Each of these solutions

were tested in terms of the quality of nanofibers that could be produced under the optimized instrumental parameters. To obtained optimal conditions for electospinning, voltage was varied from 10 to 20 kV and the distance between the spinneret and the collector was varied from 5 to 15 cm. The optimal spinning solution was produced from 12% by wt. PAA and 3% by wt. PVP dissolved in DMF yielding fine nanofibers with minimal beading. The elctrospinning conditions of polyamic acid (PAA) were; a syringe with a diameter of 0.5 mm at applied voltage of 16 – 16.8 kV, depending on the humidity. The electrospinning experiments were performed at room temperature (typically 23 ºC) with atmospheric humidity in the range of 21-24%. The flow rate ranged between 99-150 µL/hr, with the spinneret and collector distance set at 15 cm. The flow rate and spinning time had direct effect on the amount of electrospun fibers produced. The freestanding nanofibers thus produced, were dried and stored at room temperature. The resulted free-standing fibers were characterized by Fourier transform infrared spectroscopy (FTIR) and BET. The electrospinning of PAA was validated in an independent lab led by Dr. Sheila Grant in the biological engineering department (University of Missouri, Columbia, MO). The electrospinning instrument is custom design by Dr. Sheila Grant and her group. This was done through the University of Missouri South African Education program UMSAEP.

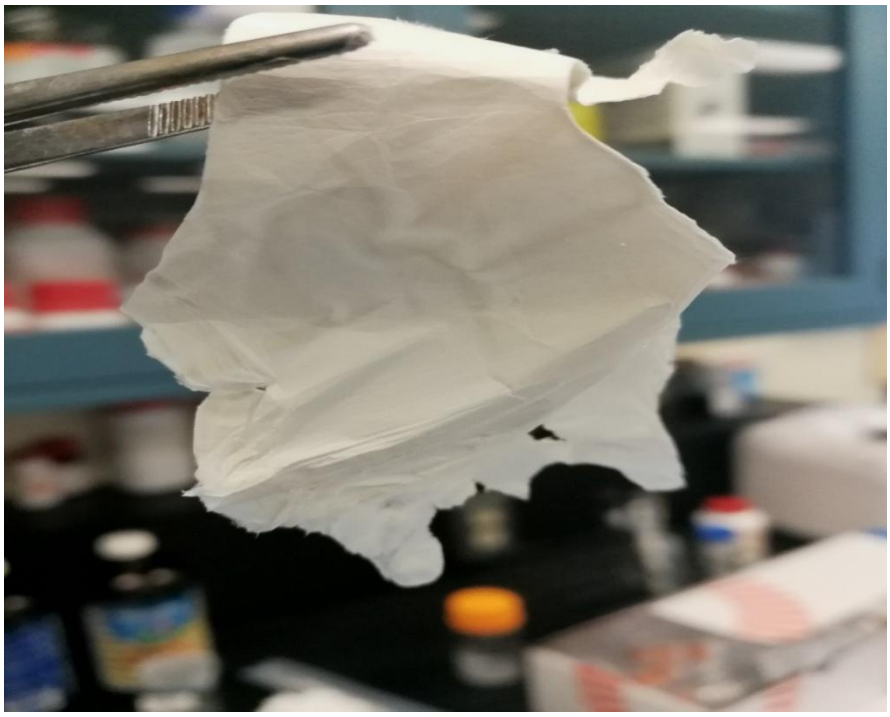


Figure 20. An image of freestanding PAA nanofibers obtained in the custom designed electrospinng instrument in the biological engineering department (University of Missouri, Columbia, MO, USA).

5.5 Fourier Transform infrared (FTIR) spectroscopy characterization.

The free-standing nanofibers were collected on aluminium foil. FTIR spectra (**Figure 1**) of PAA powder (chemically synthesized) and stand-alone nanofibers were recorded over the range of 4000 cm^{-1} to 1000 cm^{-1}, from prepared KBr pellets.

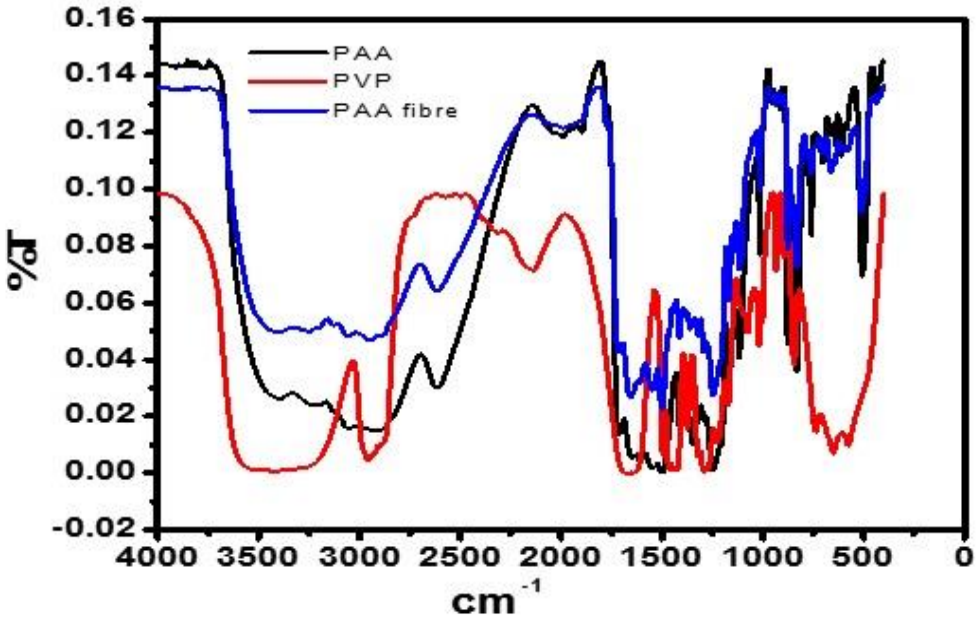


Figure 21. FTIR spectra of the synthesized PAA, PVP and PAA nanofibers.

The FTIR spectra of the PAA nanofibers and chemically synthesized PAA, were found to be in good agreements with literature reports (Noah et al., 2012; Andreescu et al., 2012); Hamnca et al., 2016; Zamfir et al., 2016). 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) were assigned to the vibrational modes of carboxylic acid. A spike appearing at 3049 cm^{-1} was assigned to the NH stretching vibration. The strong peak at around 1239 cm^{-1} was indicative of the stretching vibrations of the ether group. The FTIR spectra confirmed the integrity of the PAA prepared as nanofibers from electrospinning, by comparison with literature reports as well as with the FTIR data obtained for the chemically synthesized PAA, also done in this work.

5.6 Scanning microscopy characterization (SEM)

Images of nanostructured PAA prepared at screen printed carbon electrodes. The images represent two PAA nanostructured formats (in situ electrodesposited PAA thin films and electrospun PAA nanofibers.

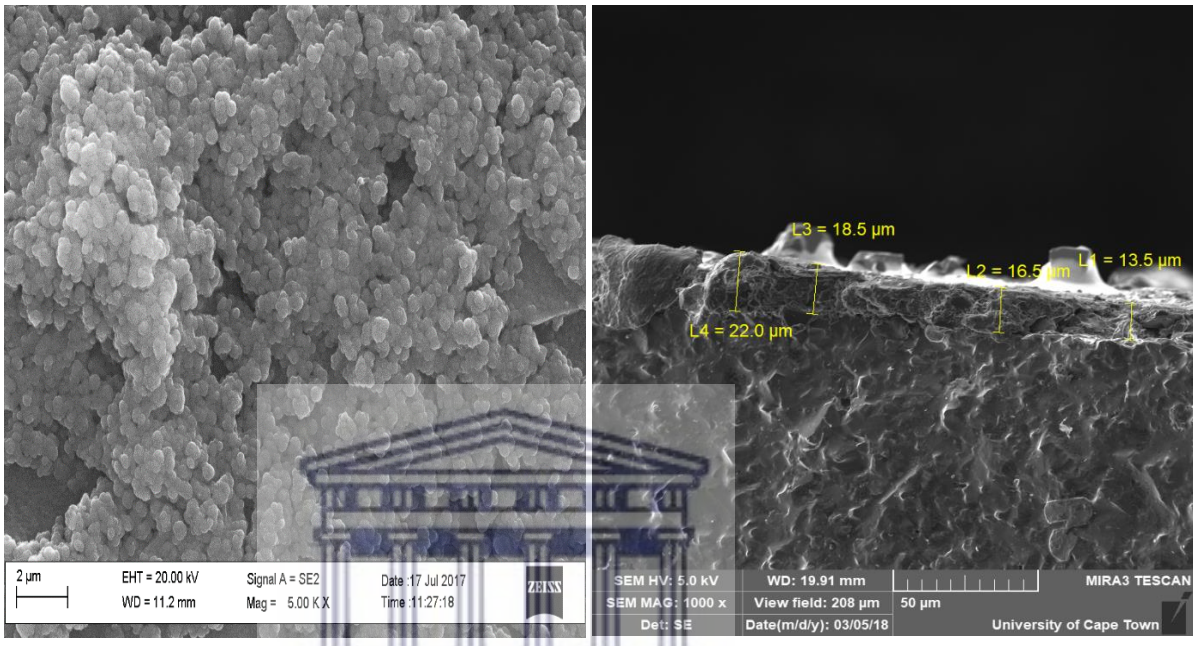


Figure 22. SEM images of PAA thin film.

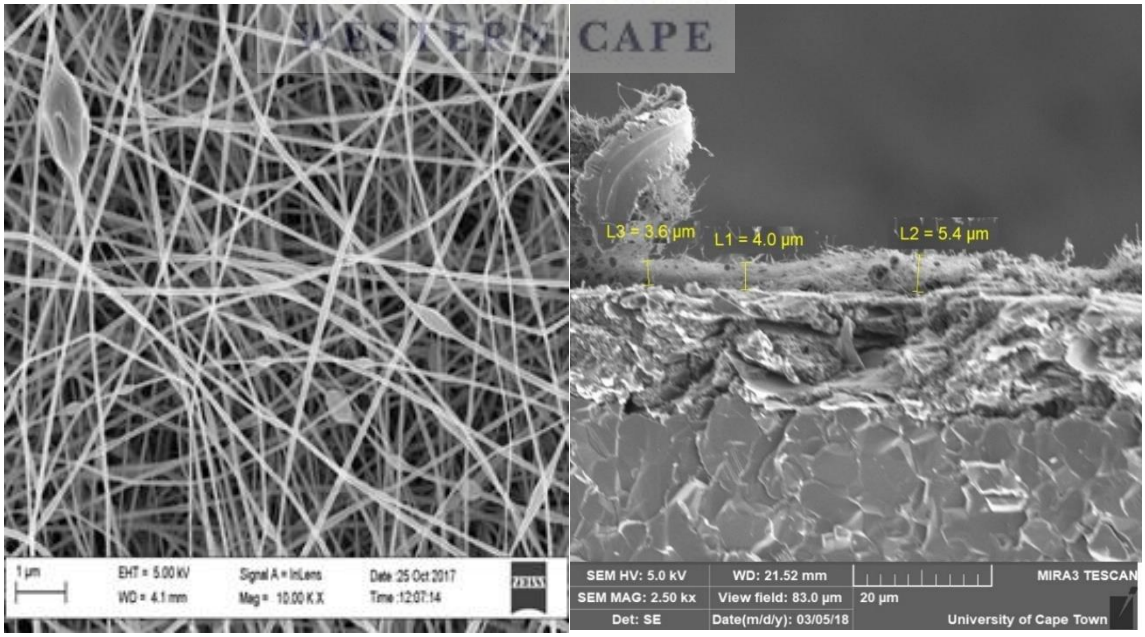


Figure 23. SEM images of PAA nanofibers.

SEM obtained for the PAA nanofibers confirmed a porous structure with fibers of nanoscale dimensions (less than 100nm). The cross-sectional SEM in **Figure 23** showed that nanofibers had an average layer thickness of 4.3 µm (n=4). Average layer thickness for electrodeposited PAA films (**Figure 22**) were measured to be 17.63 µm (n=4, 5 cycles) and 26.54 µm (n=4, 20 cycles). The decreased layer thickness is due to the space trapped within the nanofiber networks resulting in highly porous material with enhanced surface area and robustness. Thus, electrospining is able to produce intact, mechanically stable, high surface area nanofiber networks spun directly onto the working electrode surface of commercial SPCE. The efficient control over nanomaterial structure and deposition demonstrated here with the electrospinning of PAA is a major advantage in the design of highly reactive electrocatalysts for a wide range of applications.

5.7 Nitrogen adsorption-desorption

Isothermal N_2 measurements were used to study the surface structures of the freestanding polyamic acid (PAA) nanofibers.

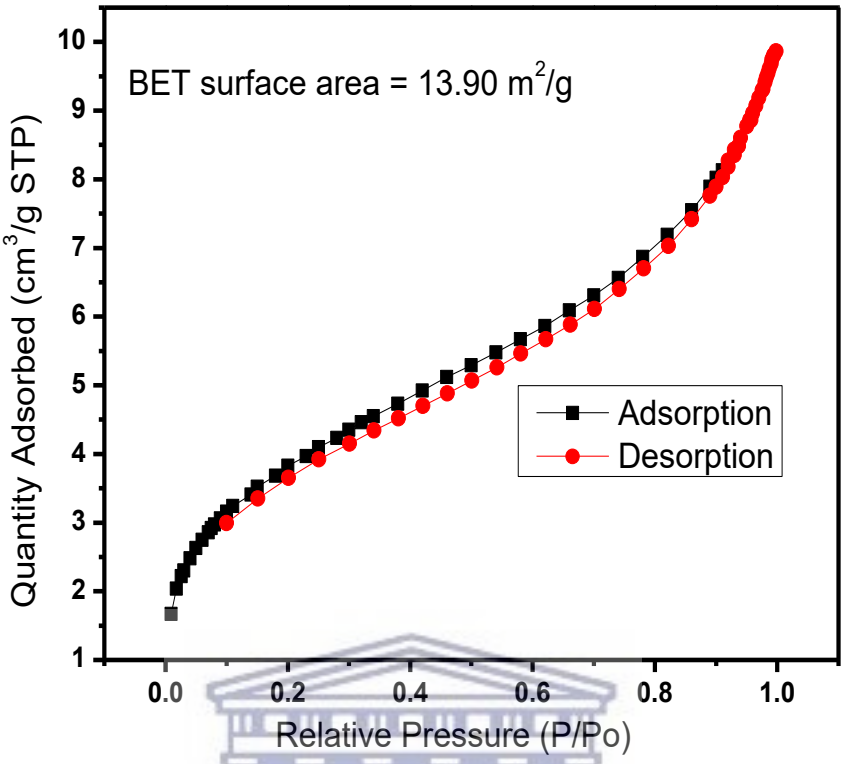


Figure 24. Nitrogen sorption isotherm of PAA nanofibers.

The adsorption-desorption isotherms of PAA nanofibers are presented in **Figure 24**. Based on the BET measurements the surface area of the PAA nanofibers was found to be 13.9 m^2/g, comparable to the surface area of synthesized carbon-based nanomaterials such as multi-walled carbon nanotubes with BET surface area ranging from 9 up to 500 m^2/g (Lehman et al., 2011; Tetana et al., 2012). The comparable BET surface area is due to the fibrous nanostructured nature of the PAA electrospun materials.

5.8 Atomic force microscopy (AFM)

The nanostructured PAA nanofibers and thin films were characterized by atomic force microscopy to study the possible correlation between their surface topography and electrochemical behaviour.

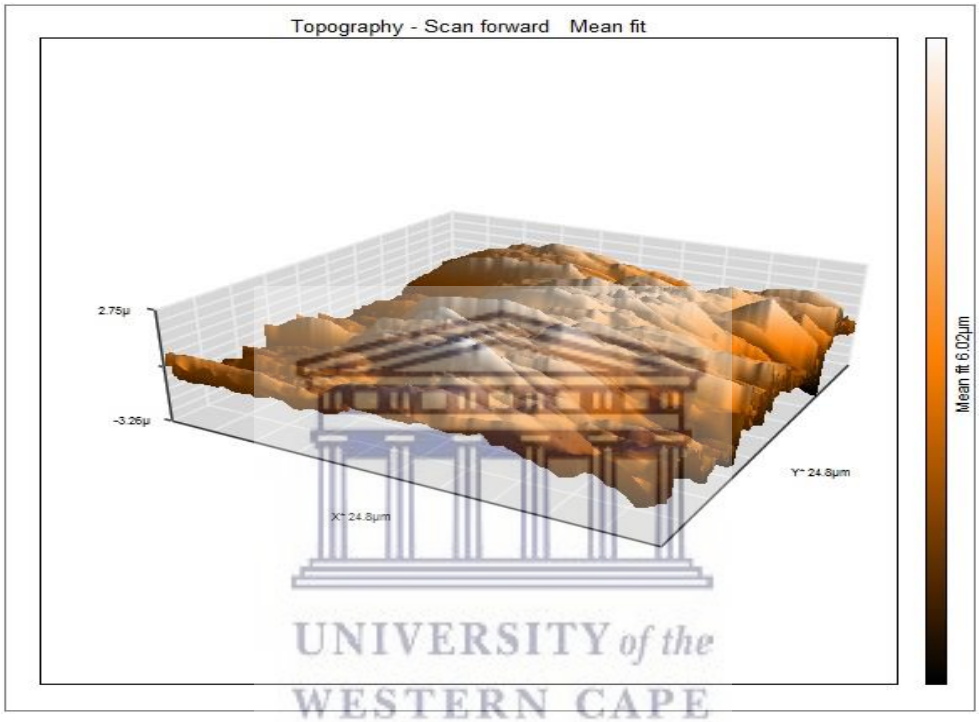


Figure 25. AFM Topographical images of PAA deposited thin films.

