Multi-array sensor technology for polycyclic aromatic hydrocarbons screening in wastewater

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by

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EXECUTIVE SUMMARY

BACKGROUND

Polyaromatic hydrocarbons (PAHs), also called polycyclic aromatic hydrocarbons, have been included in the overarching definition of persistent organic pollutants (POPs). POPs are, typically, organic compounds that are not easy to degrade using conventional methods and which pose a serious threat to the balance of natural ecosystems. The need for low-level detection and quantification of these compounds still presents serious challenges currently, notwithstanding the advances in technology materials and methods that have been made, over the years. The class of compounds known as PAHs is typically made up of multiple fused ring systems and presents as multiple species in environmental matrices. Various factors have an effect on the persistence of PAHs in the environment, such as their chemical structure and their concentration and dispersion as well as environmental properties which include soil type and structure, pH, temperature and oxygen levels. PAHs are deposited into surface water and sediments in different ways including atmospheric deposition, urban runoff, municipal and industrial effluents, and oil spillage or leakage. Due to the low solubility of PAHs in aqueous media as well as their strong hydrophobic nature, PAHs associate with particulate material in the aquatic environment and ultimately reside in underlying sediments. PAHs are well known for their strong mutagenic, carcinogenic and toxic properties and prolonged exposure poses a serious risk to humans. A detailed review of the occurrence of PAHs in water, their physical and chemical properties, and, in particular, the limited applications of electrocatalytic systems for their detection and quantification, was reported as part of this study.

RATIONALE

The analytical treatment of PAHs is challenging due to the hydrophobic nature of these compounds, their high molecular weight and low volatility. An ever-increasing list of individual analytes that need to be quantified, and increasingly stringent detection level requirements, demand a properly optimised analytical system and method. Whilst there are classical chromatographic methods which give very reliable and reproducible quantification of mixed PAH systems, these methods are laboratory based and may employ considerable volumes of organic solvents. Electrochemical methods of analysis and associated equipment development has seen the release of portable and operator-friendly equipment which may be applied for electroanalysis on site and give real-time information for monitoring ecologically sensitive aquatic systems. However, even with electrochemical methods, it is still an inherent challenge to obtain a good signal-to-noise ratio in aqueous media and to have efficient and reliable resolution of multiple analytes in composite samples. The major advantage of electrochemical analysis is that simple modification of the electrocatalysts and electrolytic environments can be implemented at very low cost and very quickly and efficiently to provide a multitude of novel electrocatalysts *in situ*. Hence, electrocatalysts can be developed for optimal detection of each of the PAHs and employed within one portable electrochemical system, for detection on site and in real time. The development of a novel class of semi-conductive polymer systems was reported on in this study, with some initial recommendations for single compound detection.

Electrochemical devices work on the principle of electron transfer and charge polarisation between the analyte and the electrode, which, in turn, provides electrical signal (current or potential). Sensors in this category include metal oxide semiconductors, organic semiconductor sensors, electrolytic conductivity sensors and electric permittivity sensors. Array-based sensing approaches discriminate between analyte species based on their individual electrochemical signatures. The strategy is derived from the responses which are acquired from the differential binding interactions of the analytes with the sensor array, through a highly selective receptor. There are different approaches to array sensing; one relies on multichannel analysis of single cells combined in an array assembly, another employs an array of wells (similar to the ELISA technique) with systematic mechanical control of solution properties, materials properties and experimental steps. The multi-array approach requires stabilised laboratory-based equipment and signal de-convolution after acquisition using principle component recognition software. The multichannel approach allows for direct intervention in the electrocatalysts preparation, solution properties and analytical detection of analyte in a step-by-step approach. The multichannel approach was preferred to realise the study objectives, and a detailed description of the approach was provided.

OBJECTIVES AND AIMS

AIM 1

The initial objectives of the current project, therefore, were to establish as a baseline the redox electrochemistry of eight PAH compounds selected from the United States Environmental Protection Agency (U.S. EPA) priority listing. Hence the redox behaviour in acidic medium (HCl) was reported. The analytical performance of the most ubiquitous sensing interface, i.e. carbon, was evaluated in terms of sensitivity and selectivity towards PAH compounds. We then set out to introduce novel polymer composites, which were produced by Schiff base synthesis, as a contribution to new knowledge as well as a potential catalyst for the redox behaviour of PAH compounds. The synthesis and characterisation of the new polymer was published as a full-length research article (M. Ward et. al., International Journal of

Electrochemical Science 9 (**2014**), 4776–4792). Work on the development of other electrocatalysts continued (X. T. Ngema et.al. Journal of Nano Research, 43, **2016**, 11-22). However, in order to achieve the overall project objectives within the project timeline, we focused all the development on the zig zag polymer system. In a future application study, the advantages of other polymer systems developed in our laboratory will be exploited.

AIM 2

The synthesis and characterisation of the Schiff base compounds, as well as their analytical performance towards PAH detection, was evaluated and reported on in deliverable 2 of this study. The novel polymer electrocatalyst followed a unique zig zag conformation induced by the selective Schiff base cross-linking, and showed enhanced electrochemical activity. A critical review of the literature pertaining to zig zag cross-linked polymer systems revealed that these types of materials were mostly used in free-standing sensing applications (in air) and that their mechanical stability and processibility were the biggest challenges to materials scientists. This review was published as a peer-reviewed, full-length research article (M Ward et.al. Mini Reviews in Organic Chemistry, 12(5) **2015**: 414–42).

AIM 3

The polymers and their electrocatalytic performance towards the detection of PAHs as single analyte species as well as within a mixed sample (cocktail analysis) was evaluated at screen printed carbon electrodes (SPCEs), a type of electrode format which is compatible with the multichannel, portable electrochemical system proposed in the final analytical design. The mixed sample cocktails were evaluated at two different concentrations to evaluate performance and interference due to chemical cross-reactivity, using both electrochemical methods as well as UV/vis spectroscopy. A multichannel protocol was proposed, based on the main research finding at that stage of the project.

AIM 4

The multichannel assembly required acquisition of components as well as in-lab assembly and testing. This process was severely affected by delays in the supply chain, unavailability of technical support as well as the 2016 “fees must fall” protest action. At the time of submission of the final report for consideration by the steering committee, the report on performance of the multichannel system and its capability as a once-off analytical system was incomplete. However, an in-principle demonstration of the available assembled technology, as to be done as a hands-on demonstration as part of the final report evaluation and recommendation by the steering committee. The verification of individual PAH analysis, and analysis within the mixed sample used for electrochemical analysis, using standardised high performance liquid chromatography (HPLC) coupled to a UV/vis spectrophotometer, was reported.

METHODOLOGY

Various electrochemical techniques such as square wave voltammetry, cyclic voltammetry and electrochemical impedