MOLECULARLY IMPRINTED POLYMERS FOR DETECTION OF VOLATILE ORGANICS ASSOCIATED WITH FUEL COMBUSTION.

By

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KEY WORDS

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Fuel combustion

Linear dynamic range

Limit of detection (LOD)

Limit of quantification (LOQ)

Polymers

Polypyrrole (Ppy)

Polymerization

Polycyclic aromatic hydrocarbons (PAH)

Pyrene

Sensitivity

Square wave voltammetry (SWV)

Scanning electron microscopy (SEM)

Ultra-violet visible spectroscopy

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ABSTRACT

Pollutants such as polycyclic aromatic hydrocarbons (PAHs) are known for their toxic effects which may lead to the cause of degenerative diseases in both humans and animals. PAHs are widespread in the environment, and may be found in water, food, automotive industry and petrochemical industries to name but a few sources. Literature reports have highlighted industrial workplace exposure to PAHs as a leading cause for development of cancer in workers. Particularly, workers in the petrochemical industry are adversely affected and the incidence of skin and lung cancer in this population group is high.

The United States of America in its guidelines developed by environmental protection agency (EPA) has identified 18 PAHs as priority pollutants. Among these are anthracene, benzo[a]pyrene and pyrene which have been selected as the focal point of this study due to their significance in the petrochemical industry.

Due to the carcinogenic and mutagenic properties reported in literature for certain PAHs, there have been monitoring procedures taken in most countries around the world. The commonly used analytical methods for the detection of PAHs from industrial samples are high performance liquid chromatography (HPLC) coupled to fluorescence detection, membrane filtration, ozonation and reverse osmosis. Analysis of PAHs from the petrochemical industry is typically performed by HPLC method as well as sono-degradation in the presence of oxygen and hydrogen peroxide.

Electrochemical sensors have emerged as a viable alternative methodology for analytical detection of a wide range for environmental pollutants due to their cost effective nature, ease of production and use, high selectivity, sensitivity and stability. By comparison HPLC methods are cost intensive and require specialized operational conditions, which limit their applicability.
In this work we evaluate the use of electrochemical sensors based on molecularly imprinted polymers. Molecularly imprinted polymers dates back to the 1970's with the incredible property of molecular memory imprinted to the polymer, during its preparation, which gives it ability to high selectively towards the target.

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Polypyrrole (Ppy) is a widely used polymer for electrochemical sensors. The polymer was electrochemically synthesized on a glassy carbon electrode using 5 cycles at a scan rate of 50 mV/s in a 0.1 M HCl solution and characterized using cyclic voltammetry. For the imprinting the template molecules (anthracene, benzo[a]pyrene and pyrene) were included during the electropolymerization and removed afterwards. The imprinted polymer was characterized using cyclic voltammetry (CV), square-wave voltammetry (SWV) and ultraviolet spectroscopy was used to characterize the target molecules. Atomic frequency microscopy (AFM) and scanning electron microscopy (SEM) were used to confirm the 'recognition cavity' created on the polymer surface.

Non-imprinted polymers (NIP) sensors were also prepared for comparison with the imprinted polymer sensor. The NIP sensors produced a limit of detection of 4.33×10^{-7} M, 1.59×10^{-7} M and 4.23×10^{-7} M for anthracene, benzo[a]pyrene and pyrene respectively (S/N =3).

On the other hand the calibration curves obtained from the MIP detection showed a limit of detection of 1.48×10^{-7} M, 2.51×10^{-7} M and 2.28×10^{-7} M for anthracene, benzo[a]pyrene and pyrene respectively (S/N=3). The use of microscopic techniques to investigate the topography of the sensor, AFM and SEM has confirmed the creation of recognition cavities from the polymer surface of the sensor.

The MIP sensors displayed a superior sensitivity towards the target analytes, due to their inherent molecular memory. The sensitivity of MIP sensors was 2.493 A/M, 2.409 A/M and 32.528 A/M for anthracene, benzo[a]pyrene and pyrene. The analytical performance of the MIP sensors compares favorably to literature reports for PAHs detection by electrochemical sensors, in petrochemical analysis.

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DECLARATION

I hereby declare that, the information embodied in this study **"molecularly imprinted polymers for detection of volatile organics associated with fuel combustion"** and the results of this investigation were carried out by me under the supervision of Prof Priscilla Baker, at the University of the Western Cape, Chemistry department, South Africa. Additionally my work has not been submitted elsewhere for the award of any degree or diploma and all the sources used in this work have been acknowledged by means of complete reference.

Olwethu Ngwanya   February 2017

Signed…………………………..

Supervisor: Prof PGL Baker

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I first would love to thank God, the almighty for all his mercies through bad and good times he has been there for me. Even when I felt hopeless and defeated, he would grant me strength, perseverance and wisdom.

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To the Sensorlab group and the chemistry department as a whole, I thank you for the love and support. For a period of more than six years you have been my home, away from home.

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I sing praises to the deceased great man and woman of the Ngwanya family, ooBhele!, ooPhutha-phutha!, ooKhuboni! ooMpofana, I say '*ukwanda kwaliwa ngumthakathi*!'

Lastly, I would like to thank the National Research Fund (NRF) along with Sensorlab group for funding my postgraduate studies, without your financial support, this may have not been possible.

DEDICATION

I dedicate this project to all my beloved close friends and family, especially;
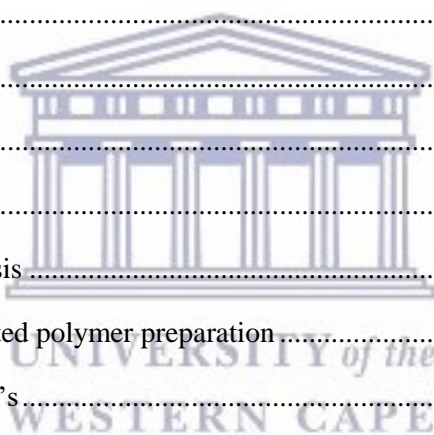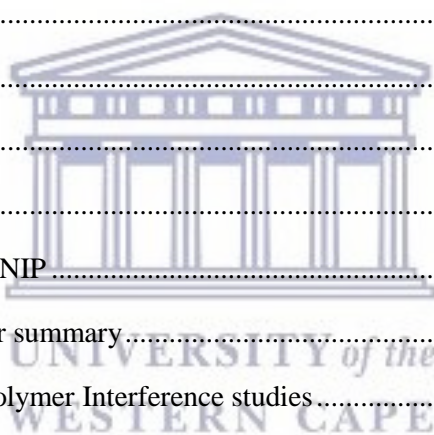
My daughter

Ayathandwa Dyubele

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ABREVIATIONS

A	Ampere
AFM	Atomic force microscopy
BaP	Benzo[a]pyrene
BPA	Bisphenol A
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
EPA	Environmental protection agency
GC	Glassy carbon
HCl	Hydrochloric acid
IARC	International agency for research on cancer
LOD	Limit of detection
LOQ	Limit of quantification
MIP	Molecularly imprinted polymers
mA	Milliampere
μA	Microampere
MIT	Molecularly imprinting technology
M	Molar
mV/s	Millivolts per second
PPy	Polypyrrole
UV	Ultra-violet
SPCE	Screen printed carbon electrode
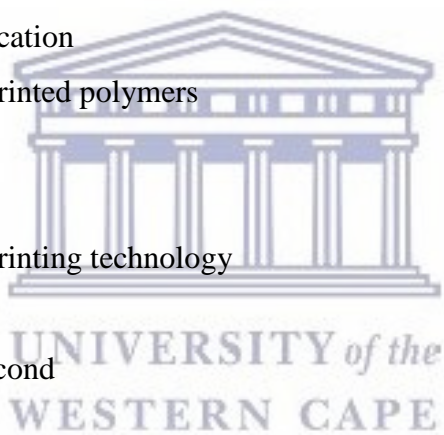
Chapter 1

Polycyclic aromatic hydrocarbons (PAH) are environmental pollutants formed at high temperatures and under pyrolytic conditions during incomplete combustion of organic matter. This chapter provides an introduction to the properties and synthetic methods associated to PAH. The impact of PAH in the environment, on both human and animal wellbeing, will be addressed.

1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) also known as polynuclear compounds are a class of complex organic chemicals, that incorporates carbon and hydrogen in a fused ring structure that have at least 2 benzene rings[1]. PAH are mostly known to be amongst the class of environmental pollutants. There are two known main sources of PAHs emission i.e natural and anthropogenic emission. Anthropogenic emissions of PAHs in the environment are established to be more significant than natural emissions. Anthropogenic emissions occurs from combustion of materials such as fossil fuel-burning, motor vehicle, waste incinerator, oil refining, coke and asphalt production, and aluminum production[2]. Natural emission of PAHs includes forest and rangeland fires and oils seeps.
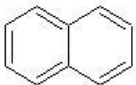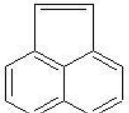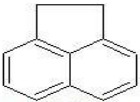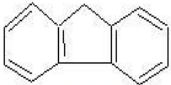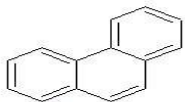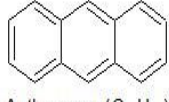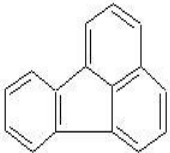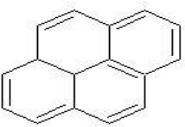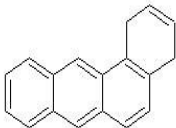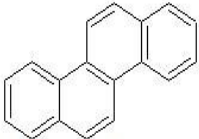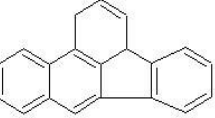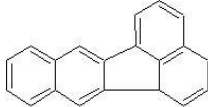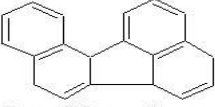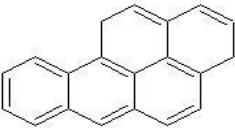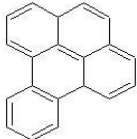
In general PAHs are formed as a result of pyrolytic process, more especially the incomplete combustion of organic materials during industrial and other human activities. In a process called pyrolysis, pyrogenic PAHs are formed where organic substances are exposed to high temperatures under low oxygen or no oxygen conditions[3]. Some of the most well-known pyrolytic processes are destructive distillation of coal into coke and coal tar, or thermal cracking of petroleum residuals into lighter hydrocarbons. The most suitable temperature conditions for pyrolysis are ranging from about 350℃ to more than 1200℃. During pyrolysis PAHs which are formed include benzo[a]pyrene, pyrene, anthracene, penanthrene, and other PAH derivatives. Most of the PAHs mentioned above are found in urban and industrial areas.

PAH are also formed during crude oil maturation and other similar processes which are referred to as petrogenic. Some petrogenic PAHs are also pyrogenic PAHs like benzo[a]pyrene and 1-

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hydroxypyrene. These PAHs are common due to widespread transportation, and use of crude oil and crude oil products. As much as it is well known that PAHs are formed due to incomplete combustion, they are also found in petroleum products[3].

Literature shows that, although there are many PAHs most regulations, analysis, and data reporting focus only on a limited due to their potent threats to both the environment and humans. The USA's environmental protection agency (EPA) have put together a list of 18 PAHs as priority pollutants [4]. This list was created to classify PAHs according to the toxicity, carcinogenicity and mutagenicity in both humans and animals.

Napthalene ($C_{10}H_8$)	Acenapthylene ($C_{12}H_8$)	Acenaphthene ($C_{12}H_{10}$)
Fluorene ($C_{13}H_{10}$)	Penanthrene ($C_{14}H_{10}$)	Anthracene ($C_{14}H_{10}$)
Fluoranthene ($C_{16}H_{10}$)	Pyrene ($C_{16}H_{10}$)	Benzo[a]anthracene ($C_{18}H_{12}$)
Chrysene ($C_{18}H_{12}$)	Benzo[b]fluoranthene ($C_{20}H_{12}$)	Benzo[k]fluoranthene ($C_{20}H_{12}$)
Benzo[j]fluoranthene ($C_{20}H_{12}$)	Benzo[a]pyrene ($C_{20}H_{12}$)	Benzo[e]pyrene ($C_{20}H_{12}$)

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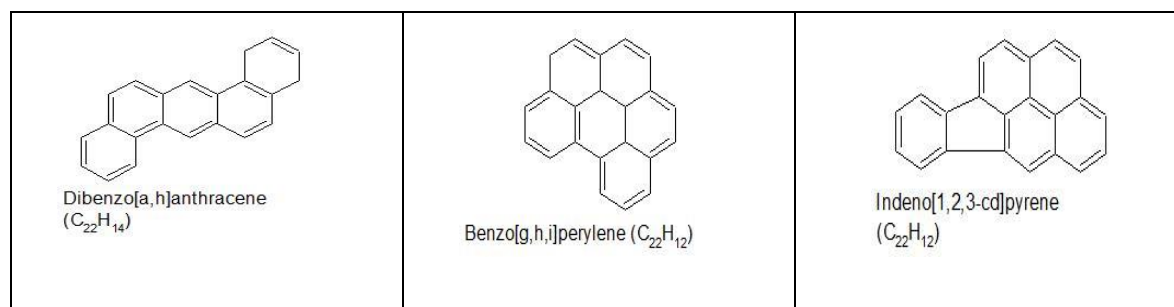
Figure 1: List and structures of PAHs identified by the USA's EPA, these are the most PAHs known to have a negative effect on the environment [4].

Polycyclic aromatic hydrocarbons containing up to six fused rings are commonly known as 'small' PAHs, and those containing more than six are known as 'larger' PAHs[3]. Low molecular weights PAHs with two to three aromatic rings are mostly present in gaseous phase, while the ones with four or more rings are linked with atmospheric particulate matter. In most instances research has been done on small PAHs due to the availability of samples. The general characteristics of PAHs are high melting and boiling point, low vapor pressure and low aqueous solubility. The latter two characteristics are said to decrease with an increasing molecular weight of the PAHs[5]. However the resistance to oxidation and reduction increases at low vapor pressure and low aqueous solubility. The aqueous solubility of PAHs decreases upon an additional ring. The temperature during formation of PAHs regulates the degree of substitution on PAHs molecules[6]. Literature shows that at high temperatures unsubstituted PAHs are likely obtained, at intermediate temperatures a mixture of both substituted and unsubstituted PAHs are formed, and then at low temperatures, typically of those of crude oil, a large amount of substituted PAHs are formed.

Out of the PAHs listed above this study's main focus is in anthracene, benzo[a]pyrene (BaP) and pyrene. These PAHs will be our target molecules since they are well-known for their toxicity resulting from incomplete combustion in petrochemical environment. The emission of these PAHs from petrochemical industry typically ends up in the waste water. Workers on these oil plants are exposed to elevated levels of PAHs in their work environment [7]. In the year 2000 the Federal Republic of Germany reported the amount of benzo[a]pyrene emitted to the air to be roughly about 30% caused by coke production, 56% by heating with coal, 13% by motor

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vehicles and less than 0.5% by combustion of heating oil and coal-fired power generation[7]. Benzo[a]pyrene is a PAH with five fused rings, and it is known to occur due to incomplete combustion of organic matter at temperatures between 300℃ and 600℃. The material is found in coal tar, tobacco smokes and certain types of foods. Benzo[a]pyrene is listed as group 1 carcinogen by the international agency for research on cancer (IARC)[8]

Anthracene and penanthrene are identified as the simplest PAHs by the IARC. In general anthracene is a solid PAHs consisting of three fused rings. It is a component of coal tar, such that coal tar remains as a major source of this material. On the other hand pyrene is a four membered ring PAHs which is also formed during incomplete combustion of organic matter. Similarly to benzo[a]pyrene and anthracene, pyrene also comes from coal tar and automobile exhausts.

1.1   Chemical and physical properties of PAH

Polycyclic aromatic hydrocarbons that consist of 4 benzene rings or more are known to have relatively low solubility in water and low vapor pressure, however they are highly lipophilic. Most of the PAHs with low vapor pressure are said to adsorb themselves on particle surfaces. For an example the well-known PAHs benzo[a]pyrene (BaP), is a 5 membered ring molecule. Because of their low vapor pressure, some PAHs are present at ambient temperature in air, both as gas and associated with particles. The lighter PAHs such as penanthrene are found exclusively in gaseous phase, whereas the heavier ones like BaP are almost totally adsorbed on to particles[9]. In the atmosphere PAHs can react with pollutants like ozone, nitrogen oxides and sulfur dioxides, which in turn will result to diones, nitro- and dinitro PAHs and sulfonic acids[5]. PAHs are often resistant to biological degradation moreover they are found not to be efficiently removed by conventional physicochemical methods such as coagulation, flocculation, sedimentation, filtration or ozonation[2]. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water and how easily they evaporate into the air. The World Health Organization (WHO) reports that some of the PAHs are said to evaporate into the atmosphere from surface waters, but most likely stick to solid particles and settle to the bottom of rivers or lakes[3]. In addition some PAHs evaporate from soil surface to air.

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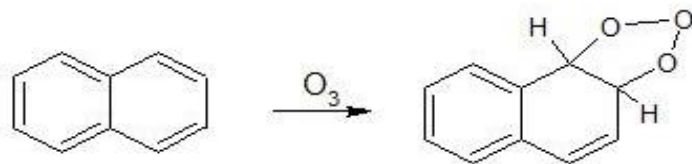
Figure 2: Illustrates the reaction mechanism of PAH with ozone in the atmosphere.

PAHs may be synthesized from saturated hydro-carbons under oxygen-deficient conditions. Moreover two other ways to account for the formation of PAHs are pyrosynthesis and pyrolysis. Low hydrocarbons form PAHs by means of pyrosynthesis. When temperatures exceed 500°C, the carbon-hydrogen and carbon-carbon bonds are broken to form free radicals. These radicals combine to acetylene which further condenses with aromatic ring structures, which are resistant to thermal degradation[9]. Figure 3 below shows the formation of such rings with ethane as a starting material.
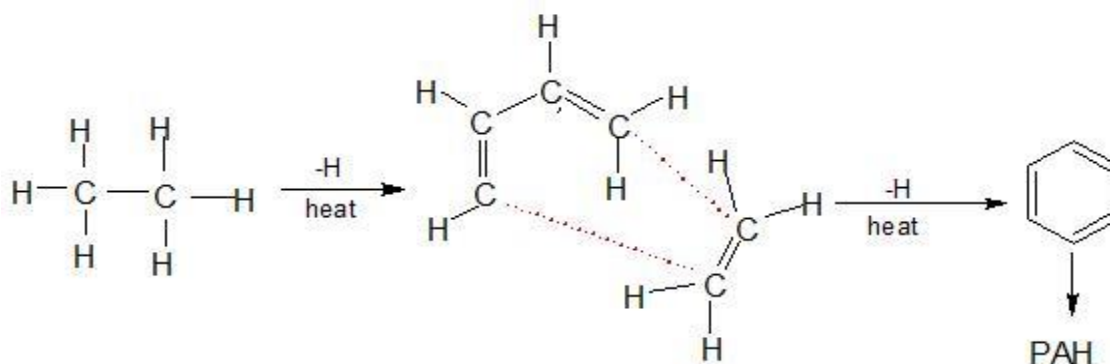
Figure 3: Pyrosynthesis reaction mechanism of PAHs with ethane as a starting material.

The ability of hydrocarbons to form PAHs structure is dependent on the structure of the starting material in the following order aromatics> cycloolefins> olefins> parafins. There are three other suggested possible mechanisms of PAHs formation during combustion, i.e slow Diels-Alder condensation, rapid radical reactions, and ionic reaction mechanism[10]. However, the radical formation mechanism is favored as the combustion process within the internal combustion engine has to occur very quickly. The spontaneous gaseous hydrocarbons radical rearrangement is providing the mechanism of PAHs formation and growth.

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1.2   Carcinogenicity of PAHs

Animal studies have shown an increase in the incidences of skin, lung, bladder, liver, and stomach cancers, as well as injection-site sarcomas, due to exposure to PAHs. As the molecular weight of the PAHs increases, the carcinogenicity of the PAHs also increases, and the acute toxicity decreases[9]. Consequently, due to the range in molecular weight of PAHs, several of the physicochemical properties that are critical to their biological activity also vary greatly[4]. The properties that influence the biological activity of PAHs include; vapor pressure, adsorption on surfaces of solid carrier particles, absorption into liquid carriers, lipid-aqueous partition coefficient in tissues, and limits of solubility in the lipid and aqueous phases of tissues. In effect, these properties are intimately linked with the metabolic activation of the most toxic PAHs hence it becomes essentials to understand the nature of this interaction especially with respect to their deposition and disposition[7].

Literature shows that PAHs exhibit carcinogenic action through adduct forming mechanism (figure 4) [7]. In general, PAHs are converted to oxides and dihydrodiols, which in turn are oxidized to diol epoxides. Both oxides and diol epoxides are ultimate DNA-reactive metabolites. PAHs oxides can form stable DNA adducts, and diol epoxides can form stable and depurinating adducts with DNA through formation of electrophilic carbonium ions. Most of the 16 listed PAHs have been shown to be initiators of skin cancer according to The International Agency for Research on Cancer. Carcinogenic effects of PAHs are due to their ability to bind to DNA, with literature showing positive correlation between levels of PAH-DNA adduct formation in different organs and PAH levels[8].