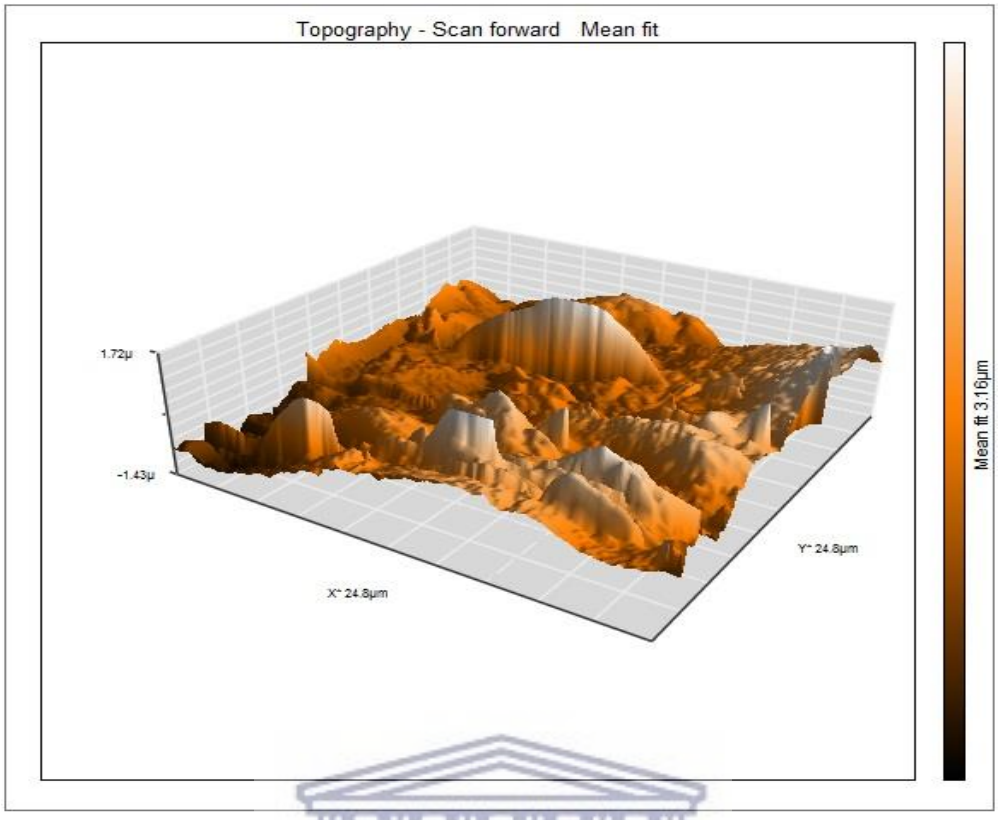


Figure 26. AFM Topographical images of electrospun PAA nanofibers.

The atomic force microscopy (AFM) was used to obtained topographical images in contact mode, of PAA thin films (**Figure 25**) and nanofibers (**Figure 26**), which report the frequency shift ($df = f - f_0$) as a function of the X–Y variation in height without regulation in the Z-direction (Hamnca et al., 2017). A sample with height and depth shows the distance variation between the tip–apex and the sample for a scan of the sample along x–y direction without height regulation in the z-direction. The average (n=3) height distribution of nanostructured PAA thin films and nanofibers was measured as 6.02µm and 3.16 µm respectively.

5.9 Electrochemical evaluation

Scan rate dependent cyclic voltammetry (CV) was used to evaluate the electrochemical integrity of the deposited PAA materials and subsequently its analytical response towards the quantification of a selection of sulfonamides. Scan rates ranged from 10

to 100 mV/s, with a potential window set between −1000 mV and 1000 mV, in a 0.1 M phosphate buffer (pH 7) electrolyte.

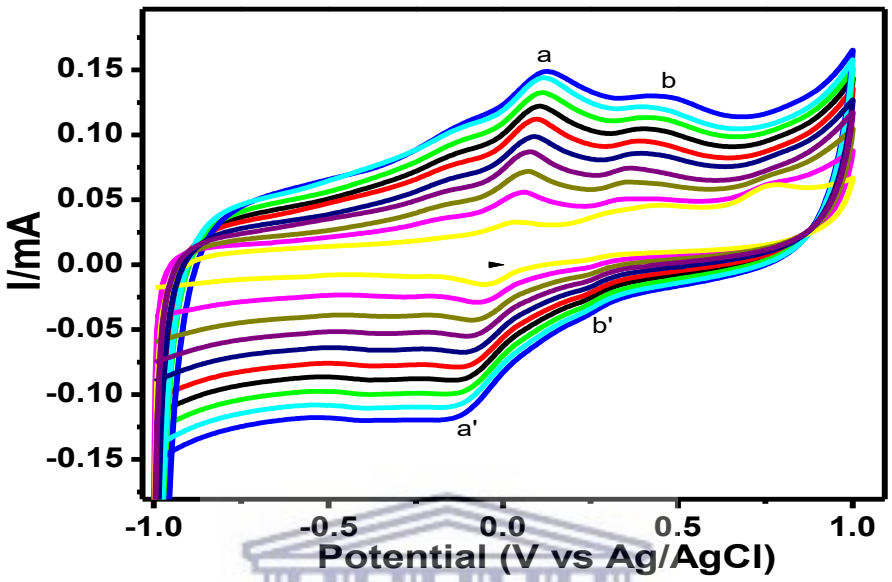


Figure 27. Cylic voltammetry (CV) of PAA thin film modified screen printed electrodes in 0.1 M phosphate buffer (pH, 7) at different scan rates (10 to 100 mV/s).

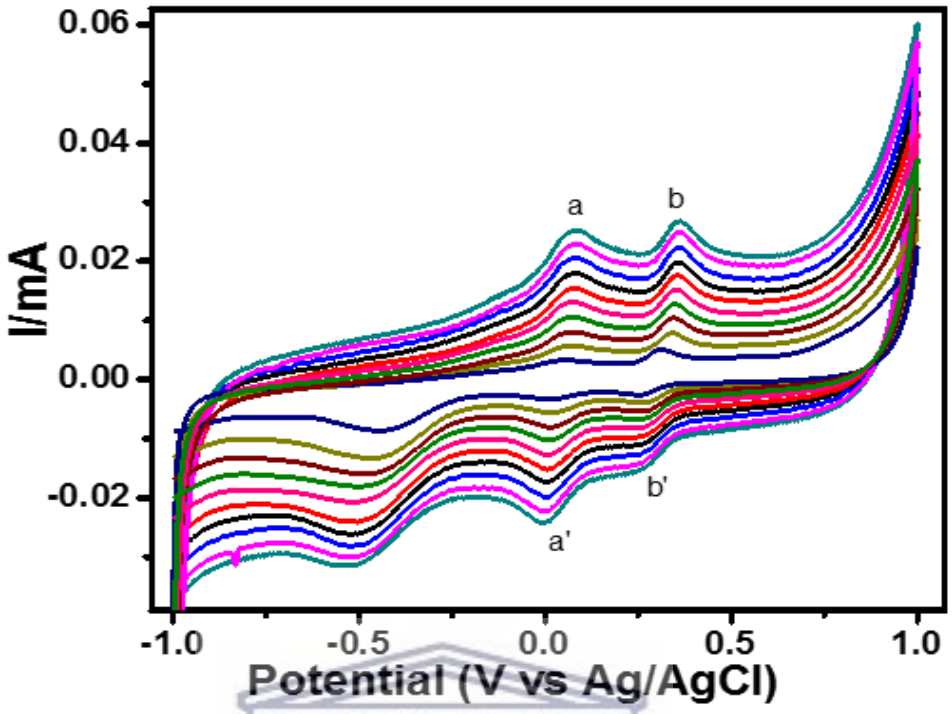


Figure 28. Cylic voltammetry (CV) of PAA nanofibers modified screen-printed electrodes in 0.1 M phosphate buffer (pH, 7) at different scan rates (10 to 100 mV/s).

Cyclic voltammetry of PAA/SPCE electrode prepared by electrospinning PAA nanofibers directly onto a screen-printed electrode (SPCE) was recorded at scan rates ranging from 10-100 mV/s. The redox behavior of PAA nanofibers were observed from CV (**Figure 28**) were characteristic of PAA electrochemistry reported for electrodeposited PAA films from chemically synthesized PAA powders (Hamnca et al., 2016) and **Figure 27** of this study. PAA nanofibers electrodes showed 2 anodic peaks at 73 mV and 350 mV respectively with 2 cathodic peaks at 11 mV and 268 mV, vs Ag/AgCl. Peak a is due to the oxidation of one electron from nitrogen atoms in the PAA structure and peak b is due to one stable quinoid type indication (Ngema, 2018). The peak currents reported for these peaks as a function of increasing scan rate

between 10-100 mV/s showed a linear dependency, with a peak separation ΔEp of 53 mV (thin film) and 27 mV (nanofibers) supporting a conclusion of controlled reversible process. The PAA nanofibers displayed enhanced peak resolution in terms of peak shape and redox current intensity, attributed directly due to the high surface area and porosity of the spun fibre networks.

Table 7. Electrochemical parameters obtained from characterization of PAA thin film.

Scan rate (mVs)	Ep,a (V)	Ep.c (V)	ΔEp (V)	E°
10	**0.018**	**-0.035**	**0.053**	**-0.0040**
20	**0.040**	**-0.033**	**0.073**	**+0.0070**
30	**0.044**	**-0.049**	**0.093**	**-0.0025**
40	**0.044**	**-0.040**	**0.084**	**+0.0020**
50	**0.049**	**-0.049**	**0.088**	**0**
60	**0.057**	**-0.053**	**0.110**	**+0.0025**
70	**0.062**	**-0.053**	**0.115**	**+0.0045**
80	**0.067**	**-0.062**	**0.129**	**+0.0025**
90	**0.071**	**-0.058**	**0.129**	**+0.0065**
100	**0.080**	**-0.071**	**0.151**	**+0.0045**

Table 8. Electrochemical parameters obtained from the characterization PAA nanofibers.

Scan rate (mVs)	Ep,a (V)	Ep.c (V)	ΔEp (V)	E°
10	**0.050**	**0.023**	**0.027**	**0.037**
20	**0.066**	**0.025**	**0.041**	**0.046**
30	**0.074**	**0.021**	**0.053**	**0.048**
40	**0.072**	**0.013**	**0.059**	**0.043**
50	**0.074**	**0.008**	**0.066**	**0.041**
60	**0.076**	**0.008**	**0.068**	**0.042**
70	**0.083**	**0.005**	**0.078**	**0.044**
80	**0.082**	**0.002**	**0.080**	**0.042**
90	**0.080**	**0.002**	**0.078**	**0.041**
100	**0.080**	**0.002**	**0.078**	**0.041**

The number of electrons transferred was calculated from peak a and a' in the CV data collected at PAA/SPCE (Fig. 6) using the equation:

$Ep - Ep_{1/2} = 2.20\ RT/nF = 56.5/n$…….. (Equation 1)

Where:

Ep: the maximum peak potential,

$Ep_{1/2}$: Half maximum peak potential,

R: Gas constant (8.314 $j.mol.k^{-1}$)

T: Absolute temperature (298 of the gas system)

n: number of electrons and

F: Faraday constant (96584 C/mol).

The a/a' redox chemistry was found to be a one-electron transfer process associated with the amine functionality of surface bound PAA/SPCE. The surface concentrations of absorbed electro active species of PAA/SPCE electrode was estimated from the plot of peak current vs potential using the equation (Brown–Anson model) (Brown and Anson as:

$Ip= n^2 F^2 I^*Av/4RT$…… (Equation 2)

The diffusion coefficient was determined using the Randles-Sevcik equation for reversible systems as follows:

$Ip= (2.69x10^5) n^{3/2}v^{1/2} D^{1/2} AC$.... (Equation 3)

Where: Ip: the cathodic/anodic peak current at a different scan rate

A: the surface area of the unmodified SPCE electrode (0.1257 cm^2),

v: Scan rate (V/s),

I^*: is the surface concentration (mol cm^{-2})

C: the molar concentration (mol/L)

D: the diffusion coefficient (cm^2/s)

F, R, and T are the same as in equation (1).

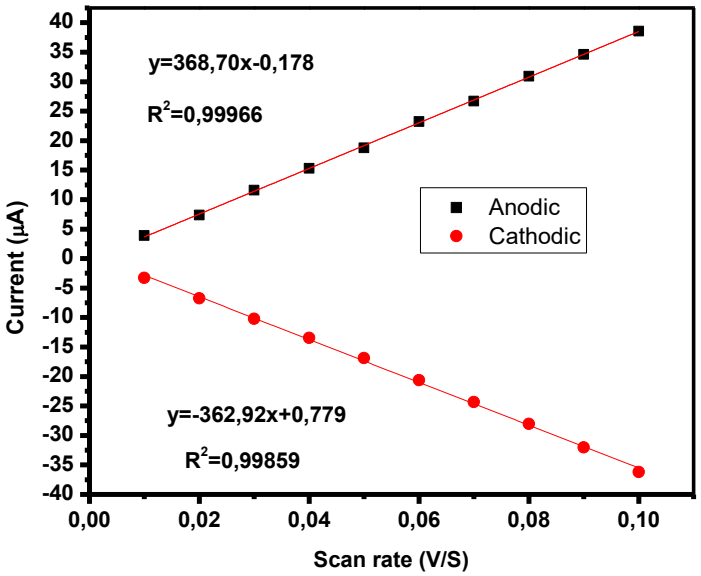


Figure 29. Nanostructured PAA thin films redox behaviour based on the Brown-Anson equation.

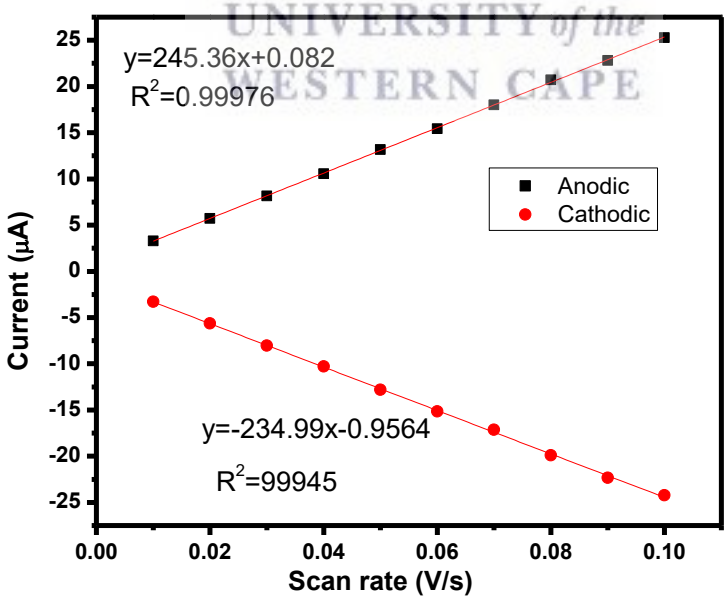


Figure 30. Nanostructured PAA fibers redox behaviour based on the Brown-Anson equation.

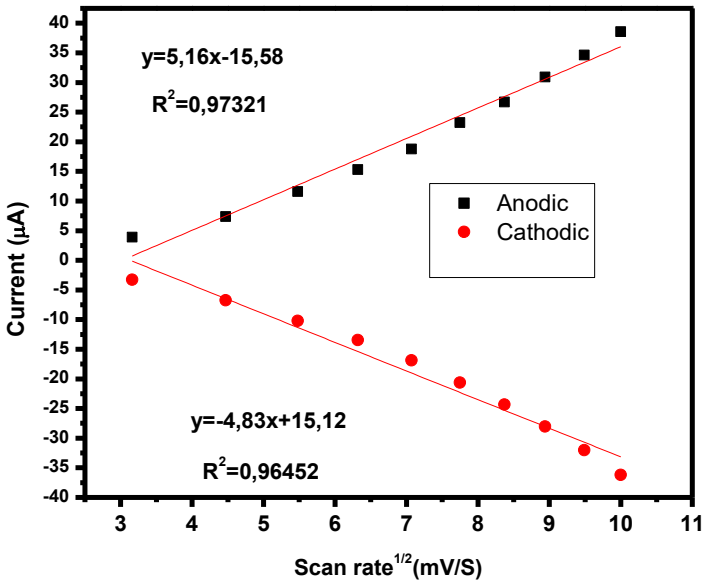


Figure 31. Nanostructured PAA thin films redox behaviour based on the Randles-Sevcik equation.

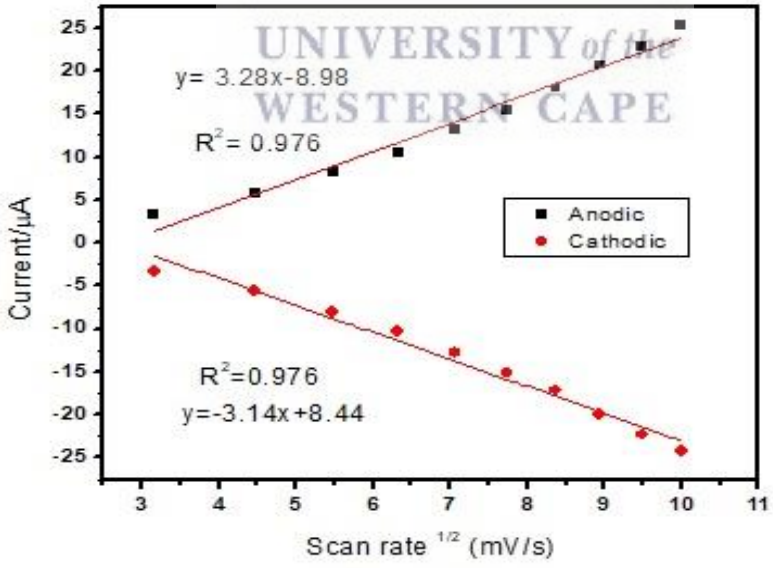


Figure 32. Nanostructured PAA redox behaviour based on the Randles-Sevcik equation.

The surface concentration absorbed electro active at electrodeposited PAA thin films electrode was $3.27x10^{-6}$ mol/cm^2 and at electrospun nanofibers on a screen-printed electrode (peak a) was estimated to be $2.14x10^{-6}$ mol/cm^2. The Randles–Sevcik plots in **Figure 31 and 32** were used to calculate the diffusion coefficient (D_e) of the screen-printed carbon –PAA thin films and PAA nanofiber electrode. The diffusion coefficient ($D_{e)}$ of the SPCE-PAA thin films and electrospun nanofiber electrode was $2.33x10^{-6}$ cm^{-2}/s and 9.43 $x10^{-7}$ cm^{-2}/s respectively, which is close to the reported value ($6.35x10^{-7}$ cm^2 /s) in the literature (Noah et al., 2012) for PAA coated electrode surface.

Table 9. Summarized electrochemical parameters determined from both the Brown-Anson and Randles-Sevcik plots and equations.

Electrode	**Surface concentration**	**Diffusion coefficient (De)**	**Formal potential**	**Number of electrons**
PAA thin film-SPCE	$3.27x10^{-6}$ mol/cm^2	$2.33x10^{-6}$ cm^{-2}/s	0.0028	1
PAA nanofibers-SPCE	$2.14x10^{-6}$ mol/cm^2	9.43 $x10^{-7}$ cm^{-2}/s	0,043	1

5.10 Conclusion

The solubility behavior and processability of polymers is crucial in its application as thin film devices such as sensors, photovoltaic cells and interpenetrating network

actuators. We have shown that nanostructured PAA could be prepared in two different formats; in situ electrochemical deposition and by electrospinning method to produce thin layers of nanofibers. The critical mass for efficient electrospinning of PAA could be achieved by minimal incorporation of a carrier polymer which resulted in highly dispersed, uniform nanofibers which could be deposited directly onto the working electrode area of commercial SPCE.

Polyamic Acid (PAA) electrodeposited thin films and electrospun nanofibers were successfully characterized and electrochemically evaluated to determine electrochemical behavior of the nanostructured PAA modified screen carbon printed electrode. The produced polyamic acid (PAA) nanofibers were characterized using Fourier Transform infrared (FTIR) spectroscopy to study integrity of polyamic acid functional groups as nanofibers by comparing them to the chemical synthesized polyamic acid. Scanning electron microscope (SEM) used to confirm the morphology of the produced nanofibers. Brunauer-Emmett-Teller (BET) was used to determine the surface area of the nanofibers. Atomic force microscopy (AFM) used to study the porousness, robustness and surface roughness of the nanofibers and study possible correlation between their surface topography and electrochemical behaviour of the developed nanostructures.

Table 10. Physical differences between the PAA thin films and nanofibers.

PAA nanostructures	**Thin films**	**Nanofibers**
Cross-sectional (layer thickness)	17.63 µm	4.3 µm
AFM (height distribution)	6.02 µm	3.16µm

Electrochemistry (inter-peak distance)	53 mV	27 mV.

Cyclic voltammetry was used to determine the number of electrons transferred in the system, the surface concentration of the deposited PAA thin film and PAA electrospun nanofibers and the diffusion coefficient (D_e) for the PAA thin film and nanofiber modified screen-printed electrode. The compact thin film showed larger, layer thickness (cross-sectional SEM), height distribution (AFM) and artificially higher peak separation peak (effect of resistance) compared to the porous nanofibers which showed a more reversible reaction. The carrier polymer (PVP) did not influence the redox electrochemistry of the PAA as evidence by the pronounced electrochemical reporting signals obtained.

Chapter 6: Analytical reporting of sulphonamides at the nanostructured PAA modified screen-printed carbon electrodes

This chapter will focus on the electrochemical determination and analytical reporting of sulfadiazine, sulfamethoxazole and sulfamethazine at graphenated commercial screen printed (SPCEs) and nanostructured polyamic acid modified electrodes.

6.1 Introduction to modified electrodes

Chemically surface-modified electrodes may exhibit unique properties or adapt a unique behaviour that can benefit electrochemical sensing (Wang, 1991). Molecular films on electrodes provides the needed flexibility to make reactions at the surface more sensitive and selective. Chemically modified electrodes can be used in broad spectrum of electrochemical investigations such as relationship between electron transfer and chemical reactivity to surface electrode chemistry, electrostatic phenomena at electrode surfaces, ionic and electron transport phenomena in polymers the design of electrochemical devices such as chemical sensors (Durst, 1997). Compared to bulk material-modified electrodes, nanomaterial-modified electrodes have advantages such as huge specific surface area for the immobilisation of more functional molecules and biocompatible nanomaterials can maintain the activity of proteins on the electrode for a longer and also accelerate electrode transfer between

the electrode and the protein (Li and Miao, 2013). Nanomaterials are widely used in the modification of electrodes for electroanalytical determination of inorganic, organic and biomolecules. In this study two types of polyamic acid nanomaterials (thin films and nanofibers) will be developed as electrocatalyts for reliable analytical reporting of sulphonamides.

6.2 Introduction to physical-chemical properties of sulphonamides

Chapter four of this thesis focused on effect of different supporting electrolyte solutions with acidic, neutral and slightly above neutral pH values in electrochemical behaviour of sulphonamides at the unmodified carbon electrode sensing surfaces. The purpose of the previous chapter was to select a suitable supporting electrolyte capable of demonstrating the true effect of the modified electrodes without the hindrances of other species formed in the solutions. This chapter aims to demonstrate the effect of both physical-chemical properties and R-group substituents in the analytical reporting of selected sulphonamides.

The sulphonamide chemical structure has amine group (-NH_3) which is basic and the acidic amide group (-NH-) which correspond to pKa_1 and pKa_2 respectively. Depending on the specific pH values the amine group can gain a proton while the amide group can release a proton (Qiang and Adams, 2004). Physical-chemical properties such dissociation constants (pKa) of organic molecules like antibiotic sulphonamides can influence the binding and interactions of these molecules with environmental matrices. Dissociation constant (pK_a) of a drug molecule is regarded as one of the key parameters as it controls the absorption, distribution, metabolism (elimination of substance) and solubility (Martell and Motekaitis, 1992: Sanli et al., 2010).

Table 11. Physical-chemical properties of the selected sulphonamides.

Sulphonamides	Molecular Weight (g mol^{-1})	pK_{a1}	pk_{a2}	Literature
Sulfadiazine	250.30	2.00	6.50	Lin et al.,1997
Sulfamethoxazole	253.30	1.85	5.60	Qiang and Adams, 2004
Sulfamethazine	279.33	2.07	7.49	Qiang and Adams, 2004

Researchers have suggested that since the R-group is the only variable involved in the N1-substituted derivatives it should be the main factor controlling the dissociation constants and sulphonamide activity. They also stated that the acidity of the amide group is influence by the electron attracting properties of the R-group (Bell and Roblin, 1942: Seydel, 1968: Soriano-Correa et al., 2003). In terms of their effect of the R-group in the electrochemical behaviour of the sulphonamides, according Braga et al. (2010) and Msingati and Ngila (2002) oxidation (-NH_3) potentials of sulphonamides are not greatly influenced by the R-group substituents compared to the reduction (-SO_2) potentials which may be varied by the R-group.

6.3 Analytical reporting of the selected sulphonamides at commercial SPCEs modified with nanostructured PAA thin film

Stock solutions SDZ (10 mM), SMX (10 mM) and SMZ (10 mM) were prepared in 0.1 M HCl. The electrochemical window was set for the oxidative SWV of SDZ, SMX and SMZ was set at -1.0 to +1.0 V. Scan rate of 50 mVs was achieved by setting the frequency at 10 Hz and the step potential at 5 mV vs Ag/AgCl. A polymer modified transducer was used in the quantitave determination of SDZ, SMX and SMZ. Consecutive concentration additions of SDZ, SMX and SMZ were added to the electrolyte respectively.

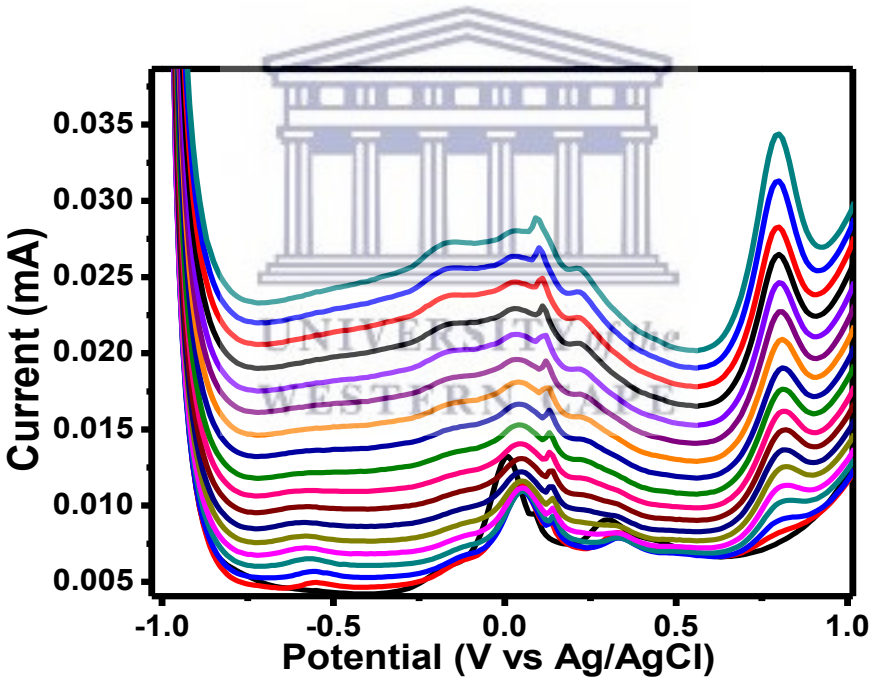


Figure 33. SWV analysis of the sulfadiazine (SDZ) at the modified PAA thin film modified screen rinted electrode in 0.1 M Tris-HCl (H 8) with concentrations ranging from (25 -300 µM) at 50 mVs.

The SWV voltammogram revealed well defined peak at 0.77 V vs Ag/AgCl attributed to SDZ. Two other peaks were identified and observed at 0.002 V vs Ag/AgCL and

0.30 V vs Ag/AgCl which are attributed to the polymer peaks. The high capacitive backround current of the polymer transducer can be attributed to the double layer at the surface of the elecrodes. Modification of the screen printed electrode with polymer materials contributed to the high capacitive current.

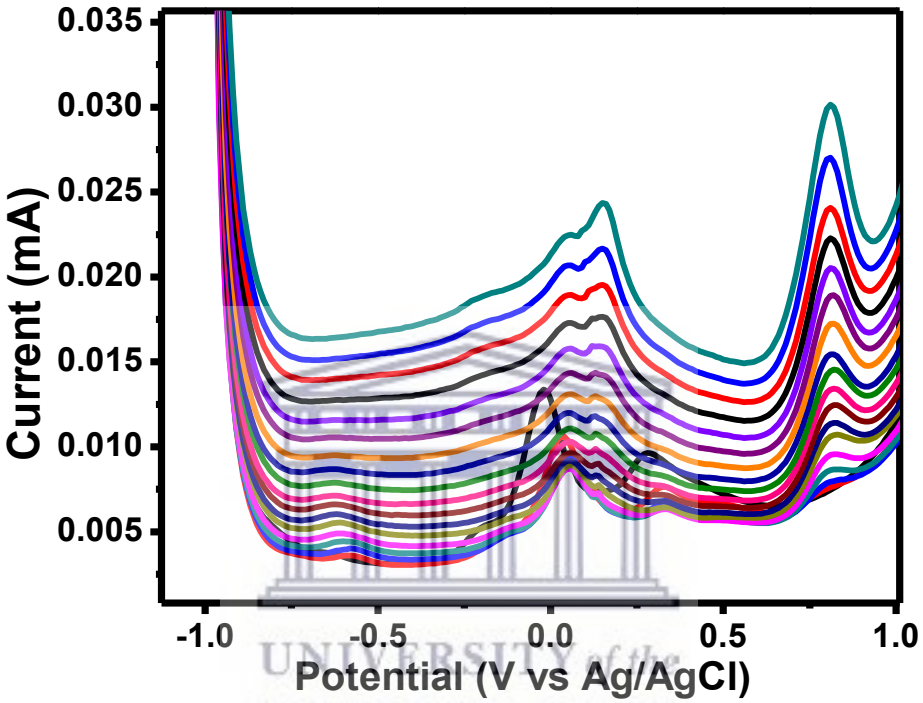


Figure 34. SWV analysis of the sulfamethoxazole (SMX) at the PAA thin film modified screen-printed in 0.1 M tris-HCl with concentrations ranging from (25 -300 µM) at 50 mVs.

During the evaluation of SMX 3 peaks were identified at potentials. The peaks were observed at -0.02 V vs Ag/AgCl, 0.29 V vs Ag/AgCl which can be assigned to the polymer redox behaviour and 0.82 V vs Ag/AgCl was attributed to the respective oxidation mechanism of SMX. Similarly, the background capacitive current due to the modified of SPCE with polymer material causing the charging and discharging double layer at the surface of the electrode. The observed change in the peak currents

occurred as a result of successive concentration additions of SMX which was used to plot a calibration of curve of current versus concentration.