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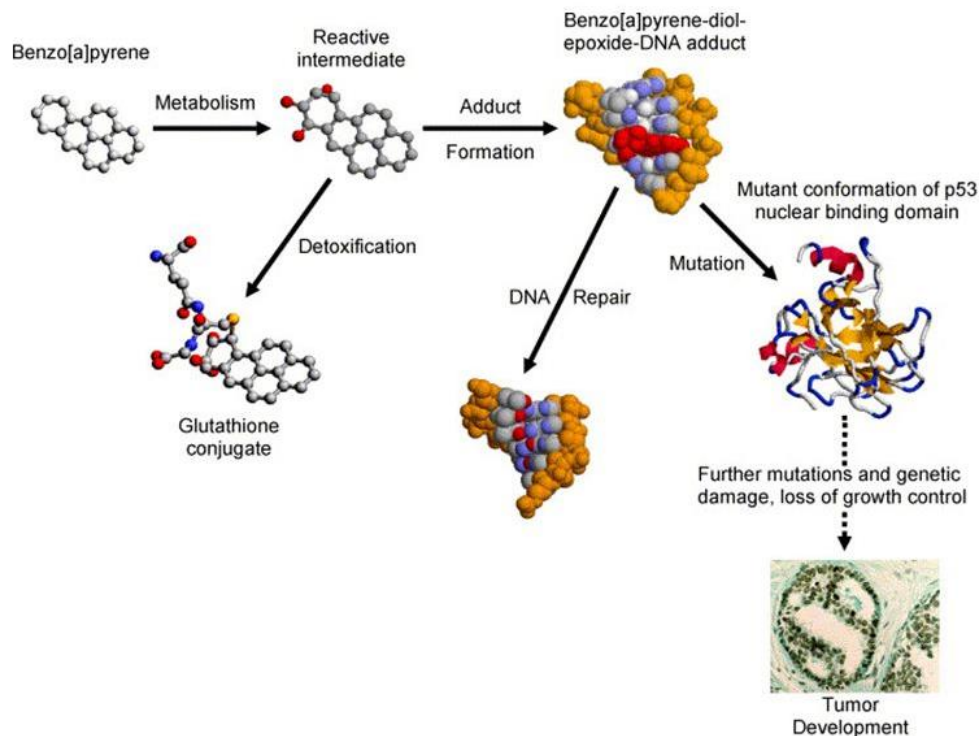
Figure 4: Schematic diagram showing the mechanism through which exposure to PAHs is thought to cause cancer.

Other PAHs which are known to be strongly carcinogenic and teratogenic (capability of interfering with development of a fetus causing birth defects) include benz[a]anthracene and chrysene [$C_{18}H_{12}$];benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene and B[a]P [$C_{20}H_{12}$]; indeno[1,2,3-cd]pyrene [$C_{22}H_{12}$]; and dibenz[a,h]anthracene[$C_{20}H_{14}$] . Animal studies show that certain PAHs can also affect the haematopoietic and immune systems resulting in reproductive, neurologic and developmental disorders. It has been reported that it is difficult to ascribe observed health effects to specific PAHs because most exposures are to PAHs mixtures[11]. The earliest human PAH-related epidemiologic breakthrough was reported in 1936 by researchers in Japan and England who studied lung cancer mortality among workers in coal carbonization and gasification processes. Thereafter USA made studies among coke oven workers which further confirmed excessive lung cancer mortality, with the suggestion of excessive genito-urinary system cancer mortality.

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There have been some studies examining the effects of exposure to PAHs via the respiratory route on patience with confirmed lung cancer. A long term inhalation of pure BaP at concentration of 10 mg/m^3, cancer of the respiratory tract was said to occur in 35% of golden hamsters, additionally a substantial number of tumours of the gastrointestinal tract were found[6]. On the other hand the tumorigenicity (tendency of producing tumors) of the inhaled benzo[a]pyrene (10 mg/m^3) in Syrian golden hamsters was enhanced by coexposure to sulfur dioxide (172 ppm)[7]. It has been discovered that the carcinogenic potencies per unit dose of tested PAHs vary widely although lack of quantitative data permits a firm evaluation. In the rat lung implantation examination using a beeswax/trioctanoin mixture as vehicle, the carcinogenic potencies of phenanthrene, chrysene, dibenz[a,h]anthracene and benzo[b]naphtho[2,1-d]thiophene relative to benzo[a]pyrene (1.00) were 0.001, 0.03, 1.91 and 0.02, respectively. Carcinogenicity of PAH-containing emission condensates was given a close look. When fractions of condensate from coal-stove and brown-coal emissions and emissions from petrol-driven vehicles were tested on mouse skin, most of the carcinogenicity originated from the fraction containing PAHs with 4–7 rings. This fraction represents only a small proportion by weight of the condensate. The amount of BaP, which varied from 0.414 to 0.702 mg per gram of condensate accounted for 6–15% of the total carcinogenicity [7][12]. Moreover it was further discovered that after implantation of condensate from coal-stove emissions, diesel exhaust and emissions from petrol-driven vehicles into the lungs of rats, the fraction containing PAHs with 3 or more rings accounted for 68–81% of the total carcinogenicity. The amount of BaP in the condensate, which varied from 0.483 mg/g in the motor vehicle condensate to 1.14 mg/g in the flue-gas condensate accounted for, respectively,1.4% and 2.4% of the total carcinogenicity[5][6][7].

Carcinogenicity of diesel particulates were also investigated and it was discovered that they caused an increased incidence of lung tumours on rats which were exposed for two or more years to high level of diesel exhaust[12]. When male and female rats were exposed for up to 30 months (7hrs/day, 5days/week) to diesel engine exhaust at soot concentrations of 0.35, 3.5 or 7.0 mg/m^3, the prevalence of lung tumours was drastically increased. Additionally it was observed that DNA-adduct levels accumulated slowly during a 12-week exposure and then levels declined rapidly after the termination of exposure[12].

1.3 Human and animal health and wellbeing due to the PAH exposure

Humans are exposed to PAHs by means of inhalation, ingestion and skin contact. Non-occupational respiratory exposure is mainly from tobacco smoke while the major sources of ingested PAHs are drinking water and cooked food. In petrochemical industries worker's urines have been found to contain BaP, pyrene and 1-hydroxypyrene which they obtained from the air during coke production. PAHs are also a threat to animals in laboratory which are exposed either by food of inhalation, because exposed animals develop tumors. On the other hand pregnant mice who are exposed to BaP experience reproductive difficulties[7]. A number of these PAHs even end up in the aquatic environment via sewage, atmospheric deposition, the discharge of municipal/industrial effluents and agricultural runoffs[13]. Once PAHs invades the aquatic environment they look like an oil spill. The aquatic life would then be subjected to similar dangers as other animals on land leading to fatality.

Hussein I et al [3] have reported on a respiratory health of 667 workers in a rubber factory. Respiratory health was evaluated and examined for workers in different sections of the factory i.e offices, outdoors and in production area. When different areas were examined results showed that workers in the compounding section were the most affected and that is due to the highest exposure to particulate matter and benzo[a]pyrene. Workers on the other parts of the factory were affected on a lesser degree and only a few were affected. Some of the workers on other areas of the factory experienced what is called sick building syndrome, some felt dizziness and fatigue which are symptoms of PAHs exposure. Other symptoms of PAH exposure includes Shortness of breath, swollen ankles or legs and fainting.

The USA's EPA science inventory [12] has proclaimed that children are at risk since PAHs have been frequently detected at their homes and day care centers and it is indicated that they may pose health concerns due to their carcinogenicity. Also PAHs are present indoors and outdoors therefore children may be exposed to chronic low levels of PAHs in these microenvironment.

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1.4 Thesis layout

In this thesis, we will explore the use of molecularly imprinted polypyrrole for the selective detection of anthracene, benzo[a]pyrene and pyrene. We will adopt an electrochemical protocol supported by atomic force microscopy (AFM), scanning electron microscopy (SEM) and Ultra-violet spectroscopy.

1.4.1 Aim 1

In this study a molecularly imprinted polymer based chemical sensor is developed for the recognition of PAHs. A non-imprinted polymer (NIP) based sensor is developed and used to detect Anthracene, benzo[a]pyrene and pyrene. Secondly a molecularly imprinted polymer (MIP) based sensor is developed with recognition cavities for the specific analytes of interest and used for the detection of the PAH, thereafter results from NIP and MIP are compared.

1.4.2 Aim 2

Evaluation of analytical performance for both the non-imprinted and imprinted polymer sensors will be done using cyclic voltammetry (CV), square wave voltammetry (SWV), atomic frequency microscopy (AFM), scanning electron microscopy (SEM) and ultra-violet spectroscopy. The performance of the sensors (MIP and NIP) will be compared in terms of their selectivity and sensitivity.

1.4.3 Aim 3

Interference of species, other than the target analytes will be evaluated as to test the selectivity of the molecularly-imprinted polymer sensor. The interference studies will be performed using species with similar structural and chemical properties to that of the target analyte.

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1.5   Study Objectives

- Polymerization of pyrrole electrochemically to obtain polypyrrole.
- Characterization of the polymer (PPy), using CV, SWV, UV-spectroscopy, AFM and SEM.
- Analytical performance of the NIP towards PAHs detection.
- Analytical performance of the MIP towards PAHs detection.
- Comparison of analytical performance of NIP and MIP and evaluation of interfering species.
- contextualization of the impact of MIP sensors for PAHs detection

1.6   Thesis Scope

This thesis is made up of six chapters;

Chapter 1: In this chapter the background of PAHs is given and a well detailed plan of action to be implemented in order to achieve the aims of this study is outlined.

Chapter 2: A comprehensive literature review of Polypyrrole, template molecules (Anthracene, BaP and Pyrene), chemical sensors and molecularly imprinted polymers is being done in this chapter.

Chapter 3: This chapter gives details about the instruments which are used in the experiment and also state all the reagents and materials used during the process.

Chapter 4: This chapter reports and discusses the results of NIP sensor towards the detection of anthracene, BaP and pyrene.

Chapter 5: This chapter gives the results of the test molecule (BPA) and results for the PAHs and makes a comparison between the NIP and MIP.

Chapter 6: In this chapter material characterization is being shown (AFM, SEM and UV)

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Chapter 2

Literature has shown that, there are various methods used for detecting PAHs, such as the use of HPLC coupled with fluorescence detection and electrochemical sensors. This chapter provides an evaluation of literature with respect to methods of detection of PAHs. Furthermore the chapter reviews the use of electrochemical sensors, particularly those based on molecularly imprinted polypyrrole for the detection of anthracene, benzo[a]pyrene and pyrene.

2   Methods for the detection of PAHs

The extensive amount of published papers on PAHs shows that, researchers have been concerned for decades about the increased exposure to PAHs in the environment and atmosphere. Due to potent threats of PAH, methods like ozonation, UV-radiation, membrane filtration, reverse osmosis, and activated carbon adsorption were considered as solutions for removal of PAHs from different matrices[13]. There are also a number of analytical extraction techniques that were used for PAHs extraction from organic matrices such as soxhlet extraction and sonication. However the use of the above methods results in increased cost because some of them require well trained personnel and some are bound to one region[14]. Therefore a cost-effective and highly selective removal method for the PAHs is needed. Chemical sensors have been successfully employed to monitor air quality such as emission control[15]. Michelle Penze et al[16] on their study of fabricating a portable chemical sensor system for urban air pollution monitoring reported that solid-state gas sensors are very promising for low-cost air quality control. That was after they managed to develop a portable sensor which was able to monitor air pollutants such as CO and NO_x in the range of ppm and ppb levels, respectively as regulated by the European Union Directive 2008/50/EC.

Chemical sensors are also used for health reason for humans. Merve Yesil et al [17] developed an electrochemical DNA biosensor for the detection of specific mycobacterium tuberculosis sequence based on poly(L-glutamic acid) modified electrode. In this study the sensor electrochemistry was studied using square wave voltammetry (SWV), which showed a linear calibration curve of detection over the range of 1.5 to 12.5 nM with a detection limit of 1.3 nM.

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Literature shows that, chemical sensors have a very high demand due to their appreciable properties. Therefore in this study we seek to make use of chemical sensors to detect anthracene, benzo[a]pyrene and pyrene in the petrochemical industry cost-effectively.

After the detection of PAHs it is important to degrade them due to their toxicity. One of the well-known earliest methods to destroy PAHs is known as the Fenton's reagent. Fenton's treatment is mostly used to oxidize contaminants in soil, sludge and wastewater[18]. It makes use of hydrogen peroxide as an oxidant, which is decomposed to hydroxyl radicals, in the presence of ferrous sulphate:

$$H_2O_2 + Fe^{2+} \rightarrow OH + OH^- + Fe^{3+}$$

In natural systems like soil for an example, ions and other metals are present in the soil therefore they assist in generating hydroxyl radicals. Fenton's reagent has shown to be a very powerful oxidizing agent[19]. However there are some species which were found to be resistant to the oxidation by Fenton's reaction. These species are small chlorinated alkanes, n-parrafins and short chain carboxylic acids

2.1 Chemical sensors associated with the detection of PAHs

A chemical sensor is simply a device that has an ability to transform characteristic chemical information into an analytically useful signal. Chemical information can either be composition (i.e. presence of functional groups), presence of particular elements or ions (i.e. radicals), concentration or chemical activity[20]. In contrary, a physical sensor provides information on physical properties of a system. Literature suggests that an ideal chemical sensor device must have at least the following characteristics; economical, portable and foolproof. Also, the device must have the ability to responds with perfect and instantaneous selectivity to a particular target chemical substrate. The reliability of chemical sensor device is such that the presence of analyte in any desired medium should produce measurable signal output at any required analyte concentration. All chemical sensors are said to consist of a transducer and a chemically selective layer. The transducer transforms the response into a detectable signal on modern instrumentation, and the chemically sensible layer isolates the response of the analyte from its immediate

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environment [21]. In general, chemical sensors can be classified in various categories amongst them are optical, electrochemical and piezoelectric sensors [20].

2.1.1 Optical sensors

Optical sensors are devices which are meant to transform changes of optical phenomena, which are the results of an interaction between the analyte and the receptor part. Moreover this group may be further subdivided according to the type of optical properties which have been applied in chemical sensors. Particularly for the detection of PAHs, types of optical sensors used are those based in fluorescence detectors. Literature has shown that most of the results in written papers based on PAHs detection, they are reported in terms of fluorescence measurements. Franz L et al[15] in their work of molecularly imprinting through Waan de Waal forces: Fluorescence detection of PAHs in water have done most of their work with fluorescence detection. Their work was specifically to detect anthracene and pyrene. Molecular cavities were created in polyutherines and coupled with microelectronics and optical detection principles.

2.1.2 Electrochemical sensors

Literature shows that electrochemical sensors have attracted vast attention in analytical chemistry due to their desired properties such as stability, ease of preparation and being unbound to the laboratory. These devices transform the effect of the electrochemical interaction analyte – electrode into a useful signal. Such effects may be stimulated electrically or may result in a spontaneous interaction at the zero-current condition[22]. This study seeks to use a voltammetric sensor in particular; in which current is measured in the DC or AC mode. This subgroup (voltammetric sensors) may include sensors based on chemically inert electrodes, chemically active electrodes and modified electrodes. This type of sensor is highly used for the detection of species in different matrices. Additionally voltammetry is one of the powerful tools used when detecting species using electrochemical sensors.

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2.1.3 Piezoelectric sensors

Piezoelectric sensors are known to use piezoelectric effect to measure changes in pressure, acceleration or force by converting them to an electrical change. These sensors are widely used for quality assurance, process control and for research and development in many industries. S Stanely et al [23] in their study of determination of PAHs using a quartz crystal microbalance. In their research they have fabricated a chemically coated piezoelectric sensor for the detection of PAHs in the liquid phase. Their results showed that, the sensor could detect anthracene with a sensitivity of 2 about ppb.

Compared to optical and piezoelectric sensors, electrochemical sensors are attractive due to their remarkable detectability, experimental simplicity and low cost. They are said to be leading in commercial stage and have found a vast range of important applications in the fields of clinical, industrial, environmental and agricultural analyses[21].

Piezoelectric sensors and optical sensors need special reaction conditions, they are not most likely to be operated under ambient conditions like chemical sensors. Therefore their use needs more money and specialists. In this study chemical (voltammetric) sensors are to be used mainly because of their selectivity and economical status. The main important parameters of this study are selectivity and sensitivity, hence chemical sensors are the best for this particular study. As far as chemical sensors are known for their selectivity, we seek to further increase our chances of selectivity and sensitivity by using a chemical sensor based on molecularly imprinted polymers.

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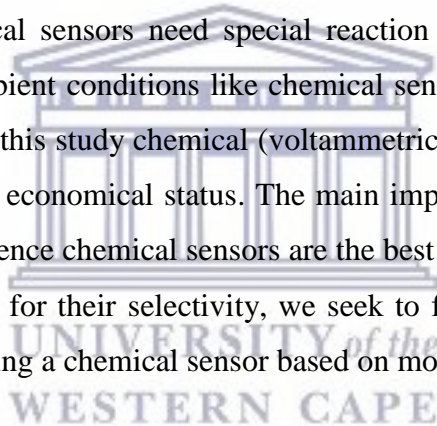
2.2 Molecularly Imprinted Polymers (MIP)

Molecular imprinting technology (MIT) offers a process of preparing materials with cavities that are able to recognize and attract a certain molecule of interest in terms of shape, size and chemical functionality[24]. One of the main advantages of the MIT is its high selective recognition. Piletsky, P et al argued that, in order to obtain high selective recognition of the molecule of interest, this molecule (analyte) needs to be incorporated during the synthesis of the material as a template[25]. When synthesis reaches completion, the molecule is extracted, therefore leaving behind its 3-D physical and chemical 'imprint' as shown in Figure 5 below. These cavities are capable of selectively rebinding the template molecule even in the presence of close structural analogues[25]
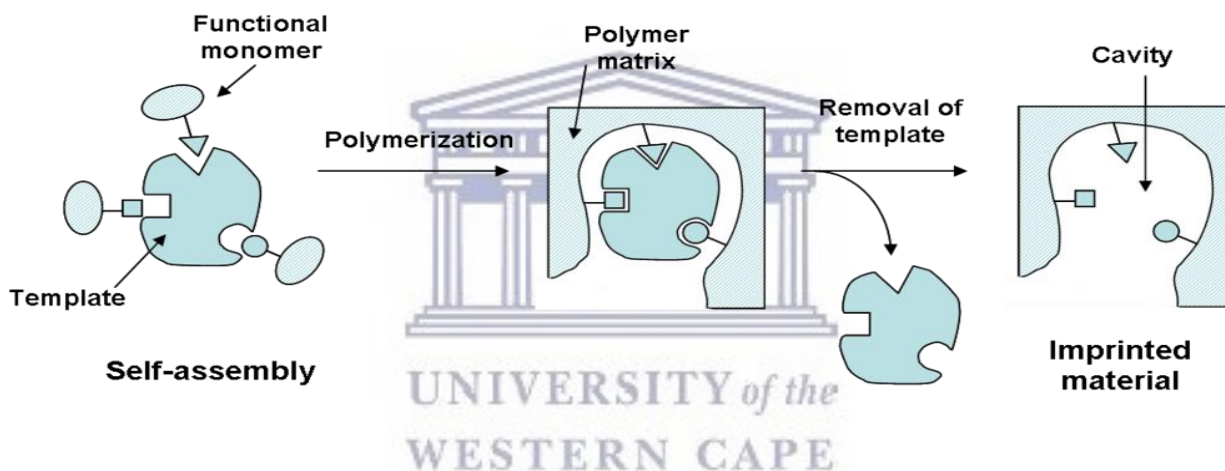
Figure 5: A schematic diagram that shows the preparation of the imprinted material for the detection of the analyte via electropolymerization.

L, Ozcan and Y, Sahin have used molecularly imprinted polypyrrole in their research for the determination of paracetamol on a modified pencil graphite electrode[26]. In their research, performance of the MIP and NIP films was evaluated by differential pulse voltammetry (DPV). Their results showed that, the calibration curves for the DPV peak current observed for paracetamol oxidation versus paracetamol concentration at MIP electrode showed two linear regressions. The first regression showed linearity over a concentration range of 5 μM to 0.5 mM with a correlation coefficient of 0.996. The slope of the second regression line was smaller than the first one, however with much wider concentration range of 1.25 to 4.5 mM with a correlation coefficient of 0.990. The detection limit of the MIP for detection of paracetamol was reported to

be 7.9 x10^{-7} M (S/N =3). These results led to a conclusion that, the modification of Ppy surface by imprinting improved the reactivity of the MIP electrode, thus making it possible to detect paracetamol in a solution up to as low as 0.5 μM. On the other hand the calibration curve observed for paracetamol on the NIP electrode also showed linearity of concentration in the range of 50 μM to 0.50 mM. Therefore this further proves that MIP detects on a much wider range than NIP, and it can detect from smallest concentrations.

Molecularly imprinting technology has also been used for the detection of PAHs from different matrices. Brylee David et al [27] used a pyrene-imprinted polythiophene sensor for the detection of PAHs. In this study a highly sensitive polythiophene sensor for the detection of ultratrace levels of pyrene in the concentration range of 0.01 – 1000 μM was developed. The recognition mechanism of the molecularly imprinting thin polythiophene films sensor for pyrene was evaluated by the use of fluorescence spectroscopy and computer-aided molecular modeling. Furthermore this study also reported on surface morphology studies for the MIP and NIP films which were evaluated via tapping mode atomic frequency microscopy (AFM). In addition, scanning electron microscopy (SEM) was used to image wider areas of the films at a relatively lower magnification than AFM. The AFM images showed the difference between the MIP and NIP morphologies, where MIP had dark shaped areas which were associated to the recognition cavities.

A morphological study of molecuralry imprinted polymers using SEM was done by Gema Paniagua et al [28]. Their paper reported a comparative study on non-covalent MIPs synthesized by bulk polymerization using digoxin as a template. Polymerization process was allowed to proceed by either UV light or thermostat-controlled water bath. Once the MIP and NIP polymers were prepared, they were evaluated with SEM. The polymers were both ground to a particle size of 100 - 200 μm. SEM images showed appreciable differences between the MIP and NIP morphologies. It was reported that NIP images exhibited uniform, smooth shape than the imprinted polymer, which showed irregular and rough morphology. The smooth and uniform surface observed from NIP were said to be due to the absence of recognition sites created for analyte, and the roughness and irregularities in MIP were due to the created recognizing sites for the analyte.

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Early studies emphasize on thin film configuration as one of the desirable MIPs formats for sensing application. In most studies, researchers prepare MIPs using bulk electropolymerization, which may decrease the number of effective binding sites. Therefore that will result to poor sensitivity and lack of repeatable selectivity in MIPs[25]. Literature reports that, extracting the template from a thick film is much more difficult than a thin film.