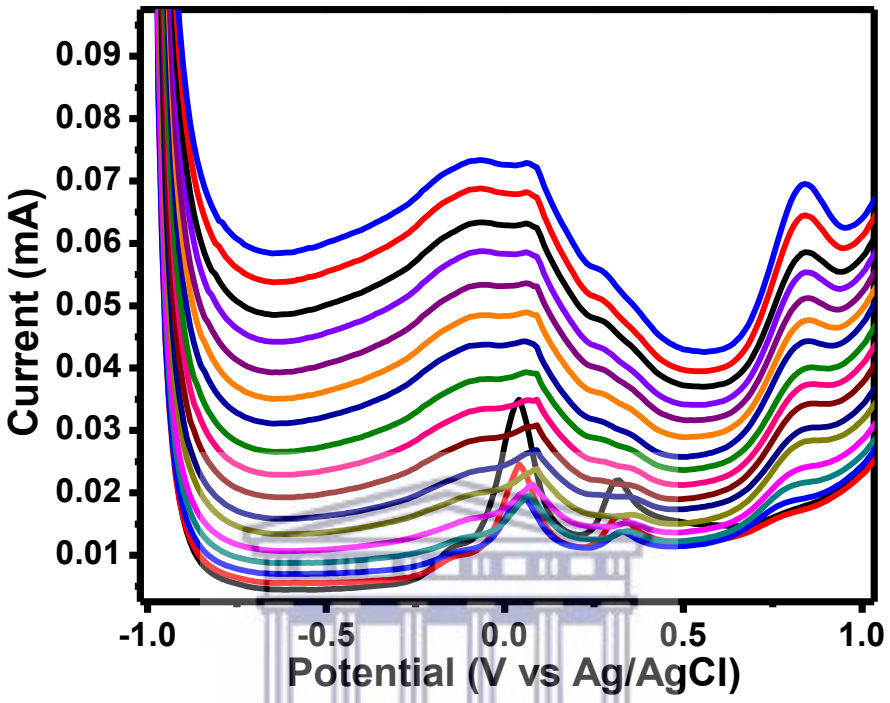
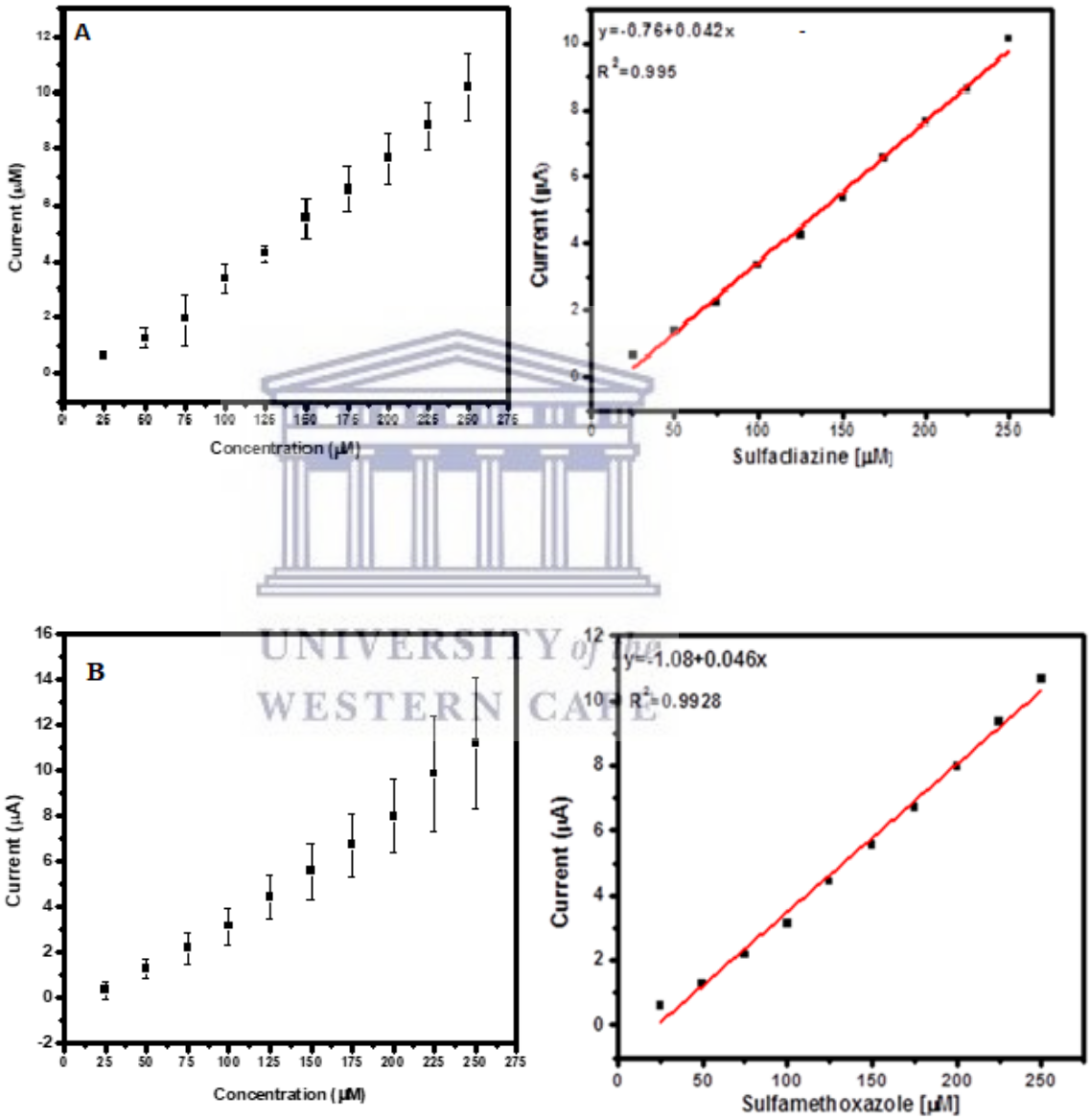


Figure 35. SWV analysis of the sulfamethazine (SMZ) at nanostructured PAA thin film modified screen printed electrode (SPCEs) in 0.1 M tris-HCl with concentrations ranging from (25 -300 µM) at 50 mVs.

The SWV voltammogram in **Figure 35** revealed a well-defined oxidative peak potential observed at 0.83 V vs Ag/AgCl is attributed to the oxidative mechanim of sulphonamides step. The two peaks observed at 0.04 V Ag/AgCl and 0.32 V vs Ag/AgCl potentials are attributed to the polymer mateials. From the SWV voltammogram SMZ and polymer peaks can be clearly distinguished. The observed change peak current is due to the consecutive concentration addition of SMZ. This change in current with concentration was used to plot a calibration curve of current versus concentration. The calibration curve of SMX provided useful quantitave

information in the determination of the limit of detection (LOD). In this work, the LOD was related to the minimum concentration that can be detected by the developed system.



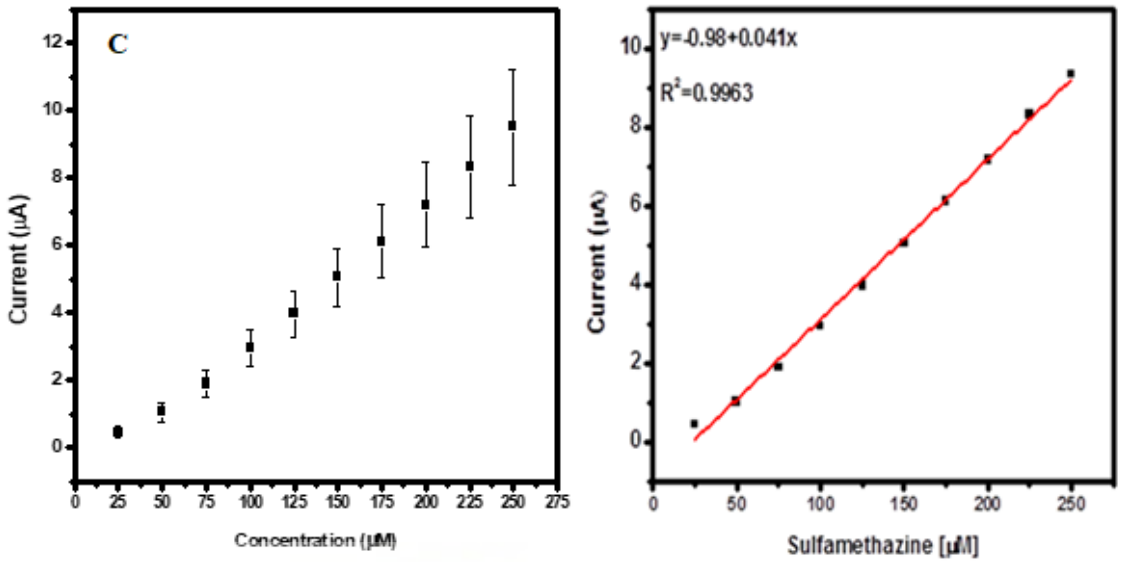


Figure 36. Calibration curves of sulfadiazine (a), sulfamethoxazole (b) and sulfamethazine (c) at a commercial screen-printed carbon electrode modified with PAA thin films.

The calibration curves provided data followed linear curve. The electrochemical paramters for detected sulphonamides and results determined from the calibration curve analysis have been tabulated below.

Table 12. Peak potentials and analytical parameters of the analytes.

Analytes	**Peak potentials (V)**	**LOD (μM)**	**LOQ (μM)**	**Sensitivity (ųA/ųM)**
Sulfadiazine	0.77	12.14	36.82	0.042
Sulfamethoxazole	0.82	14.59	44.22	0.046
sulfamethazine	0.83	10.41	31.57	0.041

A good linear range (25-250 μM) was obtained with correlation coefficients of 0.9946 (Sulfadiazine), 0.9928 (sulfamethoxazole) and 0.9963 (sulfamethazine), n=3. The equation from the straight line were expressed as y=-0.76+0.042x (sulfadiazine), y=-1.08+0.046x (sulfamethoxazole) and y=-0.98+0.041x (sulfamethazine). The y represented the current at the modified electrode (μA) and x is concentration of sulphonamides (μM). The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the previously expressed equations (LOD=3.3 x SE /slope and LOQ= 10 x SE/slope). The LOD value for the sulfadiazine was 12.14 μM. There are limited reports on the polymer thin film modified for sulfadiazine detection. A sensor based on carbon paste electrode modified with sulfadiazine molecular imprinted polymer used as recognition element. The sulfadiazine determination after its extraction onto the surface of the electrode was carried by DPV at 0.92 V vs Ag/AgCl. The LOD value for the developed sensor was 0.14 μM (Sadaghi and Motaharian, 2013). Sulfamethoxazole was detected at the PAA thin film modfied SPCE and the LOD was 14.59 μM. An electrochemical sensor based on molecularly poly(dopamine) (PDA-MIP) for sulfamethoxazole detection has been previously developed. The limit of detection of the PDA-MIP thin film sensor with anti-fouling properties was 0.8 μM (Turco et al., 2018). The LOD value at the PAA thin film electrode for sulfamethazine was 10.41 μM. Conducting polymer base nanocomposites modified screen printed electrodes are reported as sensor platforms for electroanalytical determination of sulfamethazine. The electrochemical sensor was based on a nanocomposite containing poly(3,4-ethylenedioxythiophene) (PEDOT).and MnO_2. The detection limit of the developed sensor towards the detection of sulfamethazine was 0.16 μM (Su and Cheng, 2018). The developed PAA thin film-

based sensor developed in this study showed high sensitivity towards the detection of sulfamethazine.

6.4 Analytical reporting of the selected at commercial SPCEs modified with PAA nanofibers

Electroanalytical analysis of sulphonamide analytes was conducted at the screen-printed carbon electrodes modified with PAA nanofibers. The modified electrodes were conditioned by cyclic voltammetry as described prior the analysis to stabilize the background current. Stock solutions (10 mM) of each of the selected sulphonamides were prepared in 0.1 M HCl. The electrochemical window used for the oxidative SWV of SDZ, SMX and SMZ was set at -1.0 to +1.0. A scan rate of 50 mV/s was achieved by by setting the frequency at 10 Hz and step potential at 5 mV vs Ag/AgCl. The electrospun polymer nanofiber modified transducer was used in the quantitative analysis of SDZ, SMX and SMZ.

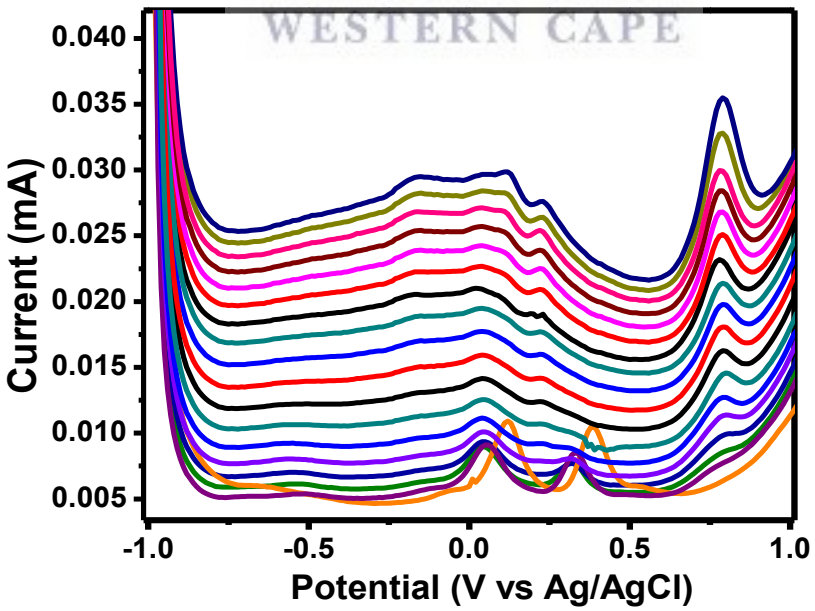


Figure 37. SWV analysis of the sulfadiazine at the PAA nanofiber modified screen rinted electrode (SPCE) in 0.1 M tris- HCl with concentrations ranging from (25 -250 µM) at 50 mVs.

In the SWV voltammogram above 3 peaks were identified at potentials 0.12 V vs Ag/AgCl, 0.39 V vs Ag/AgCl and 0.79 V vs Ag/AgCl. Peaks observed 0.12 V and 0.39 V vs Ag/AgCl are attributed to the electrospun polymer material. The well-defined peak observed at 0.79 vs Ag/AgCl was attributed to the SDZ oxidation mechanism. The high background capacitive current of platform is the contribution of both porous polymer nanofibers and SPCE.

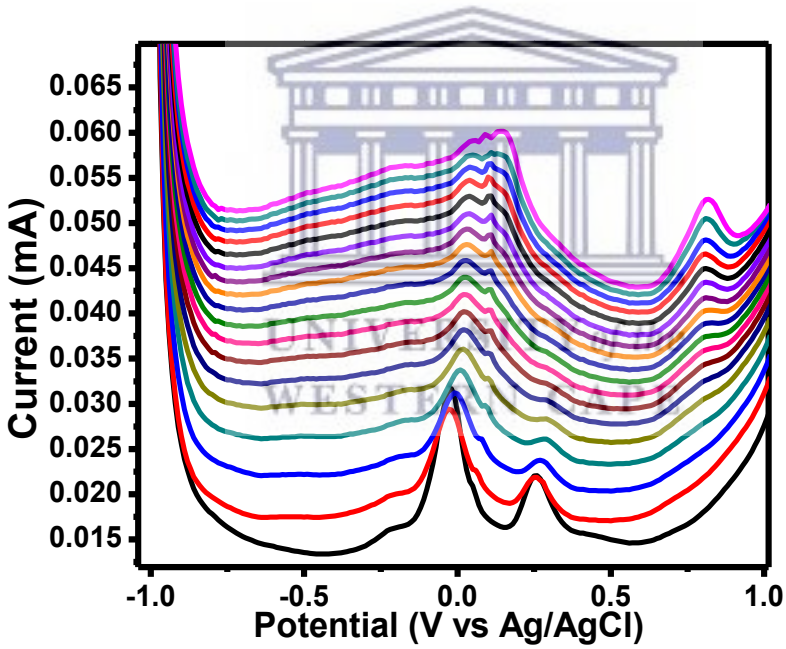


Figure 38. SWV analysis of the sulfamethoxazole at the PAA nanofiber modified screen printed in 0.1 M tris-HCl with concentrations ranging from (25 -300 µM) at 50 mVs.

Two peaks were identified lower energy potenials at -0.013 V vs Ag/AgCl and 0.27 V vs Ag/AgCl. The two peaks were attributed to the electrospun polymer material at the

surface of the electrode. The peak observed at 0.81 V vs Ag/AgCl is attributed to the SMX oxidation mechanism. The high background capacitive current and low current peak response of the SMX are due to the high porosity of the electrospun polymer platform at surface of the screen-printed electrodes. The nature of the sulfamethoxazole compound and the porous film at the electrode surface may affect the adsorption of the analyte at the electrode surface which resulted in low current reponse of the analyte and high current of the nanoporous electrode.