Moreover the template recognition may be difficult because the cavities are deeply buried in the polymer film. The other thing which may affect the sensitivity of the cavities is the solvent used to extract the template, if the imprinted polymer film is exposed for a long time to the solvent in an attempt to extract the template, the solvent may end up blocking or filling the recognizing sites.

Although molecularly imprinting technology is currently popular in sensors, it also had its shortcomings, which were minimized as more knowledge was acquired pertaining its nature. P,Spegel et al reported that during the early 1930's when molecularly imprinted technology was introduced it had some drawbacks of its own, due to the use of silica gel as a supporting matrices [24]. Silica gel was found to have limitations on stability and reproducibility of MIP sensors. Literature review showed that MIPs were vastly used in separation techniques before they were used as recognition elements for sensors.

The reason we want to employ MIPs in this study is because of their superior stability, selectivity, sensitivity, low cost and easy preparation. In addition the recognition properties of imprinted polymers are not affected by acid, base, heat or organic phase treatment hence we regard MIPs as suitable recognition element in chemical sensors. MIP based sensor have been used in different matrices and table 1 shows some of the uses of MIP in sensor applications.

Table 1: Different MIP sensors used to detect different analytes and their relevant parameters such as LOD and LDR.

Composition	Analyte	Dynamic linear range	Limit of detection	References
PPy modified pencil graphite electrode	Paracetamol	5 uM – 0.5 mM	789 nM	[14]
Chitosan-acetylene black composite film	Bisphenol A	0.0005 – 0.01 mM	2 nM	[13]
Ppy-modified pencil graphite electrode	Ascorbic Acid	0.25 – 7.0 mM	73 nM	[23]

The table above displays some interesting and wide detection range given by the MIPs for the different target molecules in different matrices. In most of these sensors based on MIPs polypyrrole is highly recognizable due to its attractive characteristics like stability and ease of preparation via electropolymerization. This study also seeks to use polypyrrole for the above mentioned reasons. We are also hoping to report results that show a wide range of detection for the MIP sensors as shown from the table above. Superiority of MIP will be tested by comparing it with NIP selectivity just like P Spegel et al [24] did on their work based on MIPs discussed earlier. This will allow us to really say with evidence that, MIP based sensor are indeed highly sensitive and selective over NIP sensors.

2.3   The use of biomarkers in monitoring of PAHs

As an alternative, some studies have used biomarkers of exposure and effect in order to monitor PAHs in the environment. Biomarkers are widely known as indicators signaling events in biological systems or samples[7]. They have further been classified as markers of exposure, markers of effect and markers of susceptibility[12]. PAHs and their metabolites have been measured in the urine of the exposed individuals. From both workers exposed to PAHs and dermatology patients treated with coal tar, the metabolite 1-hydroxypyrene has been detected in their urine. The presence of 1-hydroxypyrene in urine has also been demonstrated in workers exposed to PAHs in several different environments such as creosote-impregnating plant, road workers laying asphalt, and workers exposed to diesel exhaust fumes. 1-hydroxypyrene was used as a biomarker of exposure to PAHs due to it being constantly monitored on occupationally exposed individuals through liquid chromatography[8]. Additional studies have evaluated the usefulness of determining PAH or metabolite levels in human urine as a measure of exposure in industrial and environmental exposure settings[29]-[30].

One of the major drawbacks of biomarkers is that, it is impossible to determine from these biological media whether exposure was too high or low levels of PAH or if the exposure duration was acute, intermediate, or chronic. In this regard molecularly imprinted technology has an advantage since they can report the levels of exposure.

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2.4 Polypyrrole

In this study, polypyrrole was chosen as a suitable polymer to be incorporated during fabrication of an electrochemical sensor for PAH detection. For a very long time Ppy has attracted great amount of attention due to its low cost, conductivity, efficient electrochemical properties and stability [31]. PPy has been employed in number of chemical research fields other than molecularly imprinting such as in capacitors. In a study which was done by S Sezgin et al [31] PPy was used as an electrode in the construction of a supercapacitor.

There are lot other researches who have used polypyrrole in supercacitors since conductive polymers offer the advantages of high charge capacity and low production cost, and those are necessary conditions in industry. In molecularly imprinting Ozcan et al [26] have used PPy in the determination of paracetamol based of electropolymarized MIP modified pencil graphite electrode. Literature shows that, in molecularly imprinting technology PPy is used because of its conductive abilities.

The polymer is prepared by electrosynthesizing pyrrole through galvanostatic or cyclic voltamettry to obtain PPy. The reason behind these two methods it's because they provide a simple and rapid way of controlling the thickness of the polymer film growth since polymer thickness needs serious consideration in MIP[25]. Schweiger et al[32] in their work of sensing clofibric acid using MIP based on PPy explained that conducting polymers possess metal-like behavior due to the conjugated double bonds e.g polyacetylene, polypyrrole, polythiophene, polyphenylenevinylene, or polyaniline. Figure 4 shows the synthesis mechanism of Ppy, where it is polymerized by anodic oxidation on the working electrode. Oxidative polymerisation of pyrrole to polypyrrole proceeds via a one electron oxidation of pyrrole to a radical cation, which subsequently couples with another radical cation to form the 2,2'-bipyrrole. This process is then repeated to form longer chains.
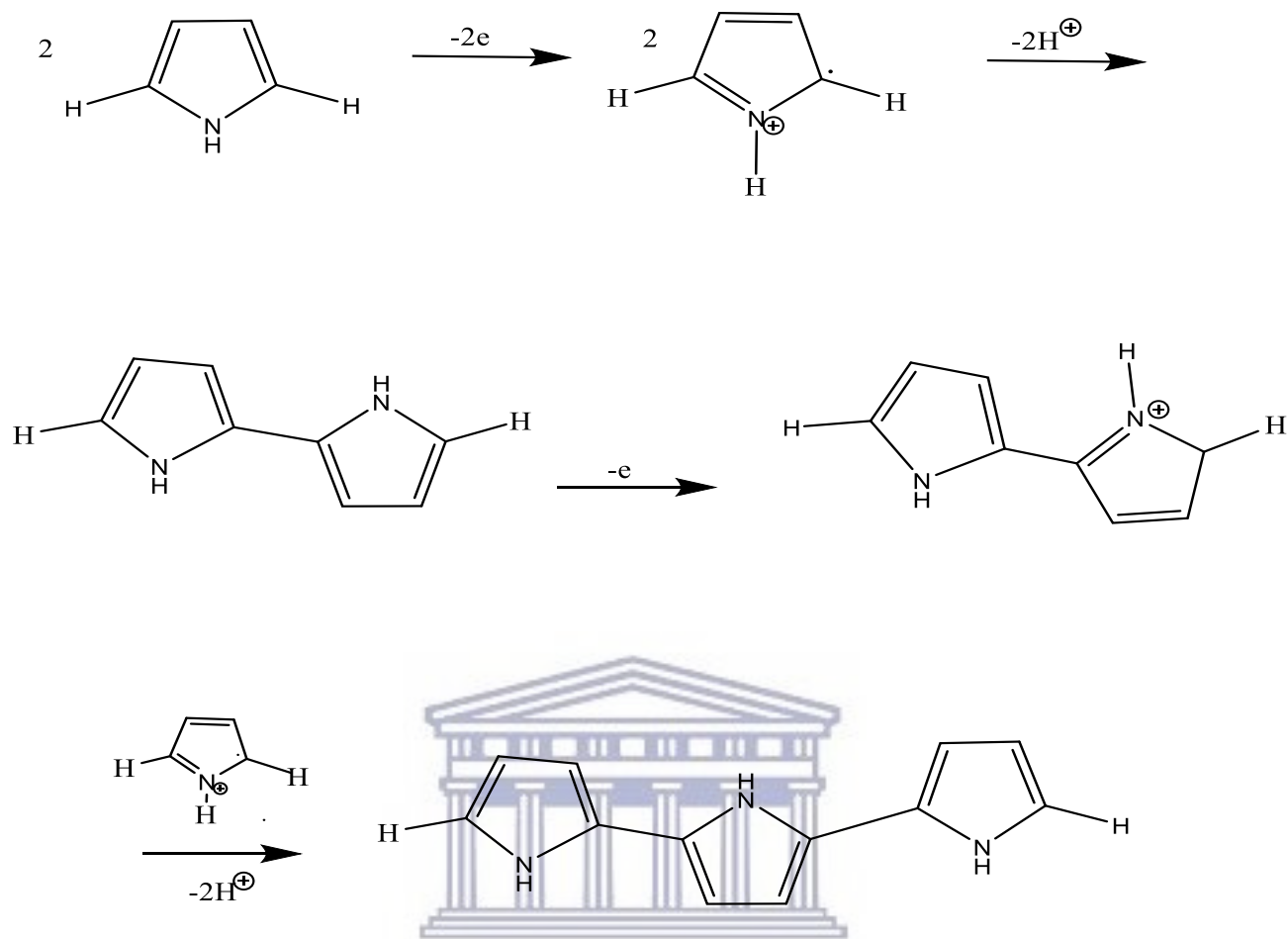
Figure 6: A reaction mechanism for the formation of polypyrrole from pyrrole via electropolymerization.

2.5 Template Molecules

This section gives more information towards the PAHs used as templates in this study, and the PAHs are Anthracene, BaP and Pyrene. Figure 7 is an illustration of their formation.
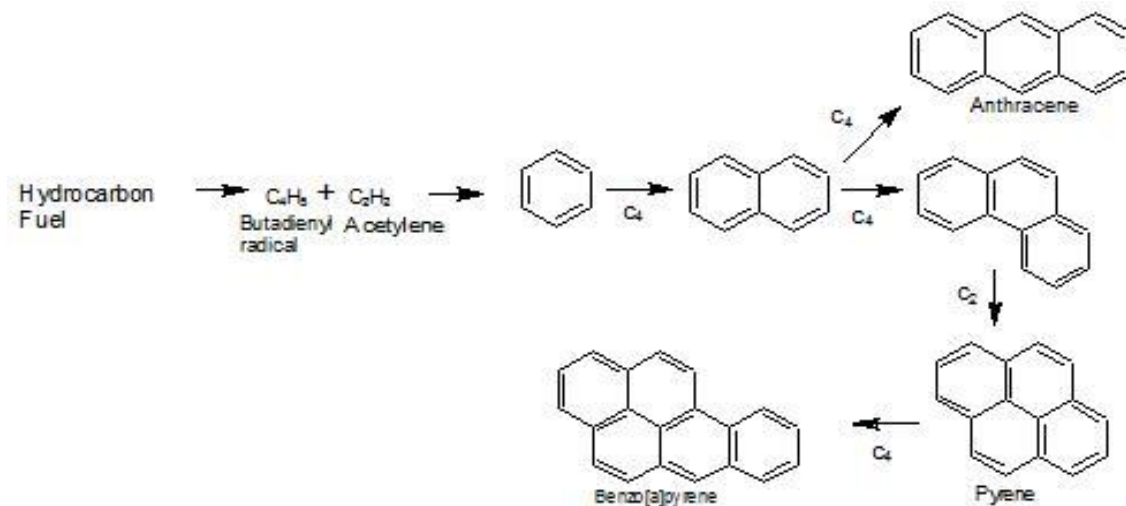
Figure 7: Reaction mechanisms towards formation of the template molecules from hydrocarbon fuel as a starting material.

2.5.1 Anthracene

2.5.1.1 Background

Anthracene, also known as paranaphthalene or green oil, is a polycyclic aromatic hydrocarbon (PAH). Anthracene is one of a number of polycyclic aromatic hydrocarbons (PAHs) on EPA's priority pollutant list. Although a large body of literature exists on the toxicity and carcinogenicity of other PAHs, toxicity data for anthracene are limited[33].

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2.5.1.2   Chemical properties and formation of anthracene

Anthracene is ubiquitous in the environment as a product of incomplete combustion of fossil fuels. It is the simplest tricyclic aromatic hydrocarbon. Anthracene is a colorless crystalline compound. It is soluble in a variety of organic solvents, including ethanol, methanol, benzene, toluene, and carbon disulfide, but is almost insoluble in water.

2.5.1.3 Uses and exposure of enthracene

Anthracene has been primarily used in the production of dyes just like pyrene. It has been known to be found in surface and drinking water, ambient air, exhaust emissions from internal combustion engines, smoke of cigarettes, and in smoked foods and edible aquatic organisms.
It has been stated that details on the toxicity of anthracene exposure in humans is very limited. Evidence indicates that anthracene is absorbed following oral and dermal exposure[33]. Targets for anthracene toxicity are the skin, hematopoietic system, lymphoid system, and gastrointestinal tract.

Adverse dermatologic effects have been observed in humans and animals in conjunction with acute and subchronic exposure to anthracene[34]. In humans, anthracene may cause acute dermatitis with symptoms of burning, itching, and edema. Long-term use of anthracene-containing laxatives produced melanosis of the colon and rectum. Human exposure to anthracene has also been associated with headache, nausea, loss of appetite, inflammation of the gastrointestinal tract, slow reactions, and weakness.

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2.5.2   Benzo[a]pyrene

2.5.2.1   Background

Benzo[a]pyrene is found in the environment and in food. It occurs in conjunction with other structurally related chemical compounds which are PAH. Moreover Benzo[a]pyrene is listed as a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), is found at 524 hazardous waste sites on the National Priorities List (NPL), and is ranked number 8 out of 275 chemicals on the Priority List of Hazardous Substances for CERCLA[12].

Literature shows that, in the 18th century a young British chimney sweeps climbed onto a chimney and suffered from what was known to be chimney sweeps carcinoma, a scrotal cancer peculiar to their profession. Frequent skin cancers were noted among fuel industry workers in the 19th century[5]. In 1933, BaP was determined to be the compound responsible for these cases, and its carcinogenicity was demonstrated when skin tumors occurred in laboratory animals repeatedly painted with coal tar and then it has since been identified as a prime carcinogen in cigarette smoke.

2.5.2.2   Chemical Properties and formation of Benzo[a]pyrene

Benzo[a]pyrene is a five membered ring PAH. It is a pale yellow crystalline solid with a faint aromatic smell. It is known for its insolubility in water and has low volatility. It is released to the air from both natural and anthropogenic sources and removed from the atmosphere by photochemical oxidation; reaction with nitrogen oxides, hydroxy and hydroperoxy radicals, ozone, sulfur oxides, and peroxyacetyl nitrate; and wet and dry deposition to land or water.

2.5.2.3 Uses and exposure

There is no known commercial use for benzo[a]pyrene, therefore it is only produced as a research chemical[5]. It is ubiquitous in the environment primarily as a result of incomplete combustion emissions. It is found in fossil fuels, crude oils, shale oils, and coal tars. Benzo[a]pyrene exposure can also occur to workers involved in the production of aluminum, coke, graphite, and silicon carbide, and in coal tar distillation. The major sources of non-occupational exposure are tobacco products, inhalation of polluted air, ingestion of contaminated food and water, and through cooking processes that involve smoke. Dermal exposure can occur through contact with materials containing soot, tar, or crude petroleum, including pharmaceutical products containing coal tar, such as coal tar-based shampoos and treatments for eczema and psoriasis[12].

2.5.3 Pyrene

2.5.3.1 Background

Pyrene is one of the well-known pollutants and it forms part of the EPA priority pollutant list[29]. Pyrene was first isolated from coal tar, where it occurs up to 2% by weight. It is also known to be produced in wide range of combustion conditions, for an example in automobiles. It is also said that since more than 20% of the carbon in the universe may be associated with PAHs, pyrene is included.

2.5.3.2 Chemical Properties and formation

Pyrene is a four membered ring resulting in a flat aromatic system. It is a colorless solid which is the smallest peri-fused PAH. Just like most PAHs pyrene is relatively insoluble in water, it is however soluble in solvents containing alcohol like methanol and ethanol. Figure 2 shows the formation of pyrene from a benzene ring through consecutive additions of carbon.

2.5.3.3 Uses and exposure

Pyrene and its derivatives are used commercially to make dyes and dye precursors, for example pyranine and naphthalene-1,4,5,8-tetracarboxylic acid[29]. Pyrene is mostly known to be pyrogenic however it is also somewhat petrogenic since it is found in many petroleum products. Human exposure to pyrene occurs primarily through smoking of tobacco, inhalation of polluted air and by ingestion of food and water contaminated by combustion effluents[33].

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Chapter 3

Research instruments and reagents are important tools to have for a viable scientific research. The instruments are used to obtain data on that particular topic of the study. The reagents are used to synthesize or prepare the material which will be used for the research experiments. This chapter reports on details of the instruments used and their principles, and material preparation with their precautions.

3   Introduction

We have prepared electrochemical sensors based on molecularly imprinted polypyrrole in order to detect benzo[a]pyrene, pyrene, and anthracene. The polymer was characterized by different electrochemical and microscopic techniques in order to be certain that indeed recognizing cavities were acquired for optimum analyte recognition. Furthermore selectivity experiments were performed in order to investigate if the sensor is sensitive only to the desired analyte.

3.1   Reagents

The *Pyrrole 98%* monomer, Pyrene 98%, Benzo[a]pyrene 98%, Chrysene, Anthracene and Bisphenol A were all purchased from Sigma-Aldrich. Methanol 99% which was used to dissolve the PAHs, ethanol used for electrodes sonication, hydrochloric acid which was used as a supporting electrolyte and saturated potassium chloride were also purchased from Sigma-Aldrich. The Micropolish alumina ($1.0\,\mu$m, $0.3\,\mu$m and $0.005\,\mu$m) used to polish the glassy carbon electrodes were purchased from Buelher chemical company.

3.2   Experimental Procedure

3.2.1   Electrode cleaning

Prior the use of electrodes it is important to make sure that they are cleaned, this was done by polishing the electrodes using the micropolish slurry of $1.0\,\mu$m, $0.3\,\mu$m and $0.005\,\mu$m

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respectively. The size of these glassy carbon electrodes was 0.071 cm^2. The electrodes were rinsed with deionized water. After polishing they were ultra-sonicated for about 20 minutes in ethanol and followed by another 20 minutes in deionized water. To test if the electrodes were clean, cyclic voltammetry of bare glassy carbon were done in 0.1 M HCl at 25 mV/s. The reference electrode Ag/Agcl had to be stored in saturated KCl solution, because the solution allows enough ionic flow to prevent salt crystals from forming in the glass pores. Furthermore the KCl solution ensures that the electrode impedance is kept low[35].

3.2.2 Polypyrrole Synthesis

The 0.05 M pyrrole monomer was first distilled in order to purify, because electrochemical experiments are very sensitive to impurities[26]. Polypyrrole was electrochemically synthesized by means of electropolymerization in the presence of 0.1 M HCl as a supporting electrolyte. An amount of 70 uL of pyrrole was inserted in a 4 ml 0.1 HCl solution. This reaction was done in a 3-electrode system where Pt-wire was a counter, Ag/AgCl as a reference and glassy carbon as working electrode. The potential window used for the polymer synthesis was from -0.4 to 0.7 V, with 5 cycles of polymerization. This reaction was done under ambient conditions at the chemistry department laboratories. Electrochemical experiments in this study were performed with Palmsense Trace 4.4.

At the completion of 5 electropolymerization cycles we expected to get a PPy/GCE sensor (non-imprinted sensor). In order to confirm that we acquired the polymer, electrochemical characterization of PPy/GCE sensor in 0.1 M HCl solution was done, and we expected to find peaks from cyclic voltammetry which are similar with those of polypyrrole found in literature.

3.2.3 Molecularly imprinted polymer preparation

This process was a crucial and challenging stage in molecularly imprinting. In this process the analytes of 0.0005 M (anthracene, benzo[a]pyrene and pyrene) were included during the electro-polymerization of pyrrole. This means, a polymer that has an analyte in it was obtained after 5 cycles. In order to create the precognitive cavity, the analyte had to be washed off so that it can

leave its print on the polymer. The electrode with the polypyrrole was immersed in methanol to wash off the analyte of interest and leave its imprint. Thereafter a polymer with an imprint was obtained. The number of cycles was kept to 5 because if the number of cycles is more it becomes a challenge to remove the template molecule in order to obtain the recognition cavities, so the lesser the number of cycles it's the easier it will be to remove the template molecule[36].

At the end of electro-polymerization we expected to achieve a MIP sensor with composition of PPy/analyte/GCE and then after the removal of analyte as explained above we expected to have a sensor with recognition cavities.

3.2.4   Preparation of PAH's

Solutions of 0.0005 M of PAH's (anthracene, benzo[a]pyrene and pyrene) were prepared by weighing the appropriate mass and dissolving each of the PAH in methanol and placed in a sonicator until fully dissolved. The samples were then kept in vials in a lab cupboard.

These analytes were expected to be fully dissolved hence they were placed in a sonicator. Moreover these materials do not need special storage so they were just kept in lab cupboards. To test their activity after sometime UV-spectrometry is a good instrument to check if they are still active. And that should be done if necessary.

After the analytes were prepared, their electrochemical behavior was investigated on a bare GCE using both cyclic voltammetry and square wave voltammetry. However, since PAHs are daunting materials to investigate, there was no positive analytical response from them in a bare GCE. Therefore the surface of the GCE had to be modified with the polymer and the imprints, only then an analytical response was acquired, and that is presented in both chapters 4&5.

3.3   Material Characterization

3.3.1   Atomic Force Microscopy (AFM)

Atomic force microscopy has been regarded as the best and yet powerful microscopy technology for studying samples at nanoscale [21]. Its importance is brought by the fact that, the atomic microscope can not only image in 3-dimension, but also provides other types of surface measurements to the needs of the user such as area roughness and line roughness[37].

AFM uses a cantilever with a very tiny and sharp tip that enables it to scan over a dry surface. As the tip approaches the surface, attractive force between the surface and the tip cause the cantilever to deflect towards the surface. However, if the cantilever is brought even closer to the surface, to a point that the tip makes contact with it, increasingly repulsive force takes over and causes the cantilever to deflect away from the surface[38].

A laser beam is used for the detection of cantilever deflections either away or towards the surface. The AFM will then generate an accurate topographic map of the surface features.

In this study a nanosurf scan 2.0 AFM was used to confirm the presence of cavities or imprints of the polymers by examining the sample topography. Three-dimension AFM images for before and after analyte removals were obtained and examined.
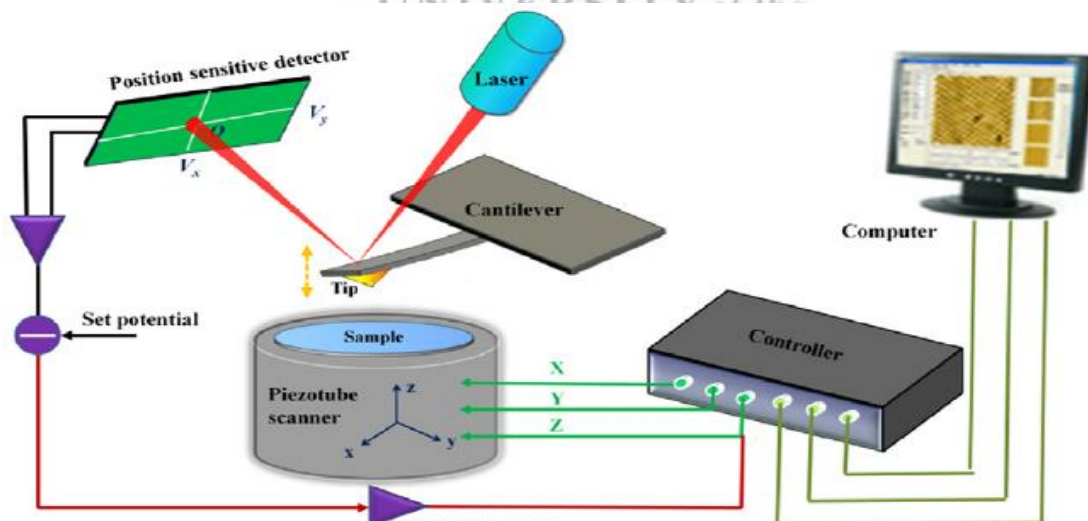
Figure 8: Complete configuration of an AFM instrument [38].

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3.3.2   Scanning Electron Microscopy (SEM)

In order to further investigate the presence of the imprints another microscopic characterization technique known as SEM was used. The SEM used was the Hitachi (X650). SEM is a type of electron based microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons are said to interact with the sample atoms, that results in production of various signals that has information about the sample's topography and composition. SEM allows scanning of the sample from different magnifications.
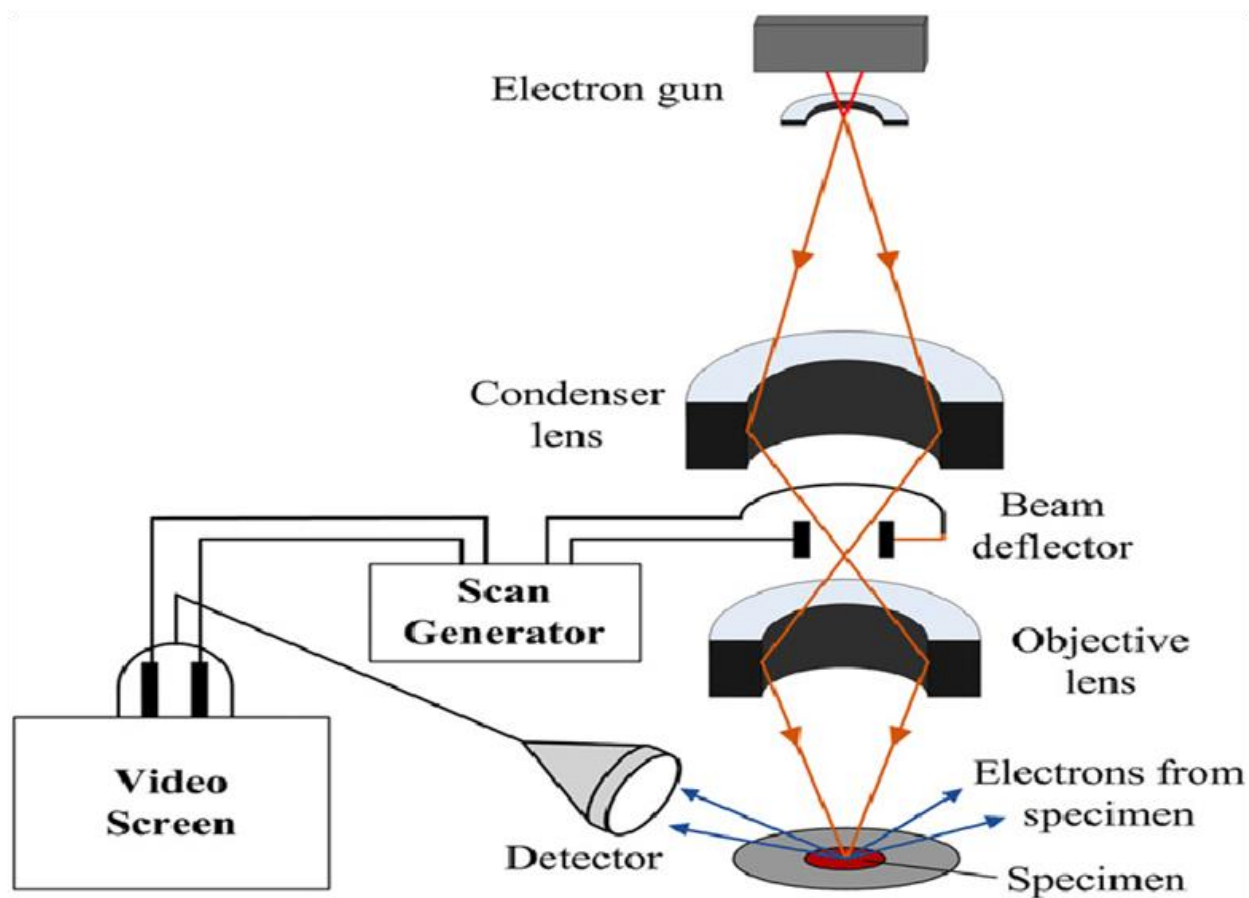
Figure 9: Configuration of a SEM instrument [27].

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3.3.3   Ultra-violet Spectroscopy

UV-visible spectroscopy is a technique that readily allows one to determine the concentrations of substances and therefore enables scientists to study the rates of reactions, and determine rate equations for reactions, from which a mechanism can be proposed[39]. UV-spectroscopy of such nature is used extensively in teaching, research and analytical laboratories for the quantitative analysis of all molecules that absorbs ultraviolet and visible electromagnetic radiation. This spectroscopic technique is used for various reasons in different industries.

In clinical chemistry UV-visible spectroscopy is used in the study of enzyme kinetics. Enzymes cannot be studied directly but their activity can be studied by analyzing the speed of the reaction at which they catalyse. In this study UV-visible spectroscopy was used to study the activity of PAHs and to degrade PAHs using the Fanton's reagent.

Spectrometry uses the absorbance of light by an analyte at a certain wavelength to determine the analyte concentration. UV-spectroscopy uses light in UV and visible part of the electromagnetic spectrum. Light of this wavelength is able to effect the excitation of electrons in the atomic or molecular ground state.
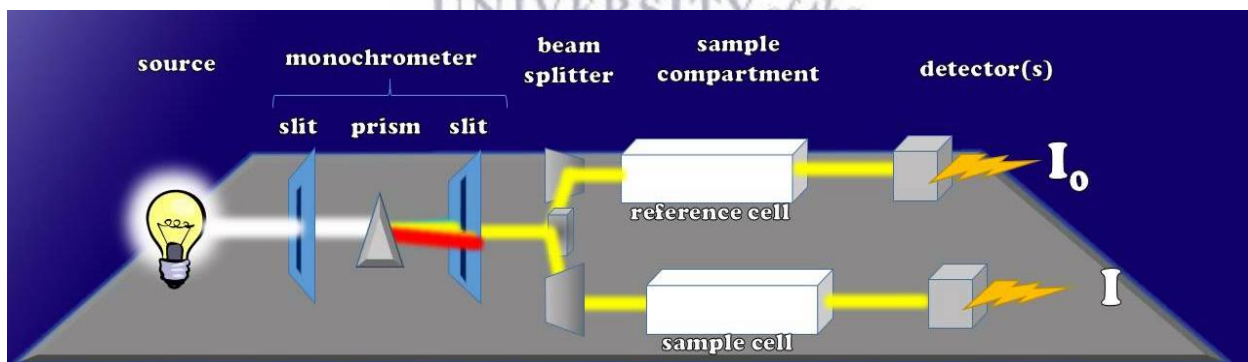
Figure 10: Schematic diagram for a UV-visible spectrometer.

3.3.4 Voltammetry

Electrochemical characterization is the study of the sample electrochemistry in different mediums. There are a number of different electrochemical techniques. In this study a form of electrochemical characterization technique which has been used extensively is Voltammetry, particular cyclic voltammetry (CV) and square wave voltammetry (SWV). Voltammetric techniques were performed using a PalmSense PTrace 4.4 using a three electrode system. The working electrode was prepared with PPy and the presence of the analytes of interest.
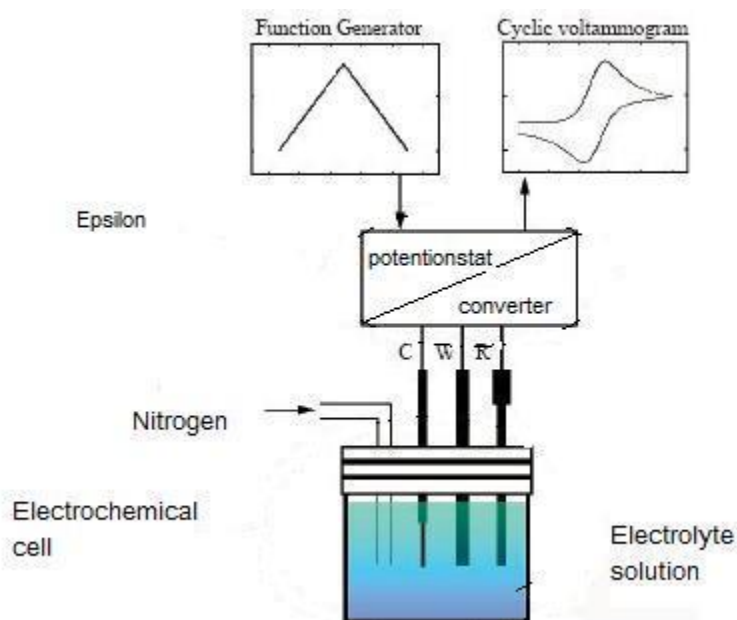
Figure 10: Configuration of a Voltammogram for both CV and SWV[14].

3.3.4.1 Cyclic Voltammetry

This is a set of linear-sweep voltammetry experiments in which anodic and cathodic scans are repeated alternately. This means that once the first scan is completed, the scan is continued in the reverse direction and the cycle can be repeated more than once[14]. CV gives information on the possible redox reaction of the system, including the Faradic insertion and extraction reaction. In this study CV has been used to polymerize pyrrole into polypyrrole and to also characterize the

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sensor. CV has further confirmed the detection process by observing the chemical behavior of the sensor. The technique allowed us to have a calibration curve based on the behavior of the sensor. Moreover the sensor sensitivity and limit of detections were obtained from CV.

3.3.4.2 Square Wave Voltammetry (SWV)

Square wave voltammetry is similar to CV because it gives similar electrochemical behavior of the analyte or sensor. The only advantage of SWV over CV is that it is much more sensitive. Also it does one cycle at a time, it can run a number of oxidation experiments and later run the reduction side. Just like in CV one can extrapolate a calibration curve. In this study SWV was used for PAH's which did not give much information on CV, then a much more sensitive technique was due, hence SWV.

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Chapter 4

As the purpose of this study is to detect PAHs (anthracene,BaP and Pyrene) generally the NIP is compared with the MIP to investigate which one possesses much better sensitivity and selectivity. This chapter reports experimental findings on the detection of the PAHs of interest using the NIP sensor and also giving the calibration curves generated from the acquired data.

4   Results and discussion of Non-Imprinted Polymers

4.1.1   Polymer synthesis

Pyrrole was electropolymerized using cyclic voltammetry in 0.1M HCl with absence of analyte. Figure below shows the polymers synthesis with the number of cycles in it.
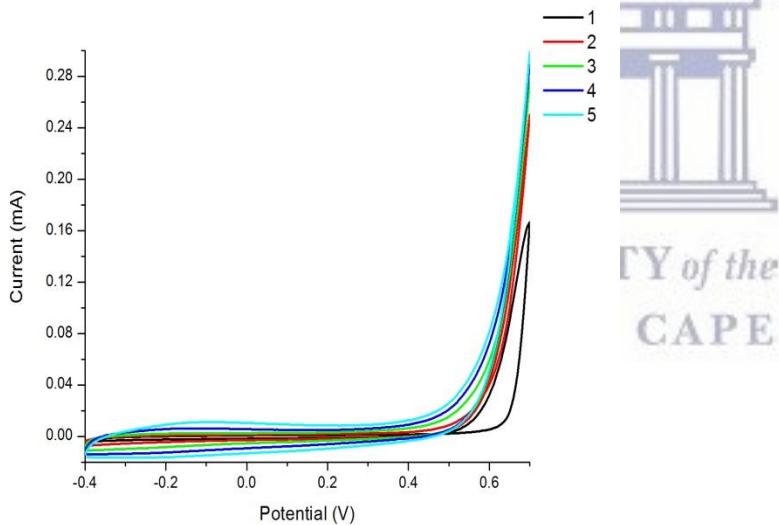
Figure 11: Cyclic voltammogram of polypyrrole synthesis in GC electrode vs Ag/AgCl at a scan rate of 50 mV/s in a 0.1 M HCl solution.

The classical three electrode system was used to electropolymerize pyrrole to polypyrrole, where GCE was a working electrode, Ag/AgCl was a reference electrode and the Pt-wire was a counter electrode. The electro-oxidation of the monomer (pyrrole) occurs at the anode. Since there is no sign of reduction peak for the backward scan, then the oxidation was irreversible. The figure above also shows a polymer film growth on the glassy carbon electrode. B Schweiger et al[32] conducted a study on electropolymerized molecularly imprinted polypyrrole (Ppy) film for sensing of clofibric acid, where they obtained similar results of polypyrrole film growing on an electrode surface during electropolymerization and the reaction was an irreversible one just like ours. They have associated the oxidation peak growth with the radical formation during polymer synthesis. The polymer build up from the electrode is proportional to the current response and that indicates that the polymer has been obtained. At the end of 5 cycles we rinsed the electrode with di-ionized water and in that manner we obtained a non-imprinted PPy/GCE sensor. The number of cycles was kept as low as 5 cycles for the ease of analyte removal when creating the recognition cavities.

It is important to understand that, PAHs have different analytical response to different electrochemical characterization techniques. In this work pyrene responded well with the use of cyclic voltammetry, therefore cyclic voltammetry was used to evaluate the analytical response of both MIP and NIP sensors. On the other hand the other two PAHs (anthracene and benzo[a]pyrene) gave a positive response in square wave voltammetry and it was therefore use to evaluate the analytical response of both MIP and NIP sensors.

4.2   Analytical response of PAHs

4.2.1   Pyrene

Pyrene was the first PAH to be investigated amongst the three. The classical three electrode system was used where the PPy/GCE was a working electrode, Ag/AgCl a reference and the Pt-wire as a counter electrode. Pyrene was added into the reaction in intervals in equal amounts and after each addition the reaction solution was stirred for about 60 seconds. Pyrene's analytical response was studied by cyclic voltammetry at ambient conditions.

Polypyrrole showed a sharp oxidation peak between 0.75 V and a second reduction peak between 0 - 0.5V. Pyrene adsorbs at about the same region as the polymer, because it has been observed that pyrene appears at 1 V without the polymer. Therefore in the presence of polypyrrole pyrene adsorbs between 0.5-1 V. Figure 13 below shows adsorption of the analyte is being observed from the additions of the analyte at different concentrations. There was a current change observed from the oxidation peak associated with pyrene and that was used as a signal peak which was used to plot the calibration curve for the NIP sensor. The calibration curve allows us to obtain the sensitivity of the sensor from the slope of the curve. In return the sensitivity also allows the calculation of the limit of detection of the sensor. The sensitivity of the sensor was found to be 14.479 A/uM with a limit of detection of 4.23×10^{-7} M (S/N =3). The calibration curve also displayed a linearity of concentration at a range of $6.2 \times 10^{-7} – 1.9 \times 10^{-6}$ M. The limit of detection for the experiments in this work was calculated using the data's standard error and slope according to the formula;

$$\frac{standard\ error}{slope}\ x\ 3.3$$
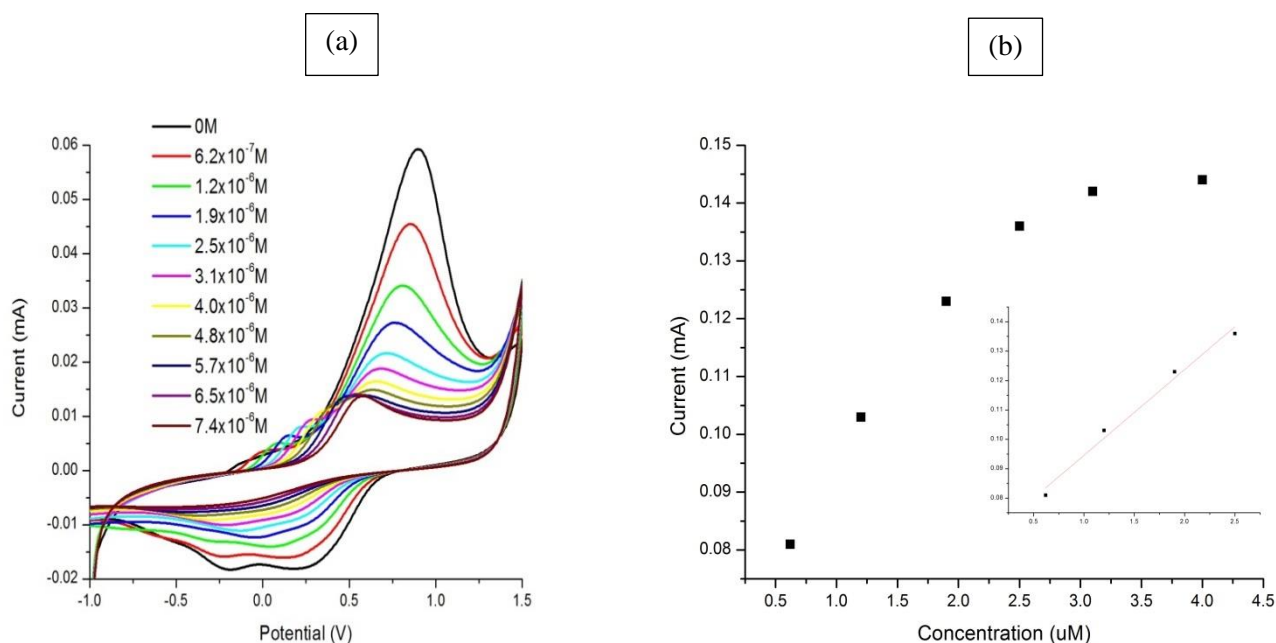
Figure 12: A cyclic voltammogram of (a) NIP sensor on GC electrode for detection of pyrene vs Ag/AgCl at a scan rate of 50mV/s in 0.1M HCl solution. (b) Calibration curve of the NIP sensor obtained from the adsorption of Pyrene in (a).

The oxidation mechanism of pyrene to pyrene-1,6-dione during the experiment is shown below
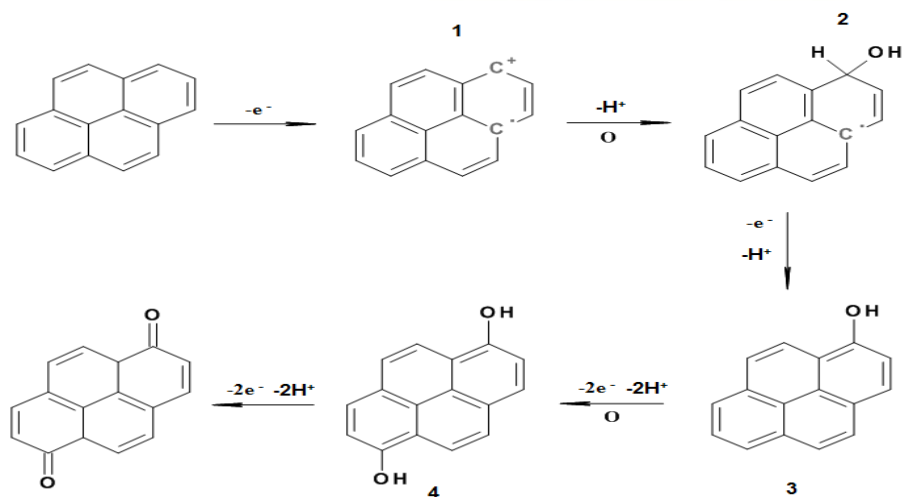
Figure 13: Electrochemical oxidation of pyrene

The square wave voltammetry was also used to investigate the analytical response of pyrene, the same standard conditions used in CV were used also for the SWV. Figure 15 shows that, pyrene a limited quantitative response for pyrene due to the fact that pyrene oxidation is not reversed during the single oxidative square wave voltammetry. The oxidation of pyrene results in product that is adsorbed onto the electrode surface that the comparative electrochemistry observed in cyclic voltammetry was not observed here, for effective quantitative response of pyrene.
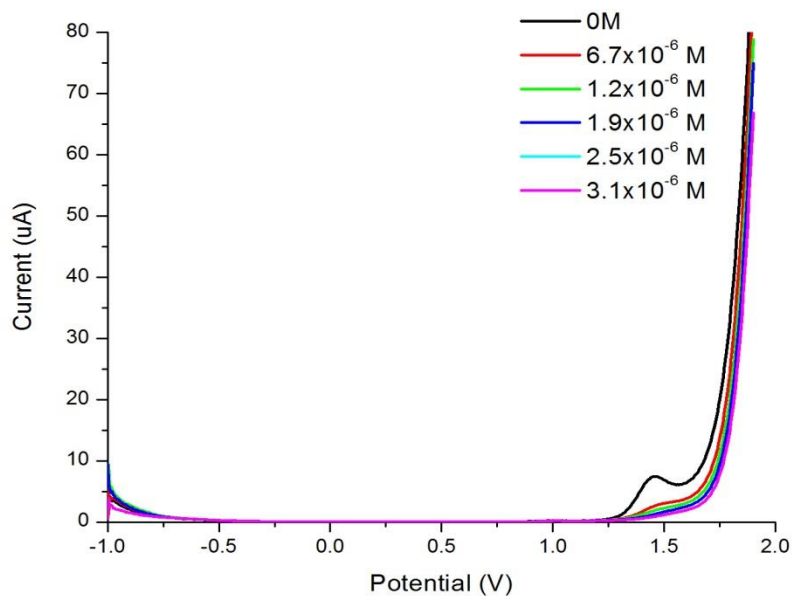
Figure 14: Square wave voltamogram of pyrene non-imprinted sensor in 0.1 M HCl solution.