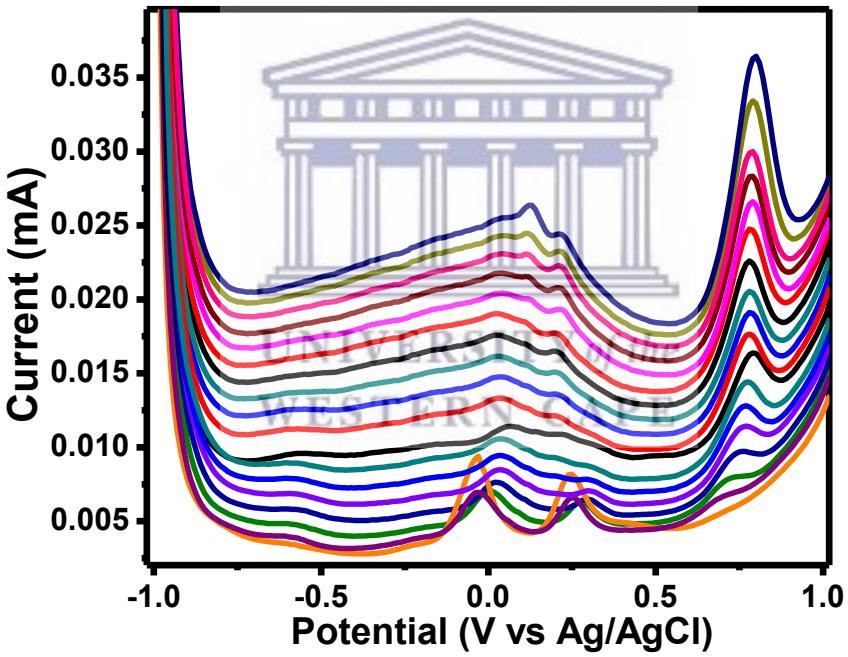
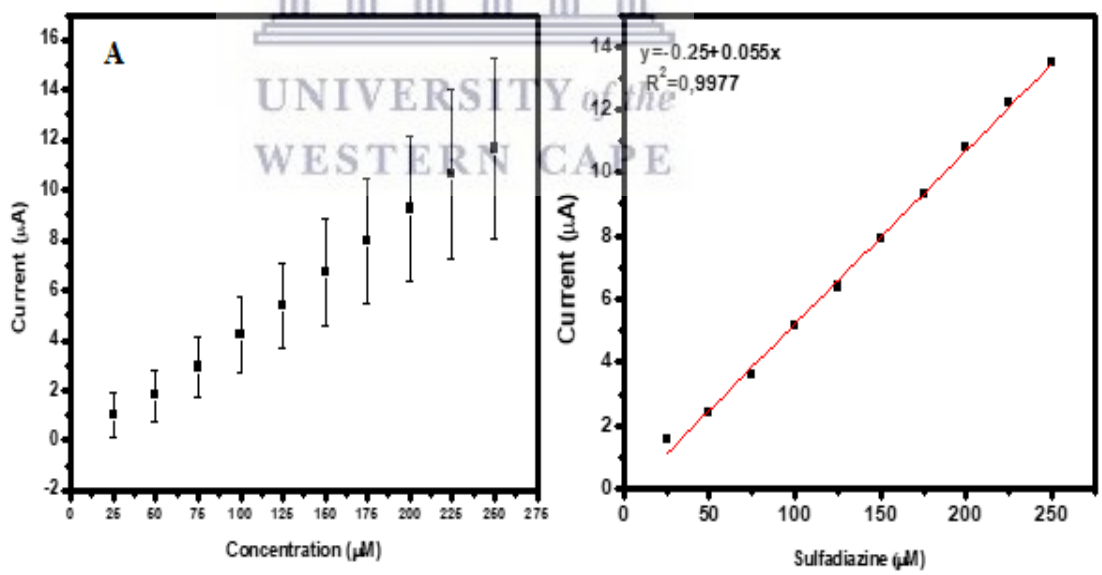


Figure 39. SWV analysis of the sulfamethazine at PAA nanofiber modified screen printed electrode (SPCE) in 0.1 M tris-HCl with concentrations ranging from (25 -300 µM) at 50 mVs.

In the SWV voltammogram represent above, a well-defined oxidative peak at 0.78 V vs Ag/AgCl which is attributed to the oxidation of SMZ, sulphonamides are typically

reported in this potential region, assigned to the oxidation of the $-NH_2$ group following the oxidation steps in the mechanism. Two peaks observed at -0.027 V vs Ag/AgCl and 0.25 V vs Ag/AgCl are attributed to the porous electrospun polymer material. The oxidation peaks observed for both SDZ and SMZ were well-defined with high current at low concentrations at the electrospun nanofiber modified electrode, which we believe is due to the binding affinity of pyrimidin functional group both compounds, higher surface, porosity of the nanofibers network enhancing the catalytic efficiency of the oxidation. The current against concentration relationship was used to developed standard calibration curves. The high disparity between the three data (n=3) at the modified electrode sensor platforms is attributed to the less controlled deposition of polymer nanofibers at the carbon surfaces by the electrospinning technique compared to the more controlled techniques (carbon printing technology and polymer electrodeposition).



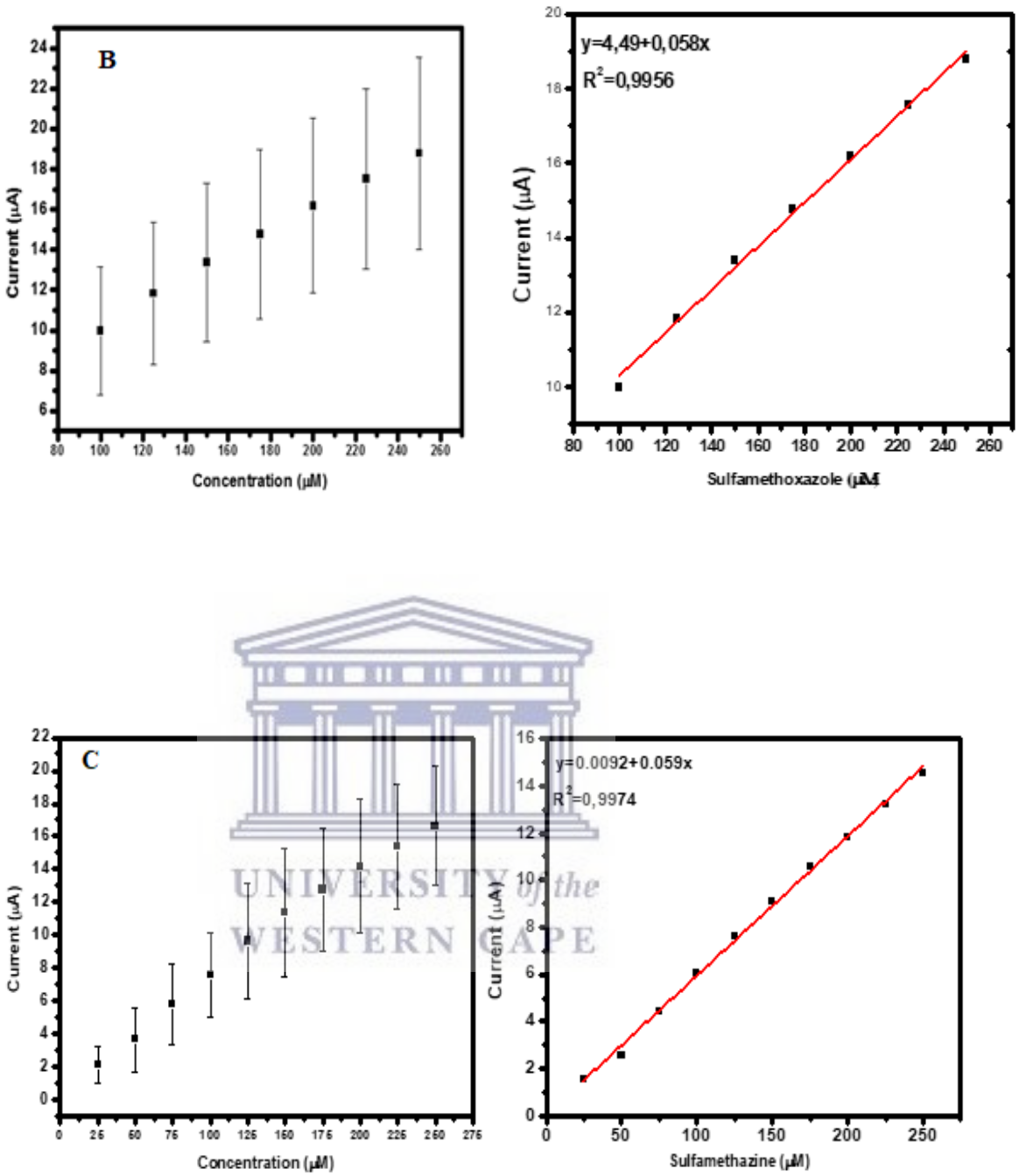


Figure 40. Linear regression plot of current against concentration of sulfadiazine (a), sulfamethoxazole (b) and sulfamethazine (c).

From the linear calibration curve it evident that the both SDZ and SMZ showed low variability compared to SMX. The similar behaviour of SDZ and SMZ is attributed to the pyrimidin functional group attached to both compounds (SDZ and SMZ).

Pyrimidin a more electron-widrawing compared to the azole increases the acidity of the N-H proton by stabilizing the resulted anion. The linear calibration analysis was used to determine analytical paramters such as limit of detection and the sensitivity of the system. Based on the linear calibration curves of the concentration against the current, the selected electroanalytical parameters have determined and tabulated (**Table 13**) below.

Table 13. Analytical parameters at the PAA nanofiber modified electrode.

Sulfonamides	Peak potentials (V)	Linear range (ųM)	Sensitivity ($\mu A.\mu M^{-1}$)	LOD (ųM)	LOQ (ųM)
Sulfadiazine	0.79	25-250	0.055	8.26	25.04
Sulfamethoxazole	0.81	100-250	0.057	16.59	49.42
Sulfamethazine	0.78	25-250	0.059	8.81	26.70

The sulfadiazine and sulfamethazine excellent linear regression response for the concentration range evaluated, at the PAA nanofiber-modified electrodes, as shown in Figure with R^2 values of 0.9977, 0.9956 0.9974 (n=3) for sulfadiazine, sulfamethoxazole, sulfamethazine respectively. The determined LOD value for sulfadiazine at PAA nanofiber modified electrode was 8.26 ųM. Nanofibrous like materials such as multi-walled carbon nanotubes have been used to modify solid electrodes in the determination of sulfadiazine. In the reported studies glassy carbon

electrode modified with multi-walled carbon nanotubes was used to detected sulfadiazine with a limit of detection 0.21 µM (Hong et al., 2010) and 7.10 µM (Fatouhi et al., 2013). At the PAA nanofiber modified SPCE sensing surface the detection limit of the sensor towards the determination of sulfamethoxazole was 16.95 µM. Reports on sulfamethoxazole determination at nanofibrous materials are limited however SPCE-Au-Tyrosinase electrochemical biosensor for detection of sulfamethoxazole is reported in the literature with a detection limit of 20.60 µM (Del Torno-de Roman et al., 2016). Multi-walled carbon nanotubes modified glassy carbon electrodes are also used as sensor platforms for the detection of sulfamethazine with a detection limit of 6.1 µM. In our study, at the PAA nanofiber modified screen-printed electrode platform the detection limit towards electroanalytical determination of sulfamethazine was 8.81 µM. The analytical performance of the developed sensor platform based on the PAA nanofibers is comparable to that of multi-walled carbon nanotubes in terms of their application in sulphonamides electroanalytical sensors. This can be attributed to their similar size and structure.

The PAA nanofiber modified screen-printed electrodes showed a good sensitivity towards the detection and following sensitivity values were obtained 0.055 µA μM^{-1} (sulfadiazine), 0.058 µA μM^{-1} (sulfamethoxazole) and 0.059 µA μM^{-1} (sulfamethazine). A comparison of the sensitivity of carbon-based nanomaterial electrodes sulphonamide detection showed that carbon nanotubes and PAA electrospun nanofibers out-performed nanoparticles, molecularly imprinted method and polymer blends used in the preparation of transducers for the detection of sulfonamides. The implication of structure as well as size as opposed to size only, in efficiency of catalysis, is therefore substantiated.

6.5 Recovery studies

The efficacy of the proposed nanostructured PAA thin film modified screen-printed carbon sensor was evaluated by performing recovery studies for the selected sulphonamides. Tap water collected from the New Chemical Science building at the University of the Western Cape provided by the City of Cape Town municipality was used as the environmental medium for recovery studies. Tap water was spiked with known concentration of the analytes and square wave voltammetry was the preferred electroanalytical technique for successful analysis of spiked tap water samples.

Table 14. Recovery tests for sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfamethazine (SMZ) in tap water samples at PAA thin film modified screen carbon electrode.

Analyte	Amount added (µM)	Amount detected (µM)	Mean of recovery (%)	RSD[a] (%)
	25	24.71	98.84	4.98
SDZ	50	29.46	58.92	5.73
	75	30.43	40.58	6.74
	25	38.54	154.17	11.00
SMX	50	65.91	131.83	15.45
	75	83.27	111.03	16.80
	25	34.61	138.44	8.19
SMZ	50	54.55	109.1	18.62
	75	67.72	90.29	18.26

[a]n=3. RSD: Relative standard deviation

The recoveries of sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfamethazine (SMZ) conducted in tap water, to simulate environmental matrix using square wave voltammetry (SWV) at PAA thin film modified screen-printed carbon electrode are tabulated above. Using intra-coefficient variabilities, three different concentration (25 µM, 50 µM and 75 µM) for each analyte were analysed in a single water sample. Using the standard addition methodology, voltammograms were recorded by adding a volume of the spiked water into the electrochemical cell once a stable background was achieved. Then successive additions were made. The recoveries of SDZ with respect to each concentration were 98.84% (RSD 4.98%), 58.92 (RSD 5.73%) and 40.58% (RSD 6.74%). For SMX the recoveries were 154.17% (RSD 11.00%), 131.83% (RSD 15.45%) and 111.03% (RSD 16.80%). The recoveries for SMZ with respect to each concentration were 184% (RSD 8.19%), 109.10% (RSD 18.62%) and 90.26 (RSD 18.26%). The inter-coefficient recoveries for the studied sulphonamides ranged from 98.84 to 154.17% with an acceptable (less than 20%) relative standard deviation of 4.98-18.62%). The high recoveries of SMX ((higher than 100%) are attributed to the cell environment contributing to the overall signal reporting due to physical-chemical properties of SMX redox.

6.6 Electroanalytical analysis of the sulphonamides by chronoamperometry

Commercial biosensors are currently based on amperometric techniques. The viability of the developed PAA nanostructured electrochemical electrodes in sensor applications was tested using the chronoamperometry. Stock solutions (1 mM) of each sulfonamide were prepared. The amperometric current-time response was recorded at the PAA thin film modified commercial screen-printed carbon electrodes to successive

additions of the studied analytes to tris-HCl (pH 8) buffer. The PAA thin film electrode was modified by electro-deposition methodology as previously described in chapter 4. Prior the amperometric detection of the sulphonamide the electrode was subjected to 5 cyclic voltammetry scans at 50 mVs to activate the electrode surfaces and to stabilize the background current. The figures (**Figure 41-43)** below show the amperometric current-time response recorded at the commercial screen-printed carbon electrodes (SPCEs) modified with PAA thin films to successive additions of the studied sulphonamides (sulfadiazine, sulfamethoxazole and sulfamethazine) to tris-HCl (pH, 8) at their respective applied potential observed in the square wave voltammetry. The amperometric response was recorded at constant gentle stirring to make sure the solutions were completely homogenous after each injection of the sulphonamides.

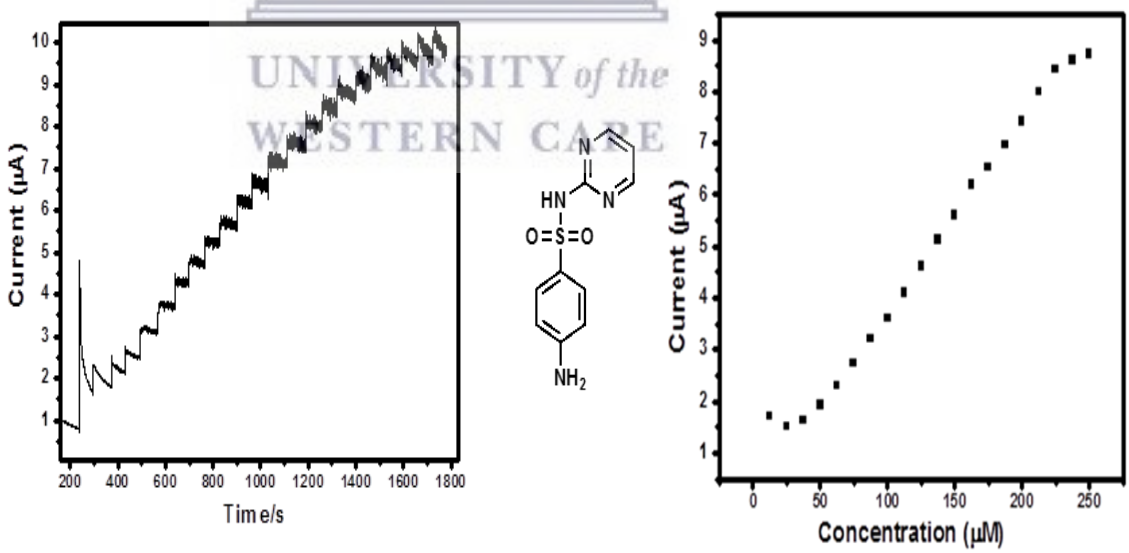


Figure 41. Amperometric response of screen-printed carbon PAA thin film modified electrodes after successive additions of sulfadiazine (12.5 to 250 µM).

The above figure (**Figure 41**) represents the time-current response of PAA thin film modified screen printed carbon electrodes after successive additions of sulfadiazine. An initial decrease in current response prior the increase oxidation current after each of the sulfadiazine forming a sigmoidal curve. The sigmoidal curve is indicative of competitive attachment to the available binding at low concentrations. The linear calibration was extrapolated from the sigmoidal curve for determination of analytical paramters such the limit of detection and the sensitivity of the system.