4.2.2 Benzo[a]pyrene

PAHs are one of the most daunting materials to work with such that it is popular for researchers to use different techniques when working with them, since they have different responses to some techniques. Standard concentration additions of benzo[a]pyrene were added into the reaction cell and stirred for 60 seconds throughout the experiment. Benzo[a]pyrene displayed a much different analytical response than pyrene because its oxidation peaks appeared at a different potential than those of the polymer.

This may even be due to the sensitivity of square wave voltammetry. Looking at the square wave voltammogram in figure 15 below, it is evident that benzo[a]pyrene oxidize at 1.5V (peak 'a'), at that region adsorption of BaP into the polymer surface is observed. Although the adsorption does not show sharp peak, but there is a small current change as concentration of BaP is added onto the reaction system and that current change has been used as a signaling peak which the calibration curve was plotted from. The sensitivity of the sensor from the slope was found to be 2.014 A/uM with a limit of detection of 1.59×10^{-7} M (S/N =3). The calibration curve also displayed a linearity of concentration at a range of $6.2 \times 10^{-7} – 2.5 \times 10^{-6}$M.

Figure 15: Illustrates a square wave voltammogram of (a)NIP sensor detection of BaP by PPy/GCE vs Ag/AgCl in 0.1 M HCl solution at scan rate of 50mV/s (b) Calibration curve constructed from the SWV when BaP adsorbs around 1.5 V.

The figure below shows electrochemical oxidation of BaP where the initial step in the oxidation process of benzo[a]pyrene is a one-electron transfer process which results into the formation of radical ion species as depicted below.
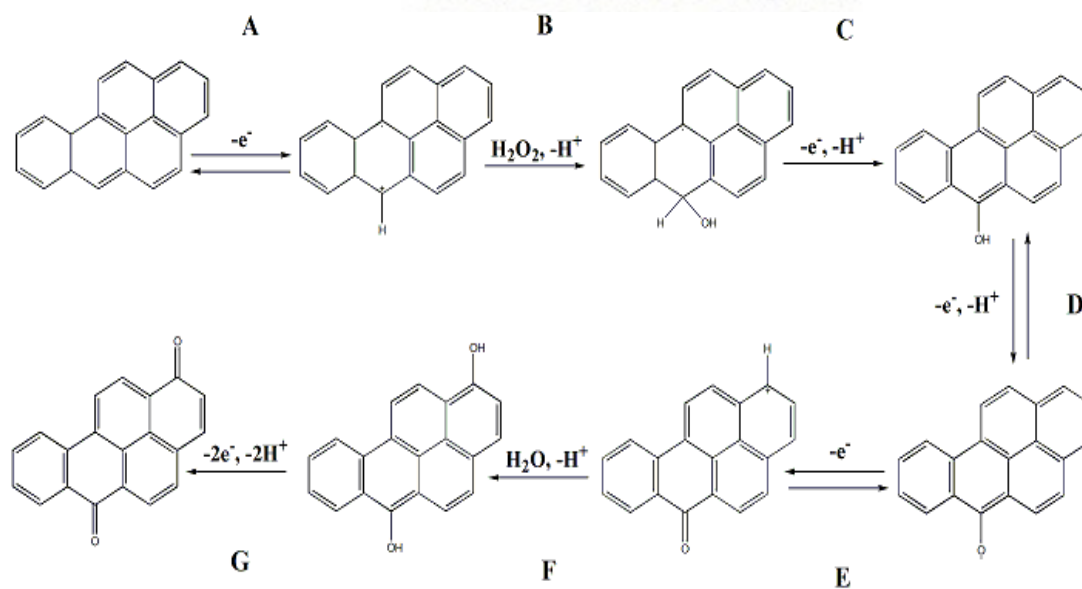
Figure 16: Electrochemical oxidation of benzo[a]pyrene

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Square wave voltammetry gave a positive response towards the sensing of benzo[a]pyrene, unlike in the previous experiment of pyrene. Cyclic voltammetry was also used to investigate the analytical response of benzo[a]pyrene and the acquired results were not as impressive as the ones obtained using SWV. In cyclic voltammetry a rapid peak disappearance as the benzo[a]pyrene was added was observed, therefore such a behavior impedes us to draw conclusions based on the analytical performance of the non-imprinted sensor such that we cannot even get a calibration curve from the cyclic voltamogramm. The analytical behavior is depicted in figure 18 below;
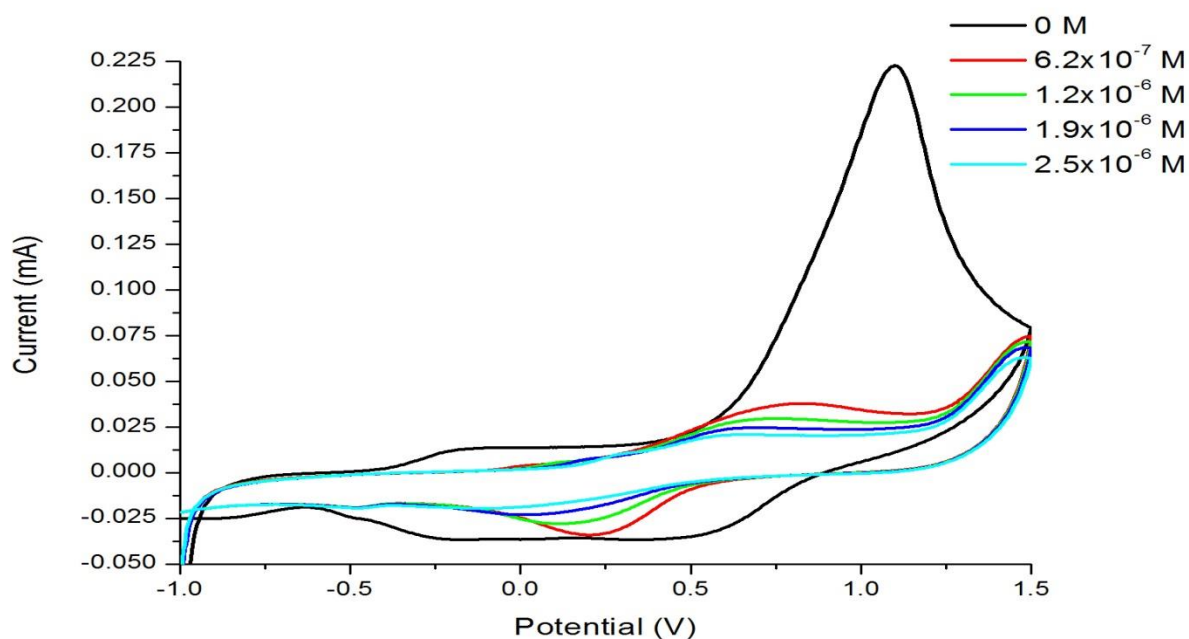
Figure 17: Cyclic voltamogram of non-imprinted benzo[a]pyrene sensor in 0.1 M HCl.

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4.2.3 Anthracene

The classical three electrode system was used for the anthracene non-imprinted sensor too. Standard concentration additions were added into the reaction system and stirred for about 60 seconds. The analytical response of anthracene was found to be similar to that of benzo[a]pyrene because even though a very sensitive voltammetric technique was used to detect anthracene, very small analytical response was observed. The oxidation of anthracene in a non-imprinted electrode was observed at 1.5 V. The response was too small, however zooming at that area there is a current change, which shows detection. The peak at 1.5 V is due to the catalytic effect of the NIP electrode towards the oxidation of anthracene. The peak at 0.45 V is a polymer peak which decreases as the analyte adsorbs onto the NIP electrode surface. The calibration curve was plotted from the small response at 1.5 V which was obtained from the sensor. The sensitivity of the sensor was found to be 0.553 A/M with a limit of detection of 4.33×10^{-7} M (S/N =3). The calibration curve also displayed a linearity of concentration at a range of $6.2 \times 10^{-7} – 1.9 \times 10^{-6}$ M.
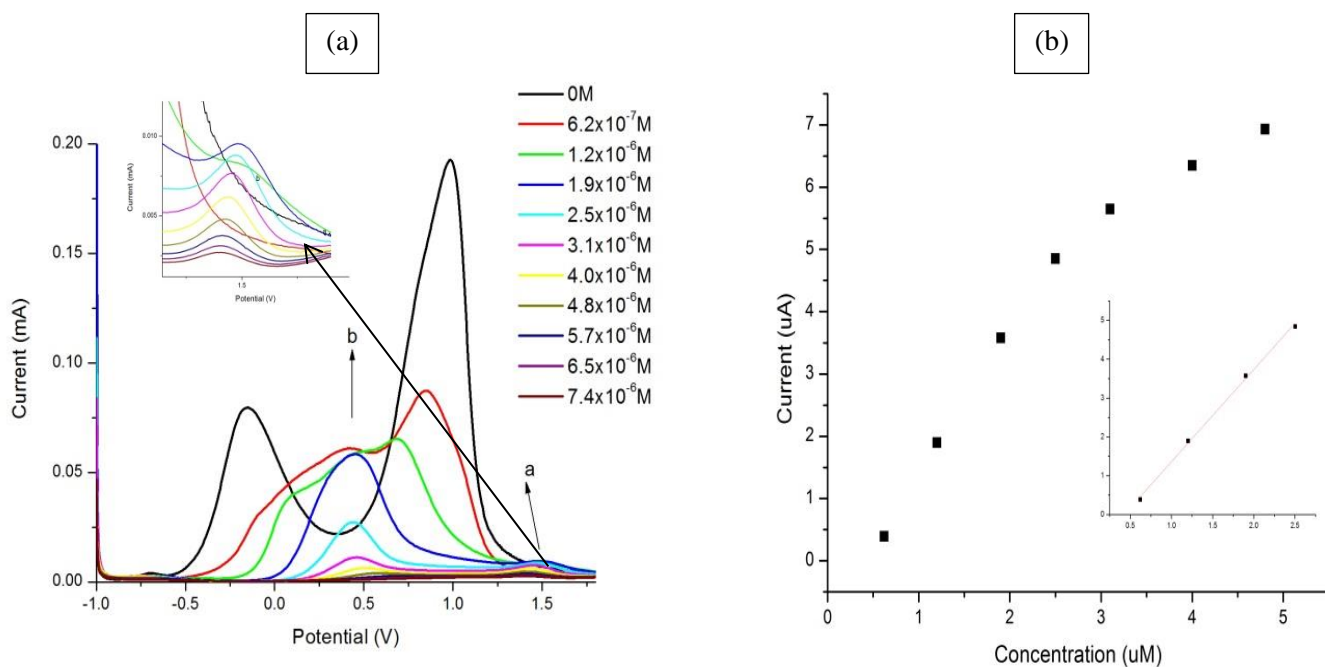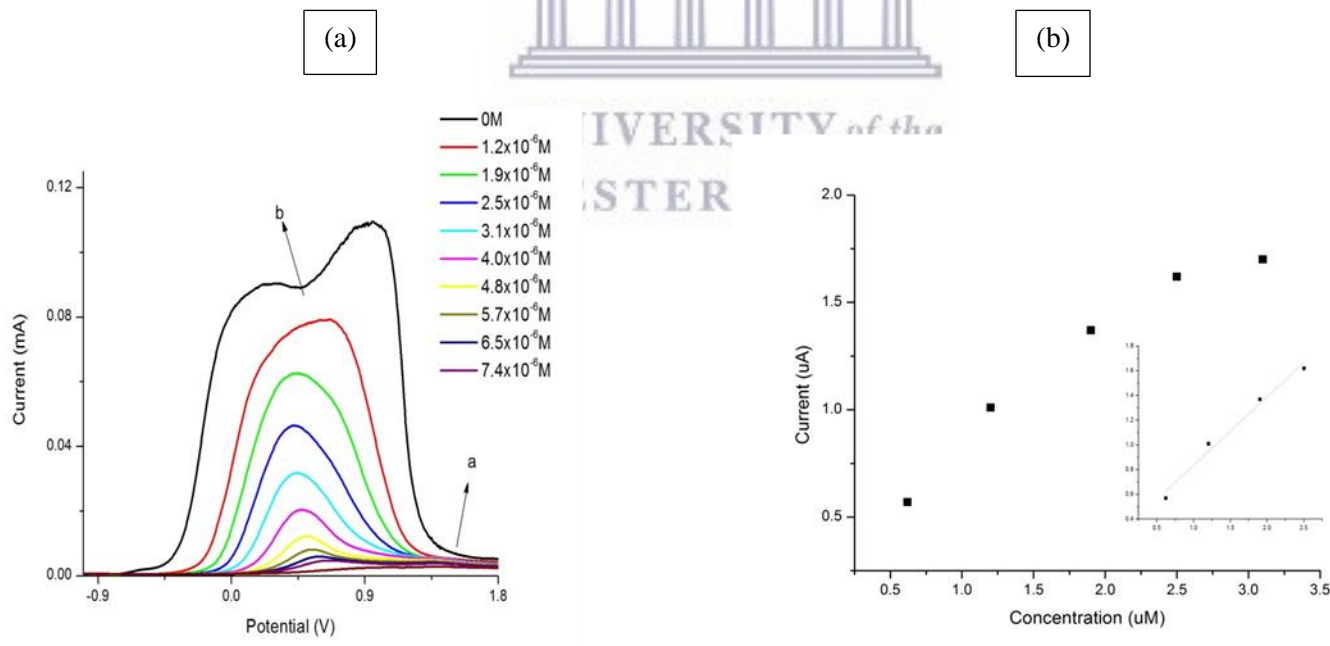

Figure 18: Square wave voltammogram of (a) NIP sensor detection of Anthracene by PPy/GCE vs Ag/AgCl in 0.1M HCl solution at a scan rate of 50mV/s (b) Calibration curve of the NIP sensor plotted from the voltammogram adsorption of Anthracene.

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The mechanism of the oxidation peak at 1.3 V can be accounted for using the oxidation mechanism below.
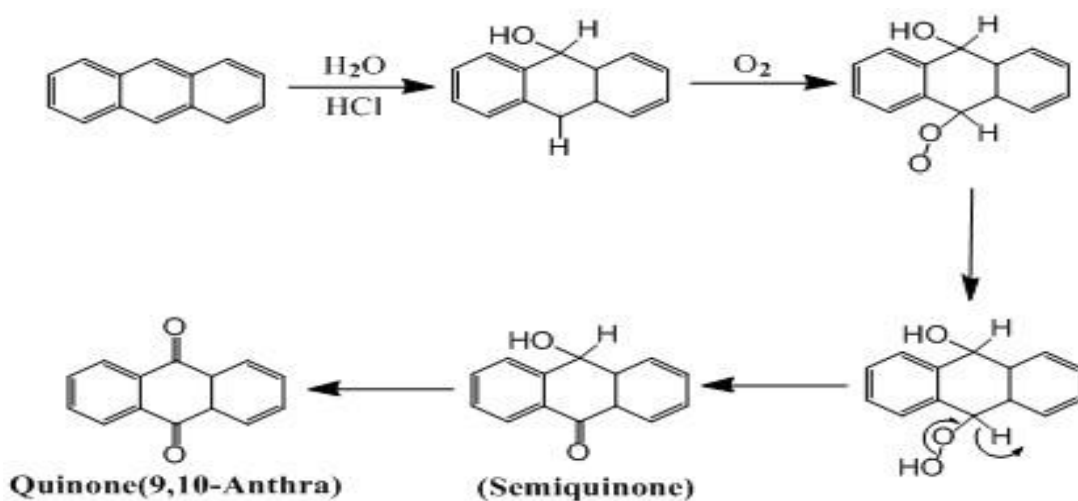
Figure 19: Electrochemical oxidation of anthracene.

Even though the sensor did not show much sensitivity towards the analyte, there were at least data points which could be taken to plot a calibration curve. Whereas with the use of cyclic voltammetry there were few points just like with the experiment of detecting benzo[a]pyrene using CV. The CV shows the polymer peak, and a disappearance of peaks upon the consecutive addition of anthracene onto the reaction.

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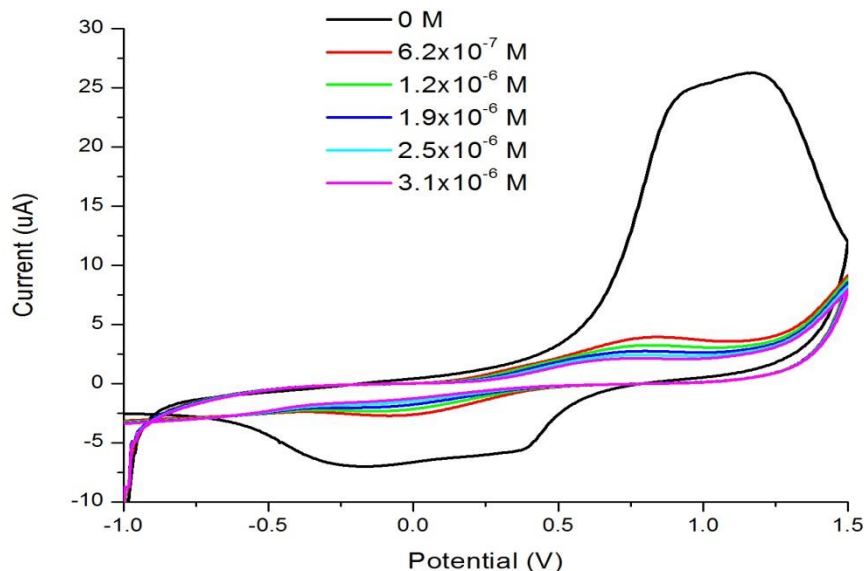
Figure 20: Cyclic voltammogram of non-imprinted anthracene sensor in 0.1 M HCl.

4.3   Electrochemical behavior summary

Literature has shown that, anthracene, benzo[a]pyrene and pyrene have been detected using the molecularly imprinting technology. The most famous polymers used for the detection of PAHs using imprinting are polythiophene and polyurethanes. Moreover, literature shows that most papers for the detection of PAHs using MIPs have been reported using fluorescence measurements.

Brylee David et al [27] in their work of pyrene-imprinted polythiophene sensor for the detection of polycyclic aromatic hydrocarbons have reported the binding capacity of the NIP sensor to be low with an analytical linear range of 0.01- 0.9 M. This low binding capacity was assumed to be caused by the absence of recognition cavities. This paper has showed the importance of the recognizing cavities for the affinity of analyte molecules. Our study showed a need for the recognition cavities for optimum analyte detection, since for anthracene and benzo[a]pyrene we obtained unsatisfactory analytical response.

The results obtained from this study for anthracene and benzo[a]pyrene were not satisfactory due to their low sensitivity, and that is depicted by the sensors analytical response while detecting the

analytes by SWV. The sensor for anthracene gave a sensitivity of 0.553 A/M and benzo[a]pyrene gave sensitivity of 2.014 A/M. Pyrene sensor showed satisfactory results and much high sensitivity of 14.479 A/M. The non-imprinted polypyrrole (NIP) sensor presented a limit of detection of 4.33 $x10^{-7}$ M, 1.59 $x10^{-6}$ M and 4.23 $x10^{-7}$ M for anthracene, benzo[a]pyrene and pyrene respectively (S/N=3).

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Chapter 5

This chapter reports findings on the detection of anthracene, benzo[a]pyrene and pyrene using the MIP sensor and then comparing the findings to the results obtained with NIP. However it is necessary to first consider the results of the test molecule (Bisphenol A) used based on a literature precedent for molecularly imprinted polymers.

5   Bisphenol A

Very little evidence of the application of molecularly imprinted polypyrrole for the detection of PAHs, exist in literature. Therefore a literature precedent was followed to establish the binding efficiency of a molecularly imprinted polypyrrole sensor for the detection of bisphenol A (BPA) first and then followed up with developing the protocol for PAH detection, by molecularly imprinting method.

5.1.1   Results and discussion

Bisphenol A imprinted polypyrrole was prepared according to the methodology reported in chapter 3, i.e. polymer electrosynthesis in the presence of the analyte molecule. Cyclic voltammetry was used to investigate the electrochemical behavior of BPA at a non-imprinted (NIP)and imprinted polypyrrole sensors (MIP). The analytical performance of the sensors was evaluated in the concentration range of $1.2 \times 10^{-13} - 8.6 \times 10^{-13}$ M using a 3 electrode system in a 0.1 M HCl electrolyte solution. A calibration curve for the detection of BPA using both NIP and MIP sensors was acquired.