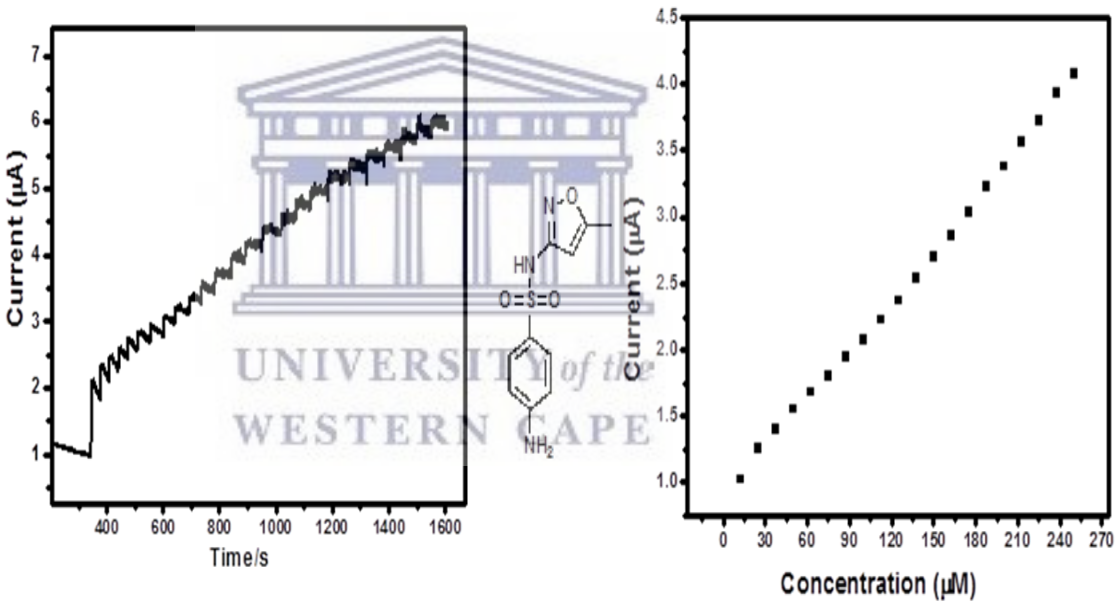


Figure 42. Amperometric response of screen-printed carbon-PAA thin film modified electrodes after successive additions of sulfamethoxazole (12.5 to 250 µM).

The figure above (**Figure 42**) represents the amperometric response of the PAA thin film modified after consecutive concentration additions of SMX. A linear current response of the PAA modified was observed after successive additions of sulfamethoxazole in tris-HCl solution. This response indicates the direct proportionality of transducer response and SMX analyte physical quantity input. The

analysis of the linear calibration curves provided useful information in the determination of the limit of detection, sensitivity and linearity coefficient of the system.

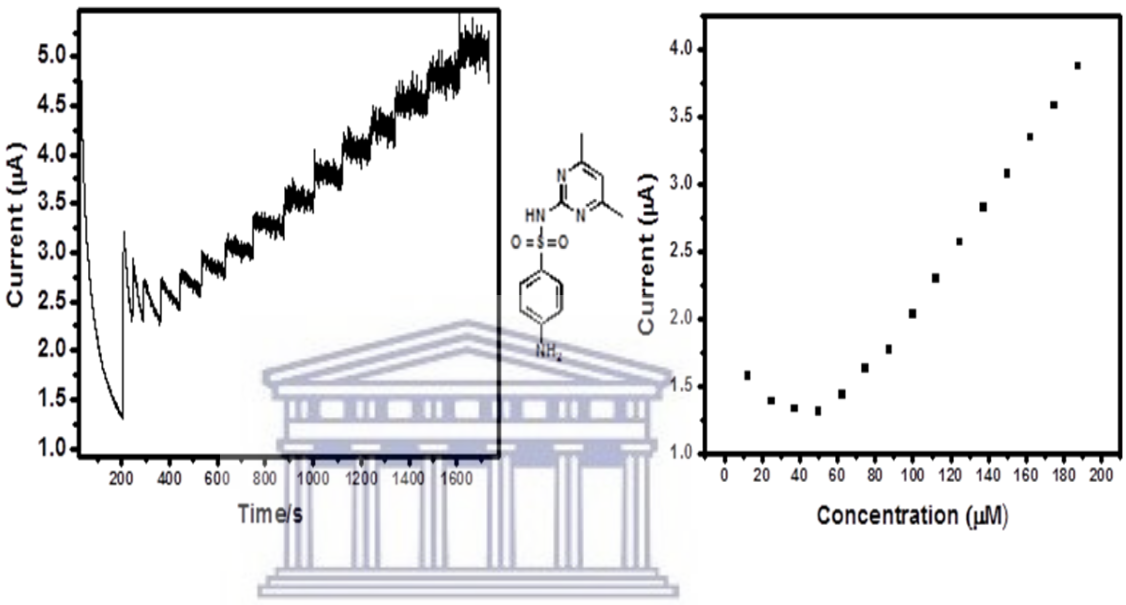


Figure 43. Amperometric response of screen-printed carbon- PAA thin film modified electrodes after successive additions of sulfamethazine (12.5 to 250 μM).

The amperometric response of the thin film modified screen printed electrode upon successive concentration additions of SMZ is represented in the above figure (**Figure 43**). Based on the staircase graph and the calibration it is evident that the data follows a sigmoidal curve, indicative of competitive binding of species for the attachment to the available binding sites at low concentrations. The observed change in peak current is the result of the consecutive concentration addition and was used to extrapolate the linear regression line which was used to determine the limit of detection and sensitivity of the system.

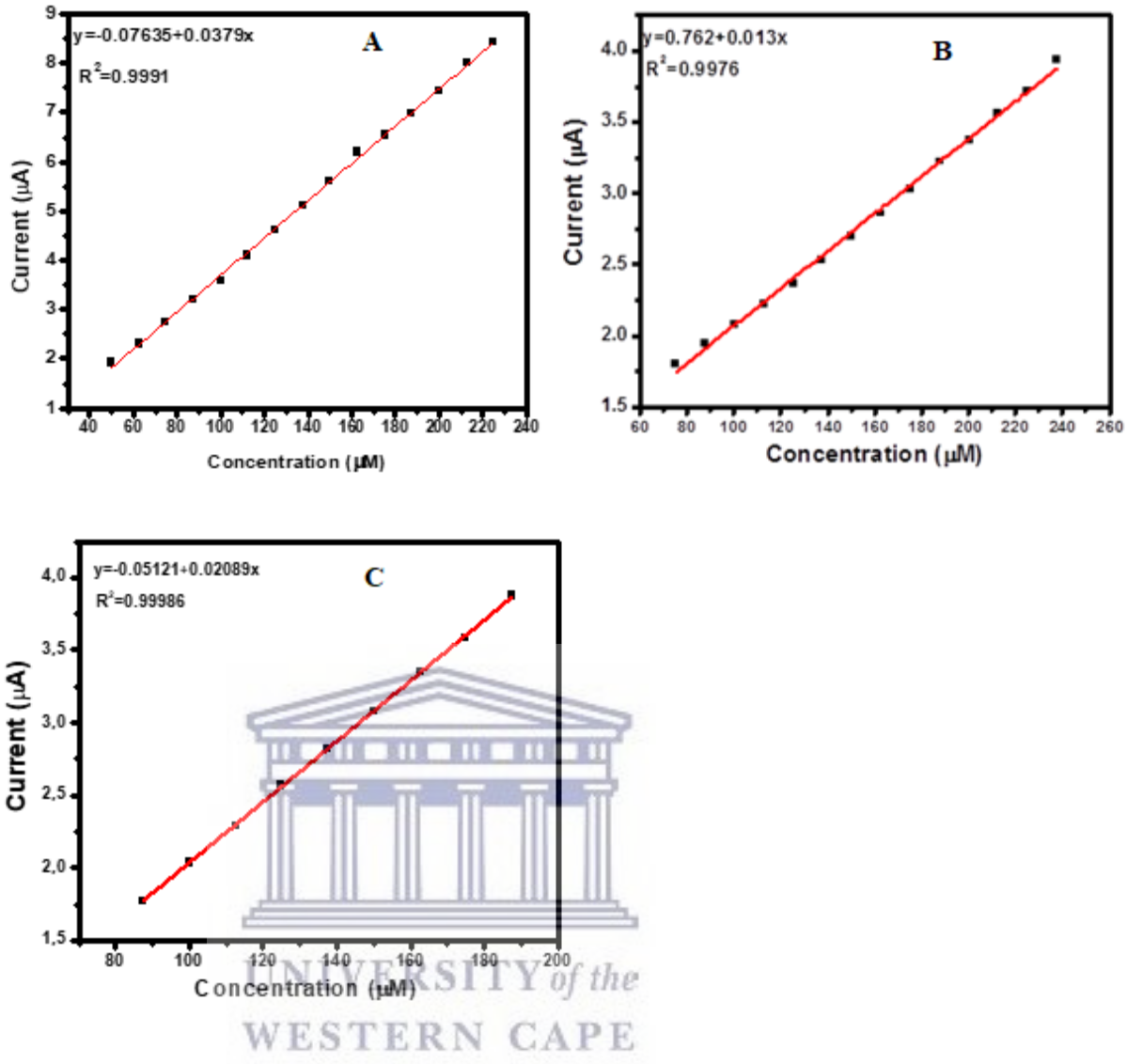


Figure 44. Linear regression plot of current against concentration of the sulfadiazine (a), sulfamethoxazole (b) and sulfamethazine (c).

The relationship between current response at the PAA thin film modified screen printed electrodes and concentration of the selected sulphonamides was used determine the linear calibration curves of each sulphonamide. The electro-analytical parameters of the sensor are summarized in the table below.

Table 15. Summarized analytical for sulphonamide detection at the PAA thin film electrode.

Analyte	Linear range (µM)	LOD (µM)	LOQ (µM)	Sensitivity (µA/ µM)
Sulfadiazine	50-225	3.80	12.16	0.076
Sulfamethoxazole	75-237.5	7.42	22.46	0.762
Sulfamethazine	87.5-187.5	1.94	5.88	0.051

The linear regression equations for detected sulphonamides were obtained from the calibration curves. The correlation coefficients were 0.9991, 0.9976 and 0.9999 for sulfadiazine (**Figure 44a**), sulfamethoxazole (**Figure 44b**) and sulfamethazine (**Figure 44c**) respectively. Analytical parameters such as the limit of detection (LOD), limit of quantification (LOQ) were obtained from the calibration and are listed in the table above. From the analytical parameters results obtained in the amperometric experiment are consistent with those obtained in the voltammetric studies. In both experiments' sulfadiazine and sulfamethazine with pyrimidine functional group had low detection limit compared sulfamethoxazole

6.6 Chronoamperometric detection of sulphonamides at PAA nanofiber modified SPCEs

The current-time amperometric experiments conducted in the previous section were also repeated at the PAA nanofiber modified SPCEs were done by additions of the respective sulfonamides at constant gentle. The stirring was very important in

homogenization of the solution. Prior the detection of the sulphonamides the modified electrodes were surface activated by 5 cyclic voltammetry.

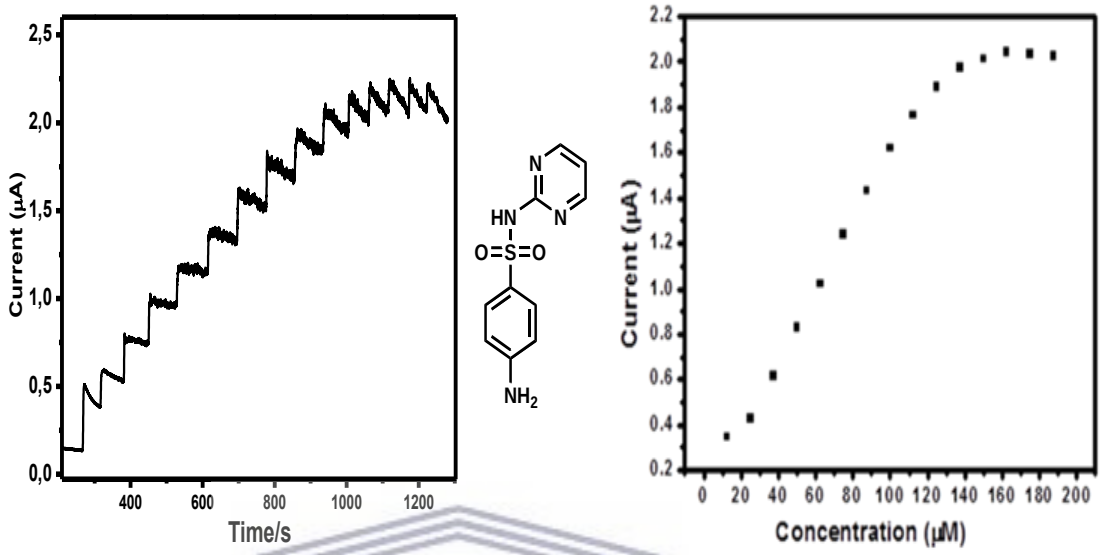


Figure 45. Amperometric response of screen-printed carbon- PAA nanofiber modified electrodes after successive additions of sulfadiazine.

The current response of PAA nanofibers modified electrode after additions of sulfadiazine shown in **Figure 45** where the amperometric oxidation current linearly increased with additions of sulfadiazine until the system reached a point of saturation. SDZ showed high adsorptive binding affinity to the porous thin film platform this attributed to the high binding affinity of pyrimidine functional group in the sulfadiazine compound.

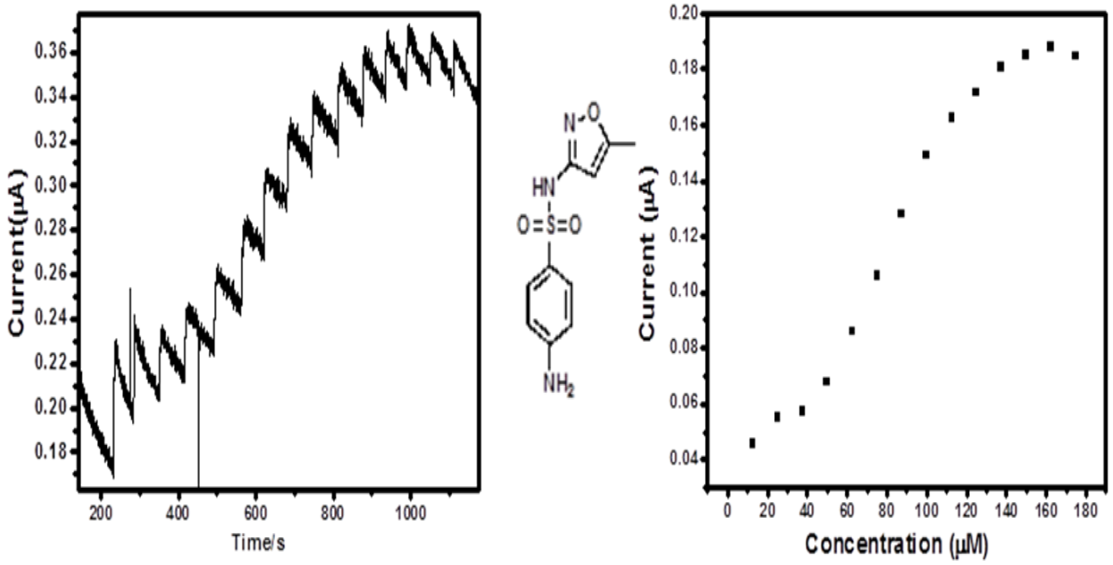


Figure 46. Amperometric response of screen-printed carbon PAA nanofiber modified electrodes after successive additions of sulfamethoxazole.

The amperometric current response of modified electrode when sulfamethoxazole was added at constant stirring showed initial increase then a decrease in current and linearly increased at higher concentrations until a point of saturation (**Figure 46)**. The curve shows S-shaped (sigmoidal shape) which is typical of a competitive binding interaction of the species at the electrode surface. This is typical in organic species with low adsorption ability therefore can cause minimal equilibrium coverage of analyte at the electrode surface. Sulfamethoxazole compound with azole functional group adsorbed poorly in the porous thin film.

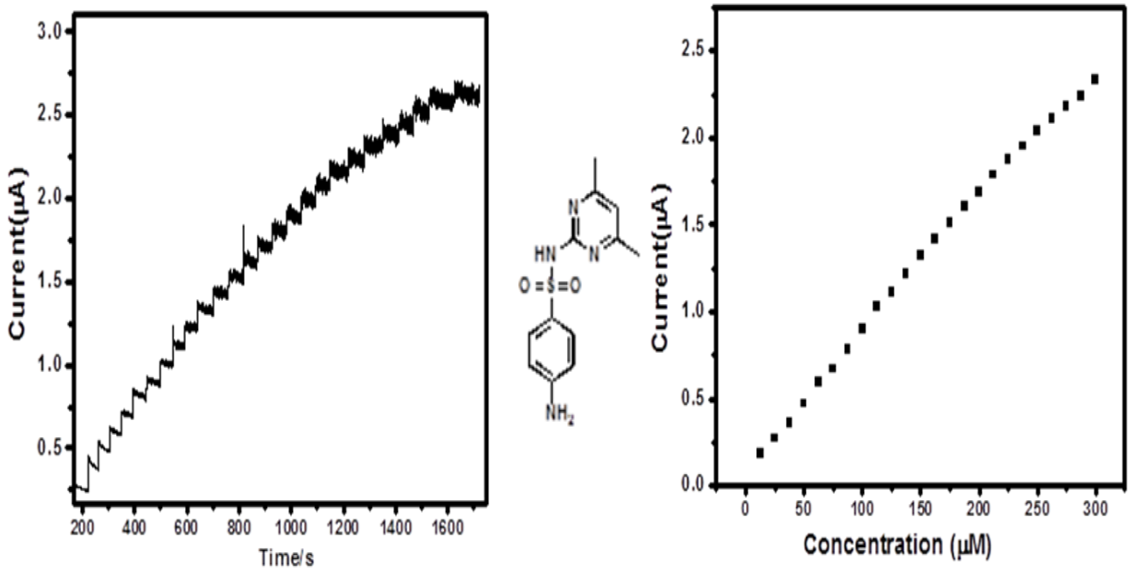


Figure 47. Amperometric response of screen-printed carbon PAA nanofiber modified electrodes after successive additions of sulfamethazine.