A slope of 0.673 A/M was determined from the calibration curve obtained for the analytical response of BPA at the MIP electrode, a slope of 0.335 A/M was obtained for the NIP electrode. The slope of the calibration curve is related to the sensitivity of the sensor response to the analyte. Hence it is evident that the MIP is two times as sensitive as compared to the NIP sensor. Additionally the dynamic linear range for the MIP sensor response to BPA was far greater than that of the NIP sensor. The linear range of the MIP sensor was from $(1.2 - 7.4) \times 10^{-13}$ M and for

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the NIP sensor, it was from $(1.2 - 3.7) \times 10^{-13}$ M. The limit of detection was calculated using the formula of standard deviation S/N, with the experiments repeated 3 times (N= 3) .The limit of detection for the MIP sensor was calculated as 1.21×10^{-14} M and 1.23×10^{-16} M for the NIP sensor, using the formula $\frac{standard\ error}{slope} \ x \ 3.3$. A novel bisphenol A sensor based on amperommetric detection was reported by J Huang et al [40] for a molecularly imprinted 2-aminothiophenol sensor for the detection of BPA. Their results showed that, the sensor gave a linearity of concentration between $8.0 \times 10^{-6} - 6.0 \times 10^{-2}$ M with a limit of detection of 1.38×10^{-7} M (S/N = 3).
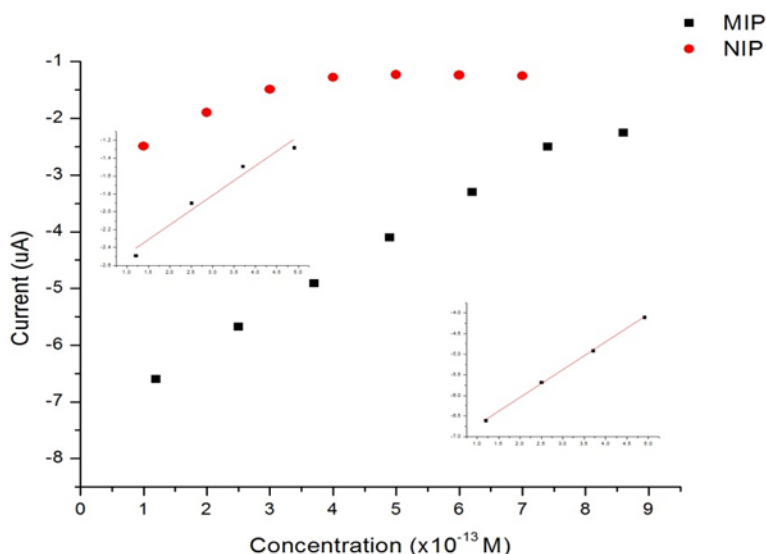
Figure 21: Calibration curve comparison for the detection of BPA by NIP and MIP sensors.

In a separate study of the detection of BPA using molecular imprinting by Y Tan et al [41], a molecularly imprinted chitosan-acetylene black composite film modified glassy carbon electrode was prepared. Their results showed that, the sensor displayed a dynamic linear range for concentrations between $0.005 - 0.20$ μM and a limit of detection of 2 nM (S/N =3).

The sensor prepared in this work is comparable to molecularly imprinted polymer sensors for BPA, reported in literature. The data reported for BPA at imprinted polypyrrole was twice as low

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as that reported in literature, however the data in this work was verified by triplicate experiments ($S = 3.08 \times 10^{-13} \pm 1.58 \times 10^{-13}$). Polypyrrole was readily functionalized with a molecular imprint using simple one pot electrochemical synthesis. The comparative study between MIP and NIP sensors has highlighted the advantages of the imprinting modification method in terms of sensitivity and dynamic linear range. It is therefore reasonable to propose the molecular imprinting methodology to develop polypyrrole sensors systems with improved analytical behavior for the detection of polyaromatic hydrocarbons. Polyaromatic hydrocarbons are notoriously challenging to quantify using simple chemical sensor systems.

5.2   Results and discussion of Imprinted Polymer sensor towards PAHs

5.2.1   Pyrene

Pyrene is one of the well-known pollutants which are part of the EPA priority list. It is a four membered ring resulting in a flat aromatic system. The structure of pyrene is shown below along with other four membered ring PAHs.
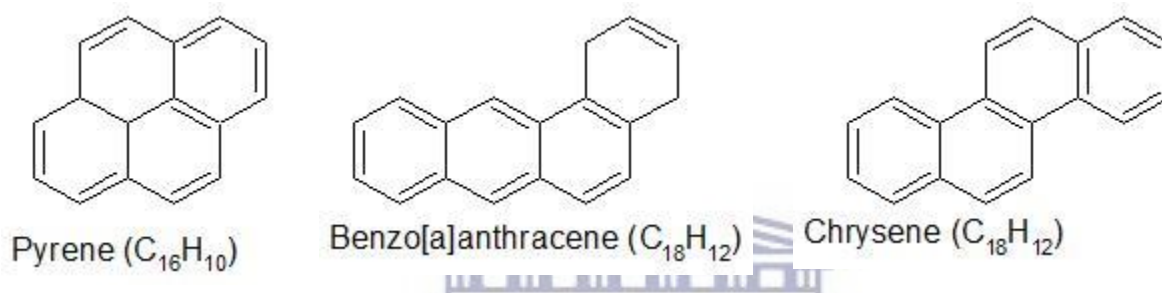
Figure 22: Pyrene and its analogous molecules

The classical three system electrode was used with pyrene-imprinted polypyrrole as the working electrode, Ag/AgCl as a reference and Pt-wire as the counter electrode. Standard additions of pyrene were added to the reaction cell and stirred for 60 seconds. Molecular recognition of pyrene was performed using cyclic voltammetry in order to evaluate the analytical response of the pyrene-imprinted polypyrrole sensor. Pyrene electrochemistry at non imprinted polypyrrole showed one oxidation and reduction peak, of which the reduction peak was associated with the oxidation of pyrene.

The cyclic voltammogram of pyrene shows an oxidation peak of the polymer between 0 – 0.5V and the reduction peak between 0 – 1 V, indicating a reversible reaction. The change in peak current between 0 – 1V vs Ag/AgCl occurred as a result of consecutive concentration addition which was then used to plot a calibration curve. The analytical response of pyrene was investigated in the concentration range of 6.2 $\times 10^{-7}$ M – 7.4 $\times 10^{-6}$ M. The results for the non-
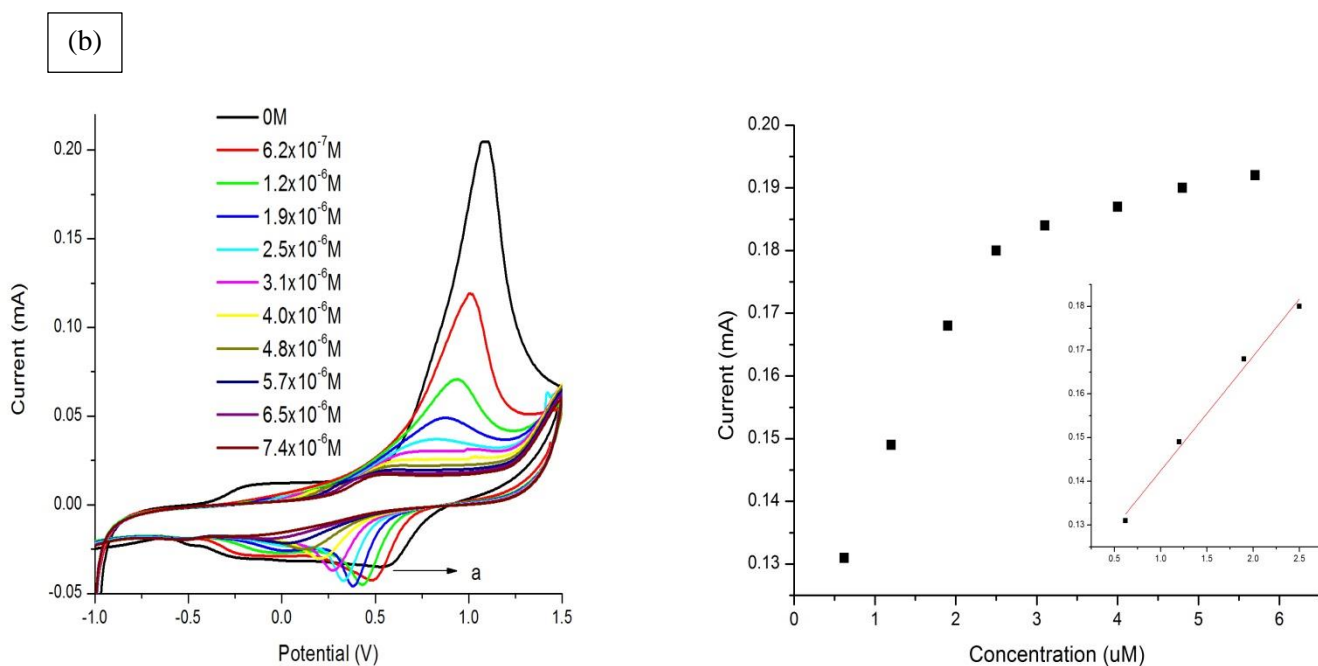
Figure 23: Cyclic voltammogram of (a) MIP sensor detection of pyrene by PPy/GCE vs Ag/AgCl at a scan rate of 50mV/s in 0.1M HCl solution (b) calibration curve of MIP sensor plotted from the adsorption of Pyrene in (a).

imprinted sensor displayed a limit of detection of 4.23×10^{-7} M (S/N = 3) and a linear concentration range of 6.2×10^{-7} M – 1.9×10^{-6} M, whereas MIP showed the LOD of 2.28×10^{-7} M (S/N = 3) and linear concentration range of 6.2×10^{-7} – 2.5×10^{-6} M.

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5.2.2 Pyrene MIP vs NIP

In a similar experiment, where an imprint of pyrene was developed in the polypyrrole thin film (i.e. MIP) the MIP electrode was used as the working electrode in the 3 electrode system. The same concentration range as for the development of calibration curve at the NIP electrode was used, for direct comparison
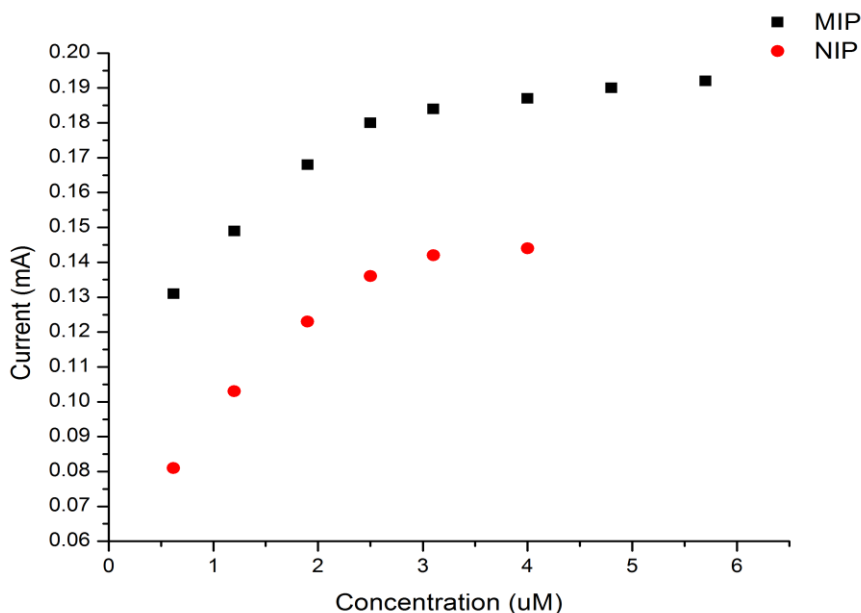
Figure 24: Shows comparison of calibration curves of MIP and NIP for pyrene detected by PPy/GCE vs Ag/AgCl at a scan rate of 50mv/s in a 0.1 M HCl.

The results displayed that, the MIP sensor has a limit of detection of 2.28×10^{-7} M while its linear concentration range is $6.2 \times 10^{-7} – 2.5 \times 10^{-6}$ M. The MIP sensor showed sensitivity that was two times that of the NIP sensor. The sensitivity of MIP was found to be 32.528 A/M and that of NIP was found to be 14.479 A/M. Therefore these results suggest that, MIP sensor is superior to NIP sensor for this experiment. In similar work reported by Levent Ozcan et al[26] for the determination of paracetamol at an electropolymerized-molecularly imprinted polypyrrole modified pencil graphite electrode. Their results showed that the sensitivity of the paracetamol-imprinted polypyrrole was 14.821 A/M and the non-imprinted sensor had sensitivity of 10.231

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A/M which is inferior to MIP. Therefore these results could be comparable with the ones obtained from our study. The advantages of the MIP sensor from this experiment are; high selectivity and sensitivity towards the template molecules.

The table below summarizes the parameters which were calculated from calibration curves of both NIP and MIP of pyrene.

Table 2: Summarizes analytical response of the pyrene MIP and NIP sensors.

Parameter	MIP	NIP
Limit of detection	2.28×10^{-7} M	4.23×10^{-7} M
Limit of quantification	6.92×10^{-7} M	1.28×10^{-6} M
Linear dynamic range	$6.2 \times 10^{-7} – 2.5 \times 10^{-6}$ M	$6.2 \times 10^{-7} – 1.9 \times 10^{-6}$ M

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5.3 Benzo[a]pyrene

Benzo[a]pyrene is found in the environment and in food. It is a five membered ring and it is a pale yellow crystalline solid with a faint aromatic smell. It is known for its insolubility in water and has low volatility. The structures shown below are of benzo[a]pyrene and other analogous five membered ring PAHs.

Benzo[a]pyrene ($C_{20}H_{12}$) Dibenzo[a,h]anthracene ($C_{22}H_{14}$) Benzo[e]pyrene ($C_{20}H_{12}$)
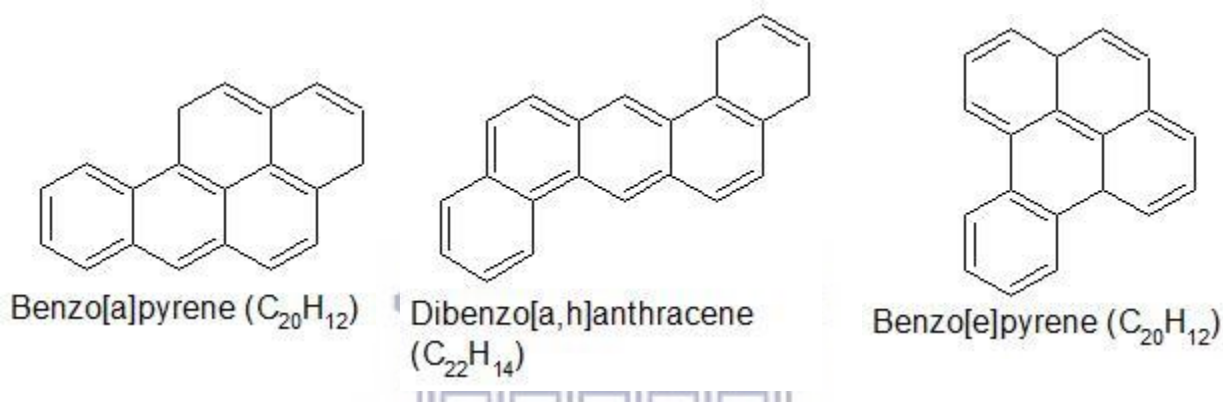
Figure 25: Benzo[a]pyrene and its analogous molecules.

The classical three electrode system was used in this experiment too, where the benzo[a]pyrene-imprinted polypyrrole was a working electrode, Ag/AgCl as the reference electrode and the Pt-wire as the counter electrode. Standard additions of benzo[a]pyrene were perfomed and after each addition the solution was stirred for 60 seconds. In this experiment we shifted from using cyclic voltammetry to using square wave voltammetry due to the observation of unrecognizable peaks from CV. Therefore a much more sensitive technique was due. Therefore the analytical response of benzo[a]pyrene was investigated using SWV.

The NIP sensor of benzo[a]pyrene displayed two reduction peaks, where the prominent one between $0 - 1.0$ V was associated with the polymer peak and benzo[a]pyrene peak was around 1.5 V. The peak which appears on the SWV (at 1.5 V) can be attributed to the respective steps in the oxidation mechanism of benzo[a]pyrene. There is peak current change at 1.5 V which is due

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to the consecutive concentration additions and that current change was used as a signaling peak which was used to draw the calibration curve. The calibration curve of the NIP sensor showed a limit of detection of 1.59×10^{-6} M and a linear concentration range of $6.2 \times 10^{-7} - 2.5 \times 10^{-6}$ M from chapter 4. The MIP calibration curve showed a LOD of 2.51×10^{-7} M and linear concentration of $6.2 \times 10^{-7} - 3.1 \times 10^{-6}$ M.

The benzo[a]pyrene-imprinted polypyrrole sensor showed similar results with that of the NIP because benzo[a]pyrene oxidize at 1.5V. The oxidation peak of benzo[a]pyrene 'a' does not illustrate a satisfactory peak current increase, that means there is no optimum detection of benzo[a]pyrene by the sensor as expected.
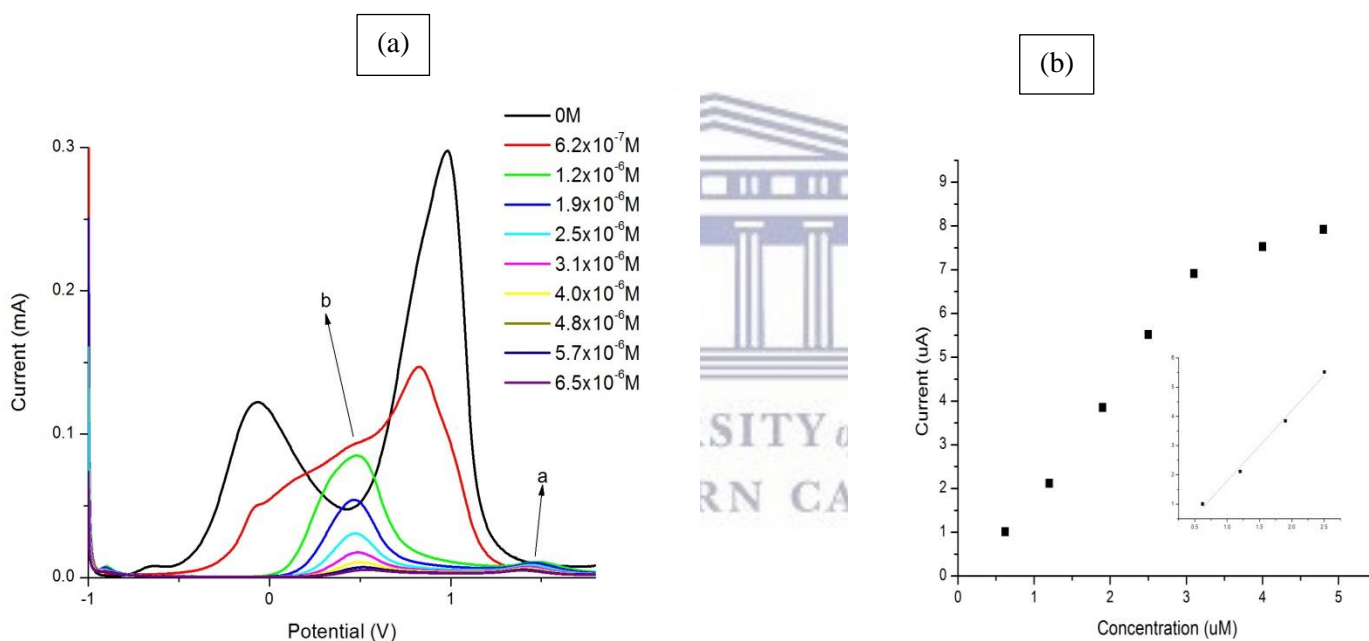
Figure 26: Shows a square wave voltamogram of (a)MIP sensor detecting BaP using SWV in at a scan rate of 50mV/s in 0.1M HC solution. (b) Calibration curve plotted from the SWV in (a).

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5.3.1 BaP MIP vs NIP

The NIP and MIP sensors for benzo[a]pyrene were performed in the same concentration range of 6.2×10^{-7} M – 6.5×10^{-6}M for comparison. The calibration curve shows that, MIP sensor detects at slightly higher current than NIP sensor, however there is no much difference in terms of sensitivity, limit of detection and linear concentration range between the two sensors. The limit of detection for the benzo[a]pyrene imprinted polypyrrole was 2.51×10^{-7} M and a linear concentration range of 6.2×10^{-7} – 3.1×10^{-6} M.
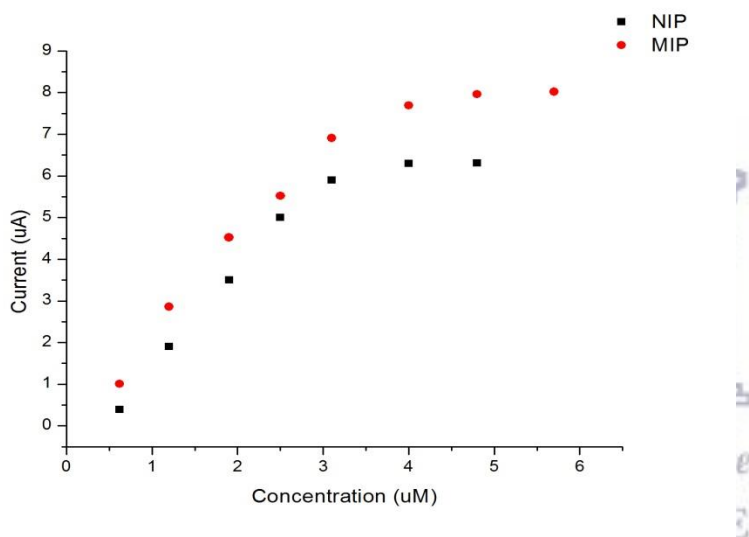
Figure 27: Shows a comparison between the MIP and NIP calibration curves obtained at a scan rate of 50mV/s in a 0.1M HCl solution.

The sensitivity of both MIP and NIP were taken from the slopes of the plotted calibration curves respectively. MIP sensor's sensitivity was found to be 2.409 A/M and NIP was 2.014 A/M. theoretically and from literature MIP is expected to have excellent sensitivity than NIP. This unusual behavior observed from the benzo[a]pyrene imprinted sensor can be accounted for in terms of the analyte molecule and recognition cavity geometry. Imprinted recognition cavities are sensitive to shape, size and chemical functionality of the target molecule, therefore any changes or disturbance on the cavities or the target molecule affects the affinity of the cavities towards the target molecule. The reason for having such poor selectivity from the MIP sensor

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may be due to the disturbance of the cavity size and shape or target molecular shape, size and functionality during the reaction. Additionally benzo[a]pyrene has a large molecular weight, therefore it takes time to fully dissolve, which may have affected the creation of the cavities while washing off the analyte.