The amperometric current response of PAA nanofiber modified electrode when sulfamethoxazole was added at constant stirring showed initial increased linearly (**Figure 47).** The smooth linear response of the SDZ at the electrode are attributed to the withdrawing -methyl groups in the substituent. Based on the above calibration it evident that the data follows a linear calibration. The calibration curve for SMZ provided informative analysis in the determination of analytical parameters such as the LOD and sensitivity of the system.

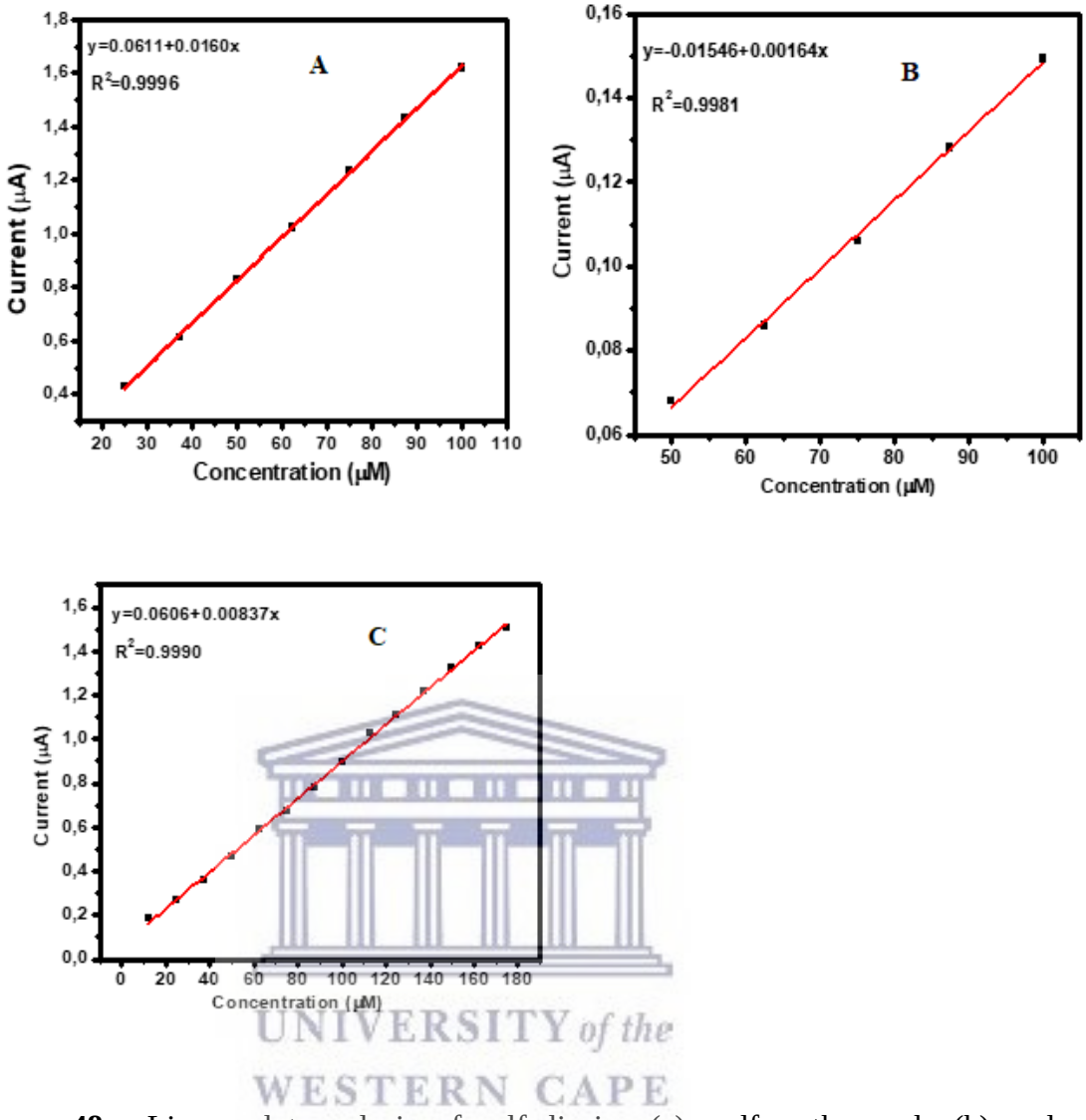


Figure 48. Linear plot analysis of sulfadiazine (a), sulfamethoxazole (b) and sulfamethazine (c).

The analytical parameters of the sulphonamides were determined based on the linear relationship between current and the concentration (**Figure 48**). The selected sulphonamides showed good linear proportionality of current and concentration with the following linear regressions: 0.9996, 0.9981 and 0.9990 for sulfadiazine, sulfamethoxazole and sulfamethazine respectively. The analytical parameters obtained from the calibration are summarized in the table below.

Table 16. Analytical data for detection of sulphonamides at PAA nanofiber sensor platform.

Sulphonamides	Linear range (µM)	LOD (µM)	LOQ (µM)	Sensitivity (µA µM)
Sulfadiazine	25-100	1.79	5.43	0.061
Sulfamethoxazole	50-100	5.49	16.65	0.0016
Sulfamethazine	12.5-175	3.06	9.27	0.061

Compared to square wave voltammetry, chronoamperometry was a more sensitive technique towards the detection of the selected sulphonamides at the PAA nanofiber modified screen-printed carbon electrodes. The results however were consistent terms of the analytical parameters obtained in both techniques, were the sulfadiazine had low detection limit of 1.79 µM followed by sulfamethazine with 3.06 µM and sulfamethoxazole had the highest limit of the detection of 5.49 µM with shorter linear range. The sensitivity determined from the slope of the calibration curves indicate that the nanofiber modified electrode was sensitive towards the detection of the examined sulphonamides. PAA nanofiber-based electrode provide a viable electrochemical sensor system with a great potential in sensor technology.

6.7 Conclusions

PAA based electro-catalysts were developed through the modification of screen-printed carbon electrodes with nano-structured polyamic acid method using two parallel approaches: (1) Electrochemical deposition of PAA thin film by cyclic voltammetry and (2) Deposition of PAA nanofibers by an electrospinning technique. The two electro catalysts were applied in parallel in electroanalytical reporting of sulfadiazine, sulfamethoxazole and sulfamethazine by square wave voltammetry (SWV). The anodic response of the selected sulphonamides occurs at the basic -NH_2 group. The analytical performance of the electro-catalysts varied from each analyte depending: (1) on the size and nature of the attached R and (2) physiochemical properties of the analyte. At the dense layered structure of PAA thin films (observed in the atomic force microscopy), the sulpha drugs electrochemical and analytical performance was similar, however the nanofiber modified electrode was sensitive with low limit of detection towards the determination of both sulfadiazine and sulfamethazine due to the adsorptive nature of the pyrimidine structure which accelerated the electron transfer between the electrode and the analyte. The analytical performance of the developed sensor platform based on the PAA nanofibers was found to be comparable to that of multi-walled carbon nanotubes in their application sulphonamides electro-analytical sensing. This can be attributed to their similar size and structure. The efficacy of the proposed nanostructured PAA thin film modified screen-printed carbon sensor was evaluated by performing recovery studies for the selected sulphonamides. The viability of the developed PAA nanostructured electrochemical electrodes in commercial sensor applications was tested using the chronoamperometry.

Chapter 7: Future perspectives; sulphonamide biosensors

This chapter discusses the preparation of the dihydropteroate synthase (DHPS) as possible enzymatic biosensor systems for sulphonamides.

7.1 Introduction

Enzymes are proteins functioning biological catalysts that speed up a biochemical reaction in living organisms by lowering the activation energy with changing its equilibrium, usually described as proteins with the ability to catalyse the conversion of substrate molecules into product molecules (Mantsala and Niemi, 2009; Robinson, 2015). Biochemical reactions *in situ* occur far from the equilibrium and do not produce mass balance between a fixed amount of substrate and the product, the added substrate is a function of time (Milanowski et al., 2013). Biological catalysts catalyze synthesis and break down of biochemical building blocks and macromolecules, transmission of genetic information and the conversion of chemical energy (Mantsala and Niemi, 2009). With development in the recombinant technology and protein engineering, enzymes have evolved as important biomolecules used in a variety of industrial and therapeutic applications (Gurung et al., 2013). Enzymes are used in various fields including technical applications, food manufacturing, feed industry cosmetics, medicine and as tools in research and development (Li et al., 2012). Microbial enzymes attract a lot of attention in commercial use due to their cost effectiveness, high yields and ease of product modification (Gurung et al., 2013). A four-part Enzyme Commission (EC) number is used to describe all known enzymes (**Table 16**). The first

three levels categorize the overall chemistry of enzyme and the fourth number is assigned to differentiate the substrate specificity. There is no correlation between the differences between catalysed reactions and numerical identifiers in the EC classification (Furnham et al., 2012).

Table 17. The six main classes of enzymes.

EC number (first digit)	Classification	Reaction
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Transfer functional groups/atoms
3.	Hydrolases	Hydrolysis
4.	Lyases	Add (or remove) elements of water, ammonia and carbon dioxide to form double bonds
5.	Isomerases	Re-arrangement of atoms with a molecule
6.	Ligases	Join two molecules

7.2 Enzymatic biosensors

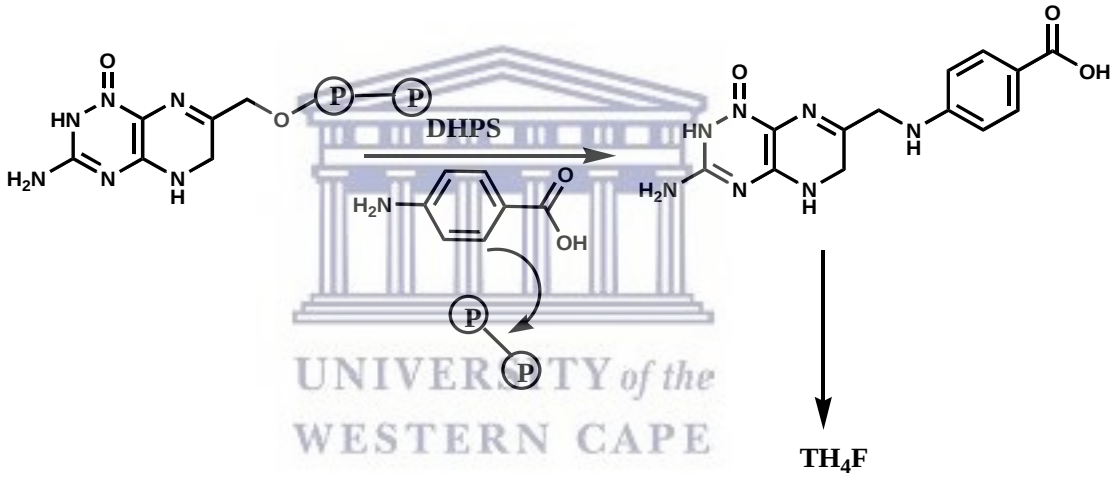
Redox enzymes also known as oxidoreductases constitute a large class of enzymes that catalyse biological oxidation and reduction reactions. Redox enzymes exhibit a wide variety of chemical and biochemical transformations involve oxidation/reduction process therefore developing practical biocatalytic applications redox enzymes in fields such as biotechnology has attracted a lot interest (May, 1999). The biological

capability of these enzymes has led to the creation of new generation of products and processes (Cock et al., 2009). The most popular product is a biosensor. A biosensor can be defined as an analytical sensing device which consists a recognition (bioreceptor) element incorporated with a suitable transducer which converts biological recognition reaction into a measurable signal (Freire et al., 2003). Oxidase type of proteins are usually used as recognition element, these proteins can selectively react with analytes thus consuming O_2 and producing H_2O_2 which can be detected. Detection of enzyme activation or inhibition by analyte and modification of the enzyme properties by analyte are two other mechanisms or processes that are based on enzyme biosensing (Hasan et al., 2009).

7.2 Dihydroperoate synthase (DHPS) as a possible recognition element for sulphonamide biosensors

Enzymatic electrochemical biosensors for sulfonamide detection have previously reported in the literature using oxidoreductases enzymes. Del torno-de Roman et al. (2015) developed enzymatic biosensor based on cross-liking tyrosinase with screen printed carbon electrode previously modified with gold nanoparticles. The biosensor was applied in sulfamethoxazole detection. The biosensor showed low selectivity and sensitivity with high concentration detection limit for sulfonamide. Carbonic anhydrase is metaloenzyme which catalyzes the hydration of CO_2 some aldehydes and hydrolysis of esters. Carbonic anhydrase is also reported in enzymatic biosensors in sulfonamide. Bourais et al. (2015) investigated the inhibition sulphonamides of carbonic anhydrase enzyme by multiphometric and electrochemical techniques. The electrochemical enzyme inhibition biosensor, based on carbonic anhydrase trapped in a carbon paste electrode using carbon black nanoparticles and solid paraffin was applied in determination of sulfanilamide.

Dihydropteroate synthase (DHPS, EC: 2.5.1.15) an enzyme which catalyzes the reaction of 6 hydroxymethyl-7,8-dihydropterin-pyrophosphate with p-aminobenzoic acid (p-ABA) to yield 7,8-dihydropteroate and pyrophosphate, is the only enzyme directly related to sulfonamide inhibition (Morgan et al., 2011). Dihydropteroate synthase (DHPS) is a key enzyme in the folate pathway of bacteria and primitive eukaryotes. Sulfonamides compete with PABA for the bacterial enzyme thereby preventing incorporation of PABA into dihydrofolic acid, the precursor of the folic acid which is required for the bacterial growth and the target of the sulfonamide class of antibacterials (Fernely et al., 2010).



Scheme 3. Schematic of dihydropteroate synthase (DHPS) catalyzed reactions within the folate biosynthetic pathway.

DHPS enzyme is described as a protein that forms α/β barrel structure, with a highly conserved binding pocket for recognition of the pterin substrate, DHPPP (6-hydroxymethyl-7, 8-dihydropterin pyrophosphate). Therefore, there is a fixed order of substrate binding: DHPPP binds first, followed by the second substrate, PABA (p-aminobenzoic acid) (levy et al., 2008). Binding of DHPPP also allows the enzyme to

recognize pABA or sulfonamide drugs, these drugs bind to the pABA substituent site and therefore inhibit product formation and/or form “dead-end” products with pterin (Henever et al., 2010), which act as pABA analogues.

Previously Vinicombe and Derrick (1999) demonstrated the fixed order substrate binding to DHPS by using PABA as substrate; in the absence of other ligands, they found that there was no detectable binding of PABA to DHPS. The equilibrium binding assays studied by the authors that showed binding of the substrate para-aminobenzoic acid (pABA) to DHPS was absolutely dependent on the presence of pyrophosphate, which acts as an analogue of the second substrate 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP). The product of the reaction, dihydropteroate, was also able to bind to DHPS. Sulphonamides were capable of displacing PABA in a competitive manner. The authors also showed that the target for sulphonamide inhibition of S. pneumoniae DHPS is the enzyme-DHPPP binary complex, rather than the apoprotein form of the enzyme. (Vinicombe and Derrick, 1999). Even though sulfa compounds take part in the DHPS reaction only to be converted to pterin adducts, they have similar reaction rates as PABA. In development of DHPS enzyme-based biosensors for screening of sulfonamides using DHPS inhibition, it is necessary to include 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) to form the DHPS-DHPPP binary complex. Wang et al. (2015) developed a highly sensitive and class-specific fluorescence assay for sulfonamide detection based on dihydropteroate synthase enzyme. Dihydropteridine pyrophosphate (DHPPP) was synthesised and used as the first substrate. In a recent study, a dihydropteroate synthase-based biosensor has been developed by Liang and co-authors. The biosensor based on was used for multi-screening of sulfonamide residues and compared to broad-specific antibody-based immunoassay by molecular

modelling analysis. The DHPS-DHPPP binary complex-based biosensor was highly sensitive towards the detection of class sulphonamides (Liang et al., 2019).

7.3 Substrate investigation

As clearly stated in the literature, 6-hydroxymethyl-7, 8-dihydropterin pyrophosphate (DHPPP) is the first substrate that binds to the DHPS enzyme and cleaved in the pyrophosphate site by PABA to form dihydropteroate leading to folic synthesis. Due to economic factors and limited research labs to source the DHPPP, in our work we investigated the possibility of cleaving thiamine pyrophosphate to form DHPS-ThPP which can be used to recognize or bind PABA or sulfonamide.

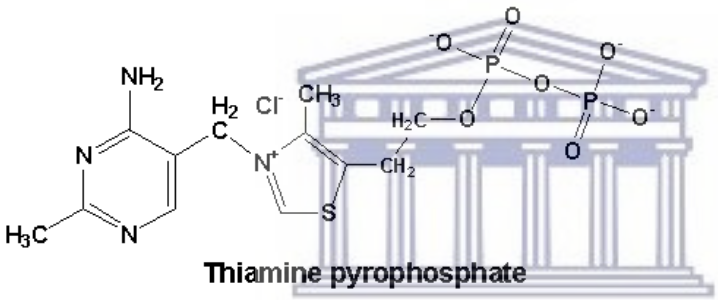


Figure 49. Chemical structure of thiamine pyrophosphate

7.3.1 Experiment details

Recombinant staphylococcus haemolyticus Dihydropteroate synthase (folp) (MBS1369200) expressed in *E. coli* in a clear liquid form was purchased from Mybiosource.com. The observed band sized (molecular weight) was 33 KDa. Long storage of the product -20 to -80 °C. For short term storage, the product was stored in 2 to 8 °C, one week from the date of receipt. The concentration of the product determined by the Bradford method was 0.3 mg/ml in 20 mM Tris-HCl, 0.5 NaCl, pH 8, 20% glycerol and was further diluted for experiments. Thiamine pyrophosphate (ThPP) was purchased from Sigma-Aldrich. Ultra-violet (UV-vis) which commonly provides information about the changes in electronic energy levels arising within the

molecule due to transfer of electrons from π- and non-bonding orbitals was the preferred technique for the investigation studies. The analysis was performed on a NICOLET evolution 100 UV-vis instrument.

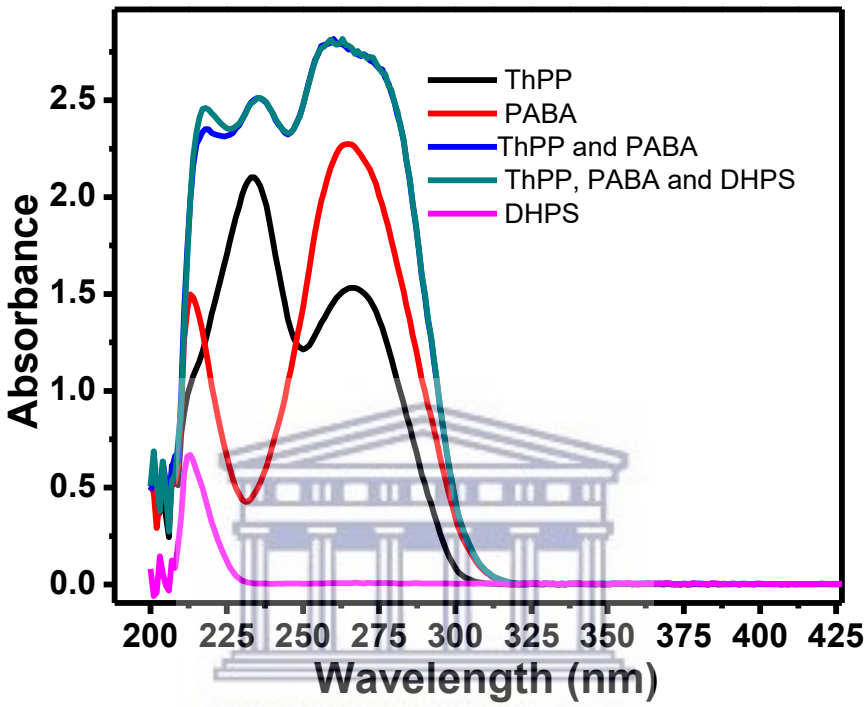


Figure 50. UV-vis spectrum representing investigation of PABA DHPS inhibition using thiamine pyrophosphate as a first substrate in place of DHPPP.

The above figure illustrates the attempt to inhibit DHPS with PABA using thiamine pyrophosphate as the first substrate in place of DHPPP. ThPP, PABA and DHPS were analysed in independently as the part of the investigation. ThPP absorbed at 233 nm and 265 nm, the absorbance peak of DHPS was observed at 212 nm, two absorbance peaks were observed for PABA at 212 nm and 235 nm. An equal mole ratio (20: 20) of PABA and ThPP were mixed in single cuvette and allowed to react for 5 to 10 minutes. No chemical changes were observed in the absorbance. The spectrum represents a physical mixture of PABA and ThPP were absorbance peaks were

observed at 217 nm, 235 nm and broad peak at 258-275 nm. The same reaction was repeated with DHPS and no chemical changes were observed.

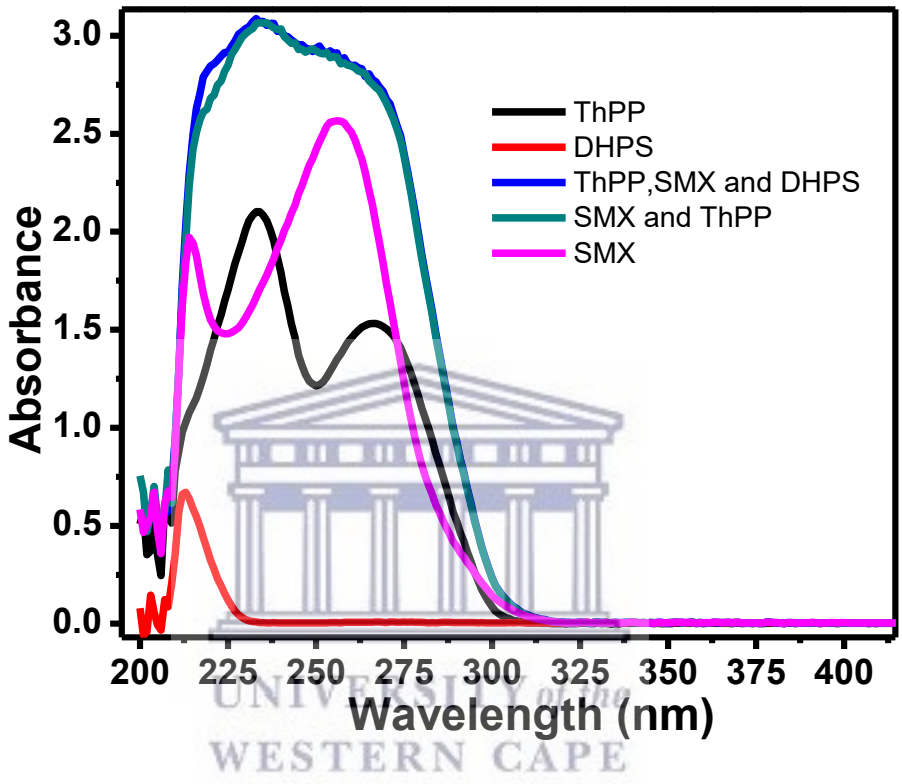


Figure 51. UV-vis spectrum representing investigation of SMX in DHPS inhibition using thiamine pyrophosphate as a first substrate in place of DHPPP.

The above spectrum represents the DHPS inhibition with SMX using thiamine pyrophosphate as the first substrate in place of DHPPP. The same procedure was followed as previously stated in this case SMX replaced its PABA analogue. ThPP and DHPS absorbs at wavelengths stated above. SMX absorbed at 214 nm and 255 nm illustrated similar absorption with its analogue (PABA). The mixture of SMX and ThPP did not yield any new peaks to indicate any chemical changes in the molecules. The same was observed when the DHPS was added to reaction. ThPP an inorganic

analogue of DHPPP however does not possess the pterin structure which plays an important role in the cleaving and displacing of pyrophosphate to form the dihydropteroate or pterin-sulfonamide adduct. DHPS based biosensors for sulphonamides detection require the use of the DHPPP substrate, therefore DHPS would not be a suitable for substrate immobilized biosensor development however as demonstrated in the literature other formats of sensor development could be explored by following the synthesis steps and incorporating the necessary substrates.

Chapter 8: Conclusion and future work

8.1 Conclusion

Analytical reporting of these pharmaceuticals will play an important role in the creation of regulations and policies for pharmaceuticals in water systems in South Africa and other developing countries, therefore there is a need to develop cost-effective analytical chemistry techniques that can able to sense and monitor the concentration levels of these type of antibiotic classes in the natural environment. Electrochemical sensors are set to provide fast, low cost, portable and reliable tools for early detection and quantification of these pharmaceuticals. A new design of nanomaterials and electrodes to analytical screen and profile sulphonamides are needed.

Nanostructured polyamic acid (PAA) materials were prepared in two parallel methodologies. PAA thin films were prepared electrochemical deposition of PAA in an aqueous solution using cyclic voltammetry. Secondly PAA nanofibers were successfully produced using PVP as supporting polymer by an electrospinning technique. Polyamic acid (PAA) electrodeposited thin films and electrospun nanofibers were successfully characterized and electrochemically evaluated to determine electrochemical behavior of the nanostructured PAA modified screen carbon printed electrode. The produced polyamic acid (PAA) nanofibers were characterized using Fourier Transform infrared (FTIR) spectroscopy to study integrity of PAA functional groups as nanofibers by comparing them to the chemical

synthesized PAA. Scanning electron microscope (SEM) used to confirm the morphology of the produced nanofibers. Brunauer-Emmett-Teller (BET) was used to determine the surface area of the nanofibers. Atomic force microscopy (AFM) used to study the porousness, robustness and surface roughness of the nanofibers. Cyclic voltammetry was used to determine the number of electrons transferred in the system, the surface concentration of the deposited PAA thin film and PAA electrospun nanofibers and the diffusion coefficient (D_e) for the PAA thin film and nanofiber modified screen-printed electrode. The carrier polymer (PVP) did not influence the redox electrochemistry of the PAA as evidence by the pronounced electrochemical reporting signals obtained. The synthesized nanostructured PAA materials were used as electrocatalytic transducers in the electrochemical evaluation of selected sulphonamides. Sulfonamides are described as class of broad spectrum synthetic bacteriostatic antibiotics. Sulfonamide antibiotic residue are frequently found in all kind of environmental water, surface water, groundwater and waste water systems due to their solubility in water and demonstrate some chelating abilities. The environmental agencies (U.S EPA and E.U EPA) and health organizations (WHO) have described sulphonamides as one of the class antibiotics that listed as contaminants of emerging concern based on the threat to human and animal health. Sulfamethoxazole is the most detected sulfonamide antibiotic in the environment. Literature reports focused on carbon-based sensors for sulfonamide using cyclic voltammetry, square wave voltammetry and differential pulse voltammetry. In this work square wave voltammetry was the preferred technique to evaluate and analytical report on the surface interaction of the sulphonamides and the PAA nanomaterials (thin film and nanofibers) platform. Screening of the selected sulphonamides was conducted by at three different supporting electrolytes 0.1 M HCl, 0.1 M PBS and 0.1 M Tris-

HCL using square wave voltammetry. The electrochemical window selected for the oxidative SWV was at -1.0 to +1.5 V. A scan rate of 50 mV/s was achieved by a frequency of 10 Hz multiplied by the step potential of 5mV. SPCE was used as a working electrode in the three-electrode electrochemical cell. The three selected sulphonamides all produced the best peaks (+0.80 Vs in 0.1 M Tris-HCl, thus this supporting electrolyte presented optimal conditions due to the well-defined peak potentials and physiological pH value, thus presented a suitable medium for biosensor development.

The electrochemical screening of sulfadiazine at the PAA thin film platform produced a well-defined analytical peak potential at 0.77 V vs Ag/AgCl. The two other selected sulphonamides, sulfamethoxazole and sulfamethazine produced well defined analytical peaks at 0.82 V and 0.83 V vs Ag/AgCl respectively. The LOD (n=3) for sulfadiazine was found to be 12.14 µM with a correlation coefficient of 0.9950. The sensitivity of the system determined from the slope was found to be 0.042 µA/µM based on the linear method data fitting. The analytical information obtained in this work was compared to literature reports and the sensitivity obtained is greater than those from the literature. The literature reports by Hong et al. and Hong and Ma report a sensitivity for sulfadiazine using polymer based electrochemical sensors of 0.04 µA/µM (Hong et al., 2012) and 0.0054µA/µM (Hong and Ma, 2017). The LOD (n=3) for sulfamethoxazole was determined to be 14.59 µM with a correlation coefficient of 0.9928. The sensitivity determined from the linear plot for sulfamethoxazole detection was found to be 0.046 0 µA/µM. The sensitivity of the system for sulfamethoxazole detection in this work was high than the one reported (Özkorucuklu et al., 2008) for polymer electrochemical sensors for sulfamethoxazole and was detected at lower potentials. The PAA thin film sensor in this work was also used in electroanalytical

analysis of sulfamethazine. The LOD for sulfamethazine was found to be 10.41 µM correlation coefficient of 0.9963. The sensitivity of the system determined from the slope of the linear plot was 0.037 µA/µM. There are limited reports in literature for polymer based electrochemical sensors for sulfamethazine. The developed PAA thin film-based sensor in this study showed high sensitivity towards the detection of sulfamethazine.

The electrospinning technique provides with an opportunity to produce very thin layer of fibers in the nanometer range with large surface areas and ease of functionalization for applications such as electrochemical sensors and biosensors. Electrospun conducting polymer nanofibers can produce nanomaterials with a wide range of favourable properties which can be employed in variety of applications.

Electrospun PAA nanofiber-based sensor platform was used for the evaluation of the selected sulphonamides in 0.1 M tris-HCl. Sulfadiazine, sulfamethoxaole and sulfamethazine displayed well defined peaks. SDZ, SMX and SMZ produced well-defined analytical signals at 0.79 V, 0.81 V and 0.78 V vs Ag/AgCl respectively using square wave voltammetry and as a result was the peaks were quantitatively determined. The calibration data for individual sulphonamide standards using linear fitted plot was used to determine the LOD values for the selected sulphonamides. The LOD (n=3) values for the individual sulphonamides were 8.26 µM, 16.59 µM and 8.81 µM for sulfadiazine, sulfamethoxazole and sulfamethazine respectively. The linear plot provides high sensitivities (from the slope of linear region) of 0.055, 0.058 and 0.059 µA/µM respectively. The linearity correlation coefficient (R^2) was determined to be 0.9977, 0.9956 and 0.9974 respectively. Linear reports for sulfadiazine included Hong et al., 2010) and Fatouhi et al., 2013 both with determined sensitivities of 0.04 µA/µM and 0.03 µA/µM respectively reported at nanofibrous sensor materials (multi-

walled carbon nanotubes). Limited reports of sulfamethoxazole determined at similar electrode platforms. Literature report of sulfamethazine detection includes Fotouhi and Zabeti. 2014 with determined sensitivity of 0.057 µA/µM. An overall comparison of the sensitivity of carbon-based nanomaterial electrodes sulphonamide detection showed that carbon nanotubes and PAA electrospun nanofibers out-performed nanoparticles, molecularly imprinted method and polymer blends used in the preparation of transducers for the detection of sulphonamides. Future work will focus on developing enzymatic biosensor for sulfonamide detection. The sensor will be based on the dihydropteroate synthase inhibition of sulphonamides. The biosensor will take full advantage of the easy of immobilisation of PAA nanofibers by immobilising DHPS-DHPP binary complex at the electrode previously modified with PAA nanofibers to enhance the detection of these sulphonamides reported on this work. In our work, the DHPS enzyme reactivity and synthetic pathway was studied as a preliminary exploration towards developing biosensor systems in the future work.

The recoveries of sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfamethazine (SMZ) conducted in tap water, to simulate environmental matrix using square wave voltammetry (SWV) at PAA thin film modified screen-printed carbon electrode. The recoveries for SMZ with respect to each concentration were 184% (RSD 8.19%), 109.10% (RSD 18.62%) and 90.26 (RSD 18.26%). The inter-coefficient recoveries for the studied sulphonamides ranged from 98.84 to 154.17% with an acceptable relative standard deviation of 4.98-18.62%) indicating reliability of analytical results. Commercial biosensors are currently based on amperometric techniques. The viability of the developed PAA nanostructured electrochemical electrodes in sensor applications was tested using the chronoamperometry. The amperometric current-time response was recorded at both the PAA thin film and nanofiber modified commercial

screen-printed carbon electrodes. In comparison to square wave voltammetry, chronoamperometry is more sensitive towards the detection of the studied analytes at the PAA thin film and nanofiber modified screen-printed carbon electrodes. The results show that the electrochemical behaviour in terms of analytical data obtained of the selected sulphonamides at both (thin film and nanofibers) developed electrocatalytic electrodes was consistent when using either of the electrochemical techniques.

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