Li Ying et al [42] encountered a similar situation while working with a MIP sensor based on graphene-oxide, where the MIP sensor showed no excellency than NIP sensor, although it had higher sensitivity. Their suspicions to these strange results were that, the recognition cavities may have been blocked by the solvent used to wash off the analyte molecue. The table below gives summary of the analytical response of the MIP and NIP sensors of benzo[a]pyrene.

Table 3: Summary of some parameters which were obtained from the two calibration curves in figure 20.

Parameter	MIP	NIP
Limit of detection	2.51×10^{-7} M	1.59×10^{-7} M
Limit of quantification	7.57×10^{-7} M	4.82×10^{-7} M
Linear dynamic range	$6.2 \times 10^{-7} - 3.1 \times 10^{-6}$ M	$6.2 \times 10^{-7} - 2.5 \times 10^{-6}$ M

5.4 Anthracene

Anthracene is a three membered ring PAH which has been identified as the simplest PAH by the USA's EPA. It is ubiquitous in the environment as a product of incomplete combustion of fossil fuels. It is the simplest tricyclic aromatic hydrocarbon. Anthracene is a colorless crystalline compound. It is soluble in a variety of organic solvents, including ethanol, methanol, benzene, toluene, and carbon disulfide, but is almost insoluble in water. The following structures shows anthracene and other three membered ring PAH.
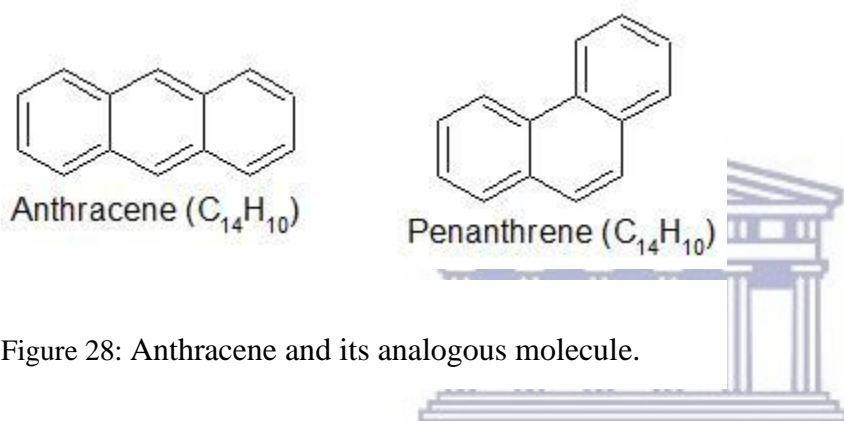
Figure 28: Anthracene and its analogous molecule.

In this experiment just like the others, the classical three electrode system was used where anthracene-imprinted polypyrrole was the working electrode, Ag/AgCl was a reference and the Pt-wire was a counter electrode. Standard additions of anthracene were inserted in the reaction cell and stirred for 60 sec. Square wave voltammetry was used instead of cyclic voltammetry for similar reasons as in section 5.2.3

The NIP sensor for anthracene form chapter 4 showed two reduction peaks, where the one between 0 – 1V was a polymer peak and the one at 1.3 V was an anthracene peak. The same behavior for the anthracene-imprinted polypyrrole has been observed. The anthracene in the MIP sensor oxidizes at 1.3 V at high current than the NIP sensor. The was a current change at peak 1.3V due to the concentration additions and that current change was treated as the signaling peak which allowed us to draw a calibration curve. The NIP results from the calibration showed a limit of detection of 4.33×10^{-7} M and a linear concentration range of $6.2 \times 10^{-7} – 1.9 \times 10^{-6}$ M. On the other hand the MIP experiments has showed a LOD of 1.48×10^{-7} M` (S/N = 3).

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Anthracene-imprinted polypyrrole sensor was fabricated in the same concentration range as NIP for the detection of anthracene. There is significantly high difference between the MIP and NIP sensor for detection of anthracene as shown on the CV below that, the analytical response of the anthracene is at high current (peak a) than that of NIP sensor.
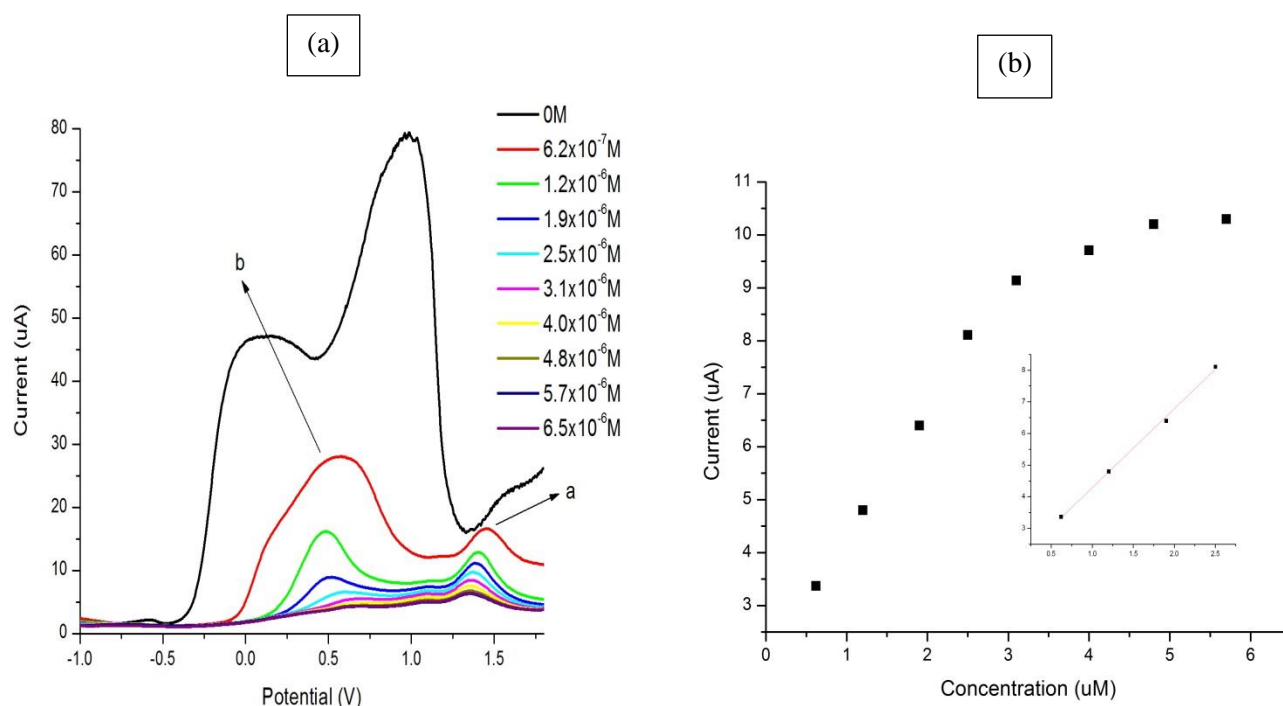
Figure 29: Shows a square wave voltammogram of (a) MIP sensor detection of Anthracene by PPy/GCE vs Ag/AgCl in a 0.1M HCl solution at a scan rate of 50mV/s (b) calibration curve plotted from the anthracene adsorption in (a).

In this experiment the MIP sensor has performed very well than NIP sensor from the previous chapter as expected and it is clear that anthracene adsorbs at 1.3 V just like with the NIP sensor. The oxidation peak 'a' shows the adsorption of anthracene and from there a calibration curve was obtained. These results are a proof of superiority of MIP sensor than NIP sensor

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5.4.1 Anthracene MIP vs NIP

The analytical response of both NIP and MIP showed that, the anthracene-imprinted polypyrrole has outdone the NIP sensor in terms of perfomance. The improvement of the surface by imprinting increased the sensors sensitivity towards the anthracene. Calibration curves obtained from both NIP and MIP are plotted below in the same concentration range of $6.2 \times 10^{-7} - 6.5 \times 10^{-6}$ M for comparison.
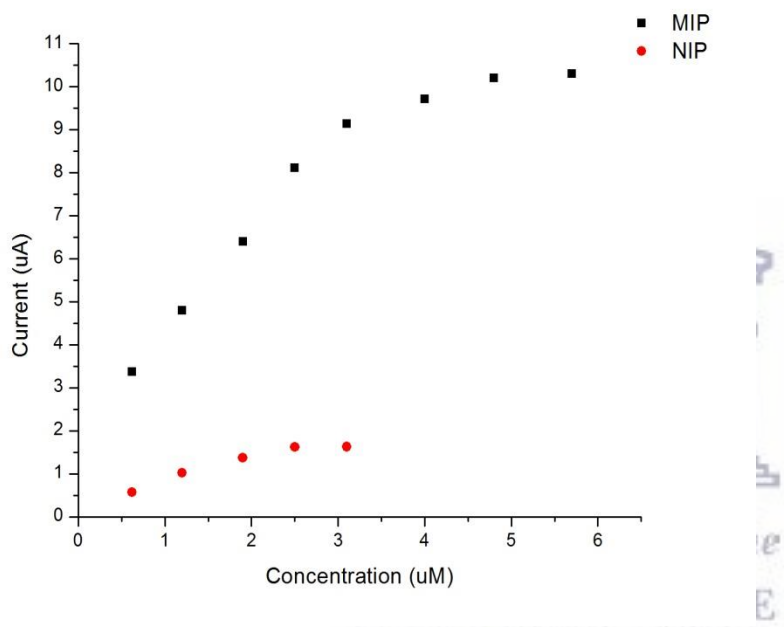
Figure 30: Shows a comparison between a MIP and NIP sensor's calibration curve in detection of anthracene.

The calibration curves of these sensors (i.e MIP and NIP) clearly show that, MIP detects at much higher current than NIP. From the calibration curve the sensitivity of the MIP was found to be 2.493 A/M whereas NIP's sensitivity was found to be 0.553 A/M. The limit of detection of the MIP sensor was 1.48×10^{-7} M`(S/N = 3) and the linear concentration range of $6.2 \times 10^{-7} - 3.1 \times 10^{-6}$M. The table that follows summarizes the analytical response of the anthracene-imprinted polypyrrole.

Table 4: Some parameters which were calculated from the calibration curves plotted in figure 22.

Parameters	MIP	NIP
Limit of detection	1.48×10^{-7} M`	4.33×10^{-7} M
Limit of quantification	4.51×10^{-7} M	1.31×10^{-6} M
Linear dynamic range	$6.2 \times 10^{-7} – 3.1 \times 10^{-6}$ M	$6.2 \times 10^{-7} – 1.9 \times 10^{-6}$ M

5.5   Electrochemical behavior summary

Molecularly imprinted polypyrrole has been widely used in sensors for the detection of different species from different matrices. L Ozcan et al [26] also used polypyrrole during their study of determination of paracetamol based on electropolymerized-molecularly imprinted polypyrrole modified pencil graphite electrode. In their study they have produced a sensor for paracetamol and reported that, the MIP sensor for paracetamol gave a limit of detection of
7.9×10^{-7} M (S/N = 3). The linearity of concentration range for the MIP and NIP were $5 \mu M -$
$0.50 \, mM$ and $50 \mu M - 0.50 \, mM$ and the current range of NIP was found to be much smaller than that of MIP.

On the other hand, Vinod Gupta et al [43] on their study of molecularly imprinted polypyrrole modified glassy carbon electrode for the detection of tobramycin reported on the excellence of MIP over NIP sensors. They have reported the linearity of concentration of MIP and NIP to be $5.0 \times 10^{-10} – 1.0 \times 10^{-8}$ M and $75 \times 10^{-10} – 5.1 \times 10^{-9}$ M. The limit of detection for the MIP sensor was calculated to be 1.4×10^{-10} M (S/N = 3).

The results of our study are comparable with those in literature. The sensitivity MIP sensors for anthracene, benzo[a]pyrene and pyrene for our study were found to be 2.493 A/M, 2.409 A/M and 32.528 A/M and limit of detections of 1.48×10^{-7} M, 2.51×10^{-7} M and 2.28×10^{-7} M respectively (S/N = 3). These results that we obtained from our study are of the same magnitude with those found in literature. The differences in the results we obtained from those in literature are due to the use of different electrolytes, electrodes and we are detecting different analyte species.

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5.6   Molecularly imprinted polymer Interference studies

The selectivity of the MIP electrode in this work was evaluated in the presence of analogous PAHs. This experiment was done with the use of the classical three electrode system, where the pyrene-imprinted polypyrrole acted as a working electrode, the Ag/AgCl as a reference and the Pt-wire was a counter electrode.

In order to investigate the interference of chrysene in a pyrene-imprinted sensor the sensor was subjected to the 4 membered ring chrysene which is similar to pyrene. This technique is used to test if the created recognition cavities are only selective to the imprinted molecule, nothing similar to it and to evaluate if there are no species that will interfere with the recognition cavities.

 L Ozcan et al[26] have made use of this technique to evaluate the selectivity of MIP based sensor for the detection of paracetamol in $LiClO_4$ solution in the presence of interfering substances like ascorbic acid and D-glucose. Their results revealed that, there were no substantial changes in the current response for paracetamol in the presence of ascorbic acid and D-glucose. This paper further confirmed the principle of selectivity in molecularly imprinting.

It has been proven from their results that, an imprinted sensor should only have affinity to the template molecule and nothing analogous to the template molecule. Our study also seeks to test if chrysene will not interfere with pyrene on a pyrene-imprinted polypyrrole sensor.
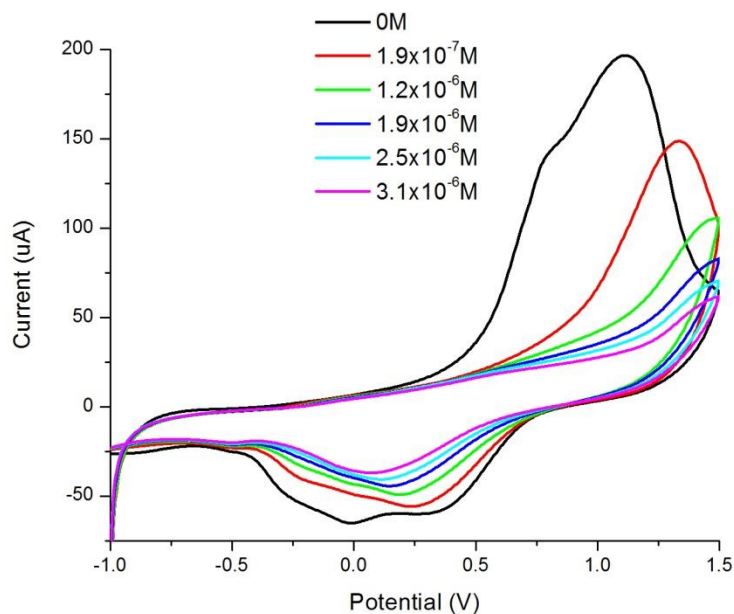
Figure 31: Cyclic voltamogram showing interference of chrysene on a pyrene imprinted sensor at a scan rate of 2mV/s.

The above cyclic voltammogram shows the analytical response of a pyrene-imprinted sensor in the presence of chrysene. The scan rate of the above experiment was brought to 2 mV/s so that the analyte can have enough time to adsorb on the electrode cavities.We have seen previously, that pyrene shows oxidation behavior between 0.5 – 1.0 V. The oxidation signal due to the presence of the chrysene is displaced outside of the expected detection range of pyrene.
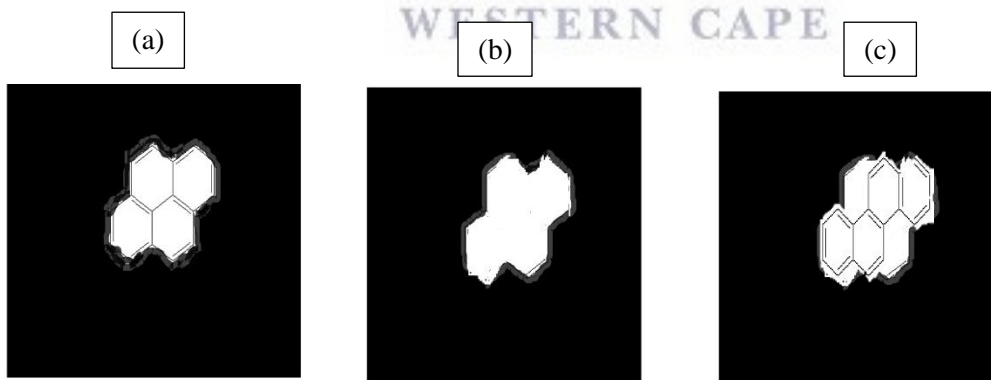
Figure 32: Shows (a) pyrene-polypyrrole, (b) pyrene-imprinted polypyrrole sensor and (c) pyrene-imprinted polypyrrole sensor subjected to chrysene for detection.

The diagram in figure 30 above shows that, a pyrene-imprinted polypyrrole sensor was prepared for the detection of chrysene. The diagram shows that, chrysene is not a perfect match for the pyrene cavities, therefore it was not detected. Moreover due to the non-complementarity between the pyrene imprint and the chrysene analyte the oxidation peak appears at a high oxidation potential or energy, indicating that the chrysene is not the preferred substrate for the imprint as illustrated in figure 30 above.

The second experiment was done by mixing solution of 0.0005 M pyrene and 0.0005 M chrysene. This experiment was to test if chrysene would interfere during the detection of pyrene.
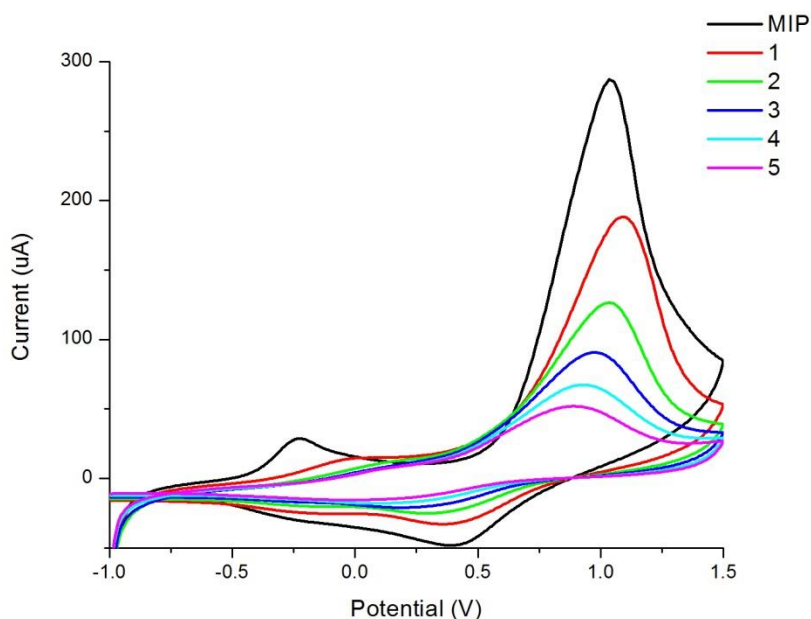
Figure 33: Cyclic voltammetry of chrysene redox behavior at a pyrene imprinted polypyrrole sensor.

The cyclic voltammogram above shows that, even though there is presence of chrysene within the mixed solution the pyrene imprinted cavities can still recognize pyrene and chrysene does not interfere with the detection. This is observed because the same oxidation peak that was observed from a pyrene-imprinted sensor is still present and the signaling peaks can allow us to plot a calibration curve as we have done before. A calibration curve of pyrene and a mixture of pyrene and chrysene were plotted, our interest from the calibration curve was sensitivity.

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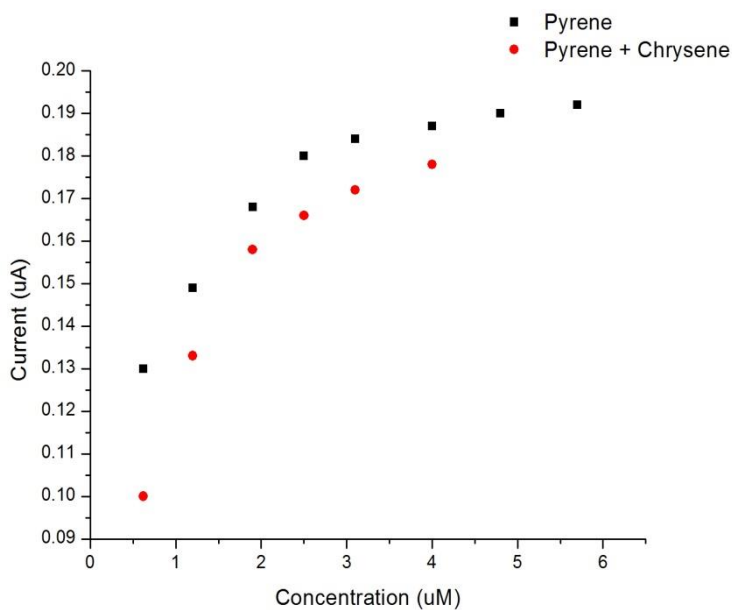
Figure 34: Illustrates a pyrene-imprinted sensor sensing a pure pyrene and a mixed solution of pyrene + chrysene.

The calibration curves show no greater difference between the pyrene and the mixture of pyrene and chrysene. The sensitivity of pyrene MIP sensor was reported as 32.528 A/M in section 5.22. The sensitivity of the mixed solution of pyrene and chrysene gave a sensitivity of 31.012 A/M. Theoretically the sensitivity of the sensor for the mixed solution (pyrene+chrysene) and pyrene should be almost the same, and that is shown by the sensitivities obtained for the two. This illustrates that, imprinted polymers are able to discriminate between analytes based on structure and are highly selective for the template molecule.

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Chapter 6

Generally when working with MIPs ones concerns should be the recognition cavity creation, and there has to be a manner in which it is tested that the cavities are created or else there will be no optimum selectivity and sensitivity. Spectroscopic and morphological information is useful to understand the nature and surface features of sensors. The stability of the analyte under experimental conditions also requires verification

6   Atomic Force Microscopy

AFM was performed on a glassy carbon electrode surface. The idea behind this technique was to study the polymer's topography to investigate if the recognition cavities are created for the MIP sensor. This technique has been used for similar purposes by Li Yings et al [42] when they made a graphene-oxide molecularly imprinted polymer based sensor for the detection of endocrine disrupting chemicals. Their study indicated that, the NIPs topography was found to be smooth and regular, whereas the MIPs topography was found to be irregular. The irregular topography from the MIP was associated with the recognizing cavities while the smoothness of the topography from NIP was associated with the absence of cavities. In this study AFM images were taken for the bare electrode, polymer with pyrene and pyrene-imprinted polypyyrole.
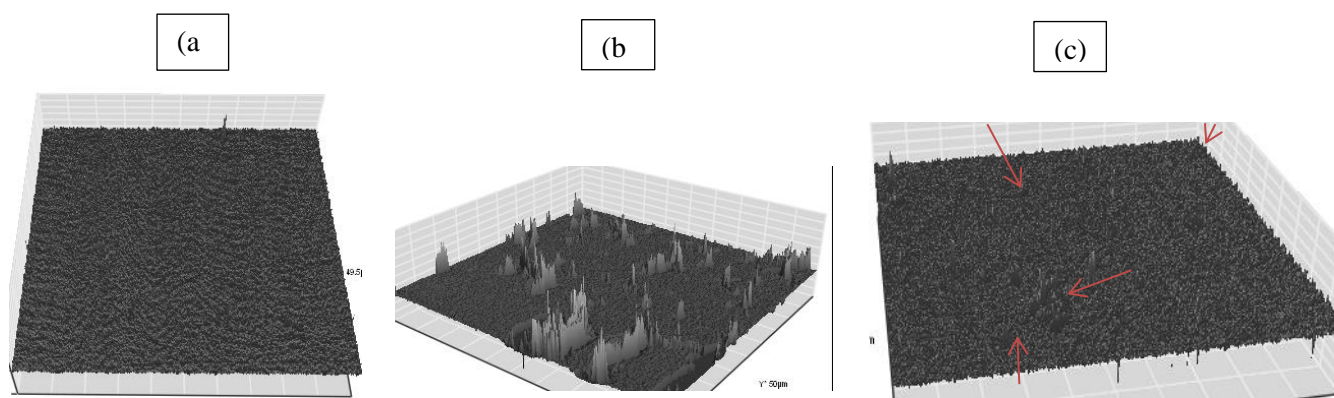
Figure 35: Shows AFM images of (a) GCE bare (b) MIP with analyte (c) MIP with cavities.

The results that we obtained also showed topographic differences between the (a) bare electrode, (b) polymer + pyrene and (c) pyrene-imprinted polypyrrole. Considering figure 31b&c it is evident that, before pyrene was washed of (figure 31b) the topography was rough and irregular, after pyrene was washed off the topography changed to a smooth surface with dark spots indicated by the arrows in figure 31c. These dark spots created after pyrene removal may be associated with the imprints of pyrene.

The results obtained for this study using AFM are satisfactory because we expected to see topographic changes on the electrode surface between the pyrene-polypyrrole and pyrene-imprinted polypyrrole. Although the cavities are not as clear since AFM does not scan the whole surface of the electrode at once. Moreover the recognizing cavities are not created evenly on the electrode surface.

6.1 Scanning Electron Microscopy (SEM)

SEM was used to study the surface morphology and the particle size of the NIP and MIP, to further confirm that the recognition cavities have been created as the AFM confirmed. G Gonzalen et al [28] have used the same technique to study molecularly imprinted polymers morphology. In their study they have reported that, the NIP electrode showed narrow and clustered particles whereas the MIP electrode showed much thicker particle size and there were spaces created in between the particles. Additionally the spaces created in the electrode surface were irregular and they were said to form due to the cavities. The images below show SEM images before and after the removal of pyrene.
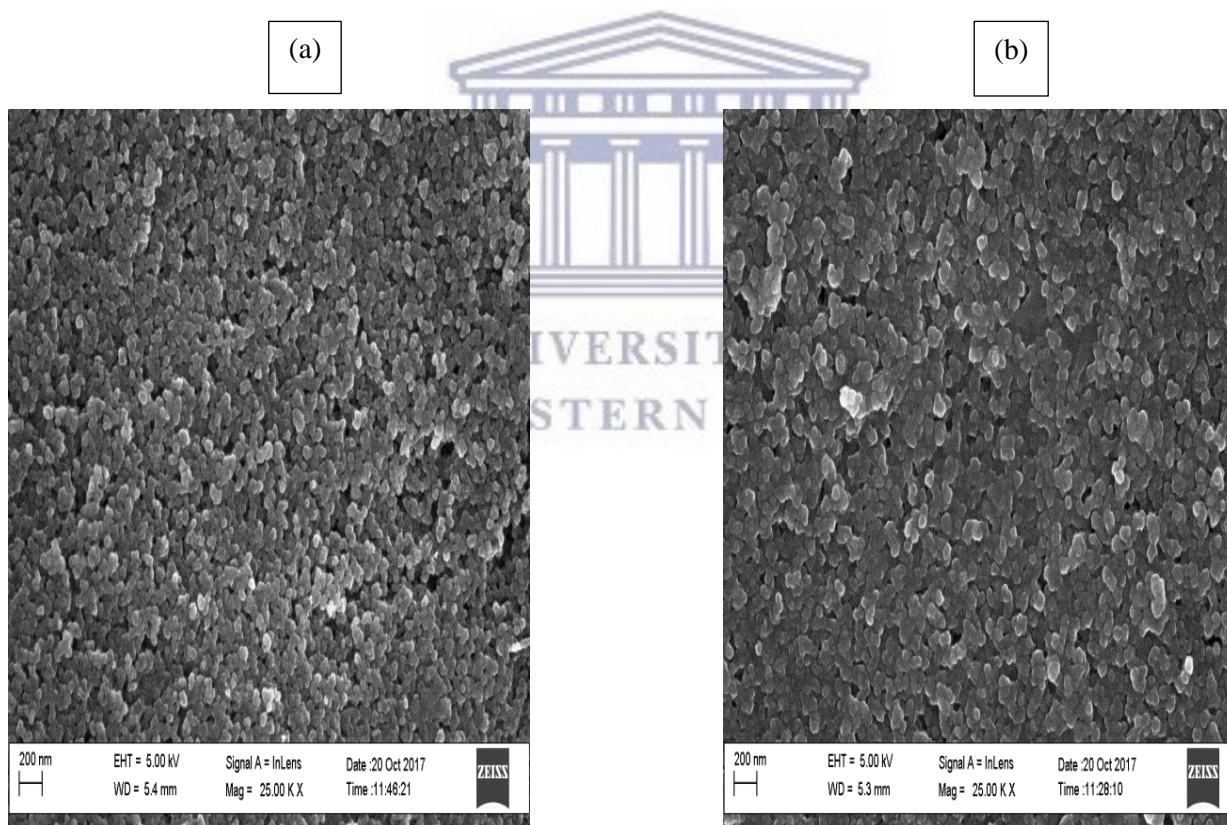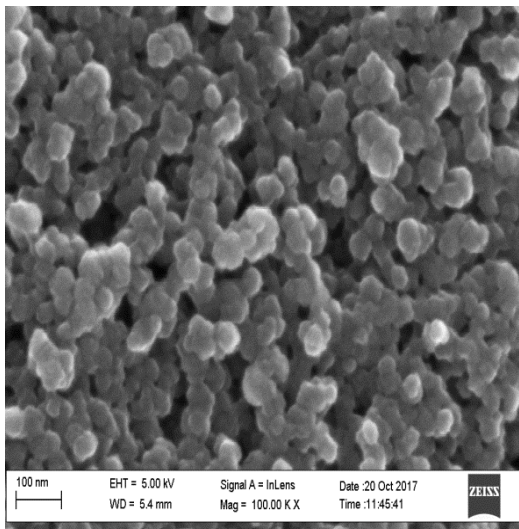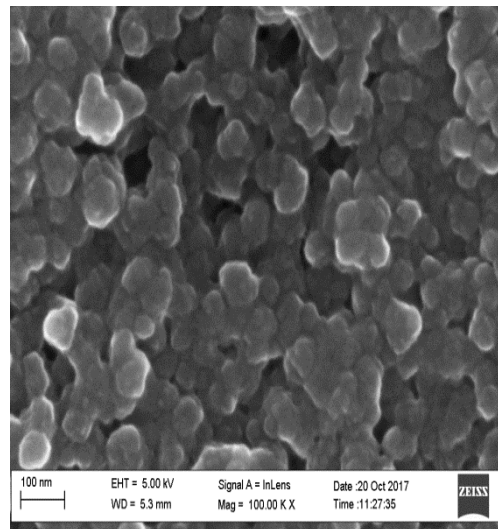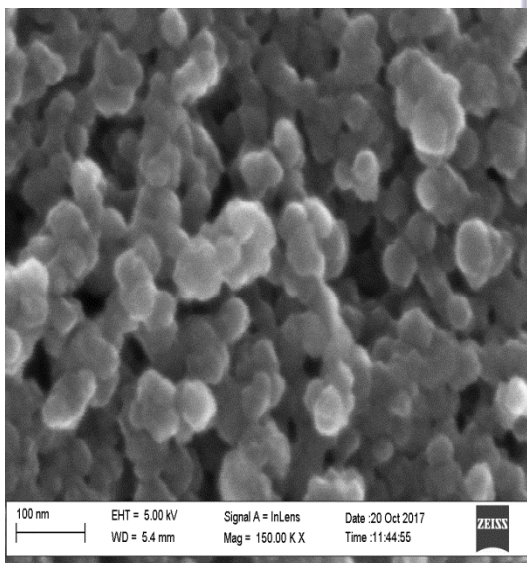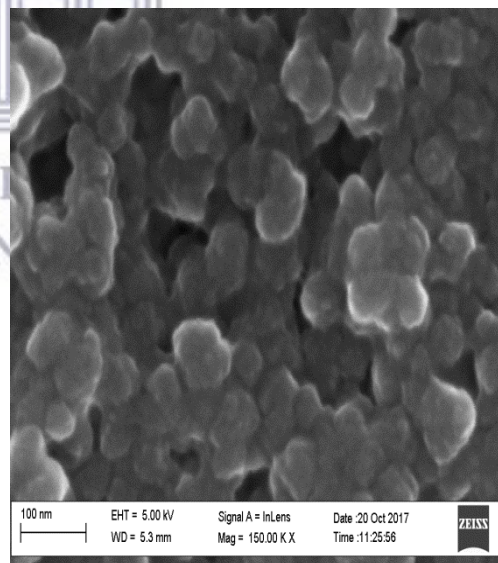
Figure 36: SEM images before (a, c, e) and after (b, d, f) pyrene removal.

The results which we obtained for the SEM experiments are in agreement with the ones reported by G Gonzalen et al [2]. Prior the removal of pyrene, the particle size was small and regular and then when pyrene was removed the particle size changed to thick and irregular. The MIP looked rather rougher and irregular those properties may be attributed to the formation of the recognizing cavities.

Considering figure 31e and 31f it is prominent that, the particle size has changed from small to much bigger particle size. The image in figure 31f shows a hollow space which may be associated as one of the potential cavities for the recognition of pyrene. Additionally the roughness of the pyrene-imprinted polymer surface can lead to higher surface are than that of the non-imprint and therefore MIP can attract and enclose pyrene better than NIP.

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6.2 UV-visible spectroscopy of anthracene, benzo[a]pyrene and pyrene.

UV-visible spectroscopy has been widely used to monitor pollutants which are contaminants in the environment. In this study we have used UV- spec to characterize anthracene, benzo[a]pyrene and pyrene.

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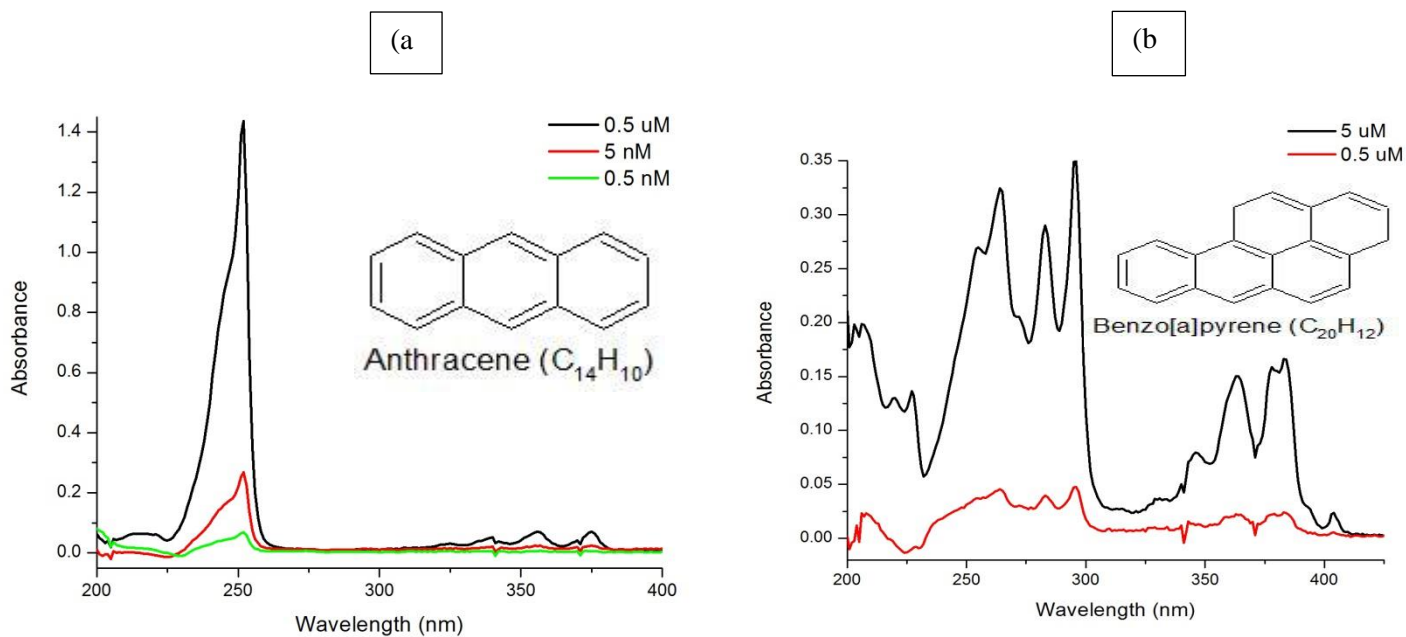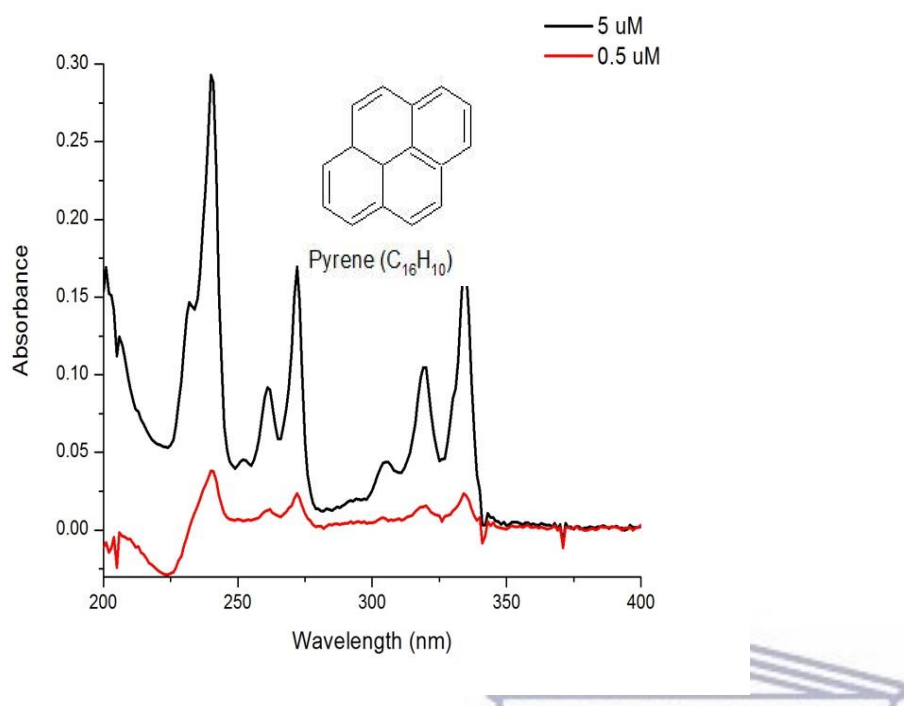
Figure 37: Shows UV images of (a) anthracene (b) benzo[a]pyrene and (c) pyrene

The results above show fingerprints of each of the PAHs i.e anthracene, benzo[a]pyrene and pyrene. However there are too many peaks in all three spectrums for the PAHs, therefore it would be difficult to clearly identify the individual PAHs using UV-vis. This brings us to a conclusion that, the use of MIP based electrochemical sensor would be viable for the detection of the PAHs. Moreover literature shows that UV-visible spectroscopy has only limited application when working with PAHs, because UV can only distinguish between pure PAHs not mixtures. In most cases in the environment we get mixed PAHs either from the soil or waste water from industries.

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Chapter 7

7   Conclusion

Electrochemical sensors have attracted vast amount of attention since they are viable, robust, inexpensive and easy to prepare. Electrochemical sensors that incorporate molecularly imprinted polypyyrole for the detection of PAHs have been reported in small amount in literature.

In this study we have managed to successfully develop highly selective and sensitive electrochemical sensors based on molecularly imprinted polypyrrole for the detection of anthracene, benzo[a]pyrene and pyrene. However there were two sensors who displayed excellent selectivity and sensitivity towards the PAHs and they are; anthracene-imprinted polypyrrole and pyrene-imprinted polypyrrole. The benzo[a]pyrene-imprinted sensor did not show much satisfactory analytical response.

The anthracene-imprinted polypyrrole displayed satisfactory analytical response with the LOD of $1.48x10^{-7}$ M`(S/N = 3), the linear concentration range was $6.2x10^{-7} – 3.1x10^{-6}$M and the selectivity of 2.493 A/M.

The second excellent sensor was the pyrene-imprinted polypyrrole which showed an analytical response with the LOD of $2.28x10^{-7}$ M (S/N =3), a linear concentration range of $6.2x10^{-7} – 2.5x10^{-6}$ M and the selectivity of 14.479 A/M.

The sensitivity of a MIP sensor is highly dependent on the shape and size of the molecule or recognizing cavities [1]. Benzo[a]pyrene displayed unsatisfactory results where the MIP and NIP sensors showed almost the same sensitivity of 2.409 and 2.014 A/M respectively. This analytical response may be influenced by changes on the shape of the cavities after the removal of benzo[a]pyrene. Once there are disturbances or changes (physically and chemically) on the shape or size of either the molecule of interest or cavities, there will be no optimum detection because the analyte molecule will not fit on the designated imprinted cavity. Moreover benzo[a]pyrene

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has a larger molecular weight, therefore it takes time for it to dissolve, and that must have caused incomplete template removal during the creation of the benzo[a]pyrene-imprint.

Selective recognition towards the template molecule is an important capability for a MIP sensor [2]. Selectivity of the pyrene-imprinted sensor was evaluated by cyclic voltammetry in the presence of its analogous molecule i.e chrysene. The results showed that, the pyrene-imprinted polypyrrole sensor exhibited higher recognition selectivity towards pyrene with no interference to chrysene.

Microscopic techniques such as AFM and SEM were used to study the morphology, topography and particle size of the sensors. The results successfully confirmed the creation of the recognizing cavities which were associated with the rough and irregular morphologies of the sensors. AFM further showed dark sports that were almost circular in shape and they were described as the potential recognizing cavities. The roughness of the MIP particles has proved to lead to high surface area than that of NIP and thus MIP can adsorb better than NIP.

The imprinted polymer sensors displayed strong favorable properties such as having a wide linear concentration range, low limit of detection, high sensitivity and selectivity than the non-imprinted polymer sensors. Additionally these types of sensors (i.e MIP) are easily reproducible and stable.

Apart from anthracene, benzo[a]pyrene and pyrene, 1-hydroxypyrene is one of the famous PAH in petrochemical industry. Albers PH et al [3] conducted a study based on exposure to PAHs in petrochemical industry by measurement of urinary 1-hydroxypyrene. This study was carried out because workers who were involved in manufacture and maintenance operations in oil refineries got sick with lung cancer. The sickness was then attributed to the exposure to 1-hydroxypyrene.

Urinary samples from the oil company employees were collected and analysed using HPLC. Their results showed that high levels of 1-hydroxypyrene were present to workers who work directly on the oil plant than those who are in offices. In each worker an amount of about 0.99

mM of 1-hydroxypyrene was found whereas an amount of 0.22 mM was found on those working in offices and other parts of the company far from the plant.

The Agency for Toxic Substances and Disease Registry (ATDSR) gave a report from their study at a hazardous waste land treatment site for refinery that, the limit of detection of anthracene in soil was to be 0.1 – 2 mM[29]. This limit of detection was acquired from UV-visible spectroscopy and it is not in the same range as the one acquired in this study. The reason behind such difference is that, in the soil there were other PAHs present, therefore UV is not capable to detect when PAHs are mixed.

In this study we have developed sensors that have illustrated to be detecting on a wide concentration range. These sensors detects at low concentrations as close to nano scale. Their application in petrochemical industry could be viable as they can save time since they are easy to prepare, to use and they are not laboratory bound just like HPLC. Their ability for specific recognition is paramount, since other techniques such as UV-vis cannot detect specific PAHs of interest in the presence of other analogous PAHs. Additionally literature does not have much studies reported based on the use of molecularly-imprinted polypyyrole for the detection of PAHs, therefore this study comes as one of the rare studies.

In the near future, it would be viable to have the sensors that we developed tested in detecting PAHs from actual samples collected from the petrochemical industry. The samples could be waste water, soil and urine of workers in the petrochemical industry. This would allow us to see the actual application of our sensor in real world. HPLC would also be incorporated in that study where MIP based sensors do the detection of the present PAHs in that particular sample and HPLC works with the extraction of the PAHs of interest from the sample. Additionally since these PAHs are also found in the air, it would be good to develop a sensor that would be able to detect gaseous PAHs just like we have done for liquid phase PAHs.

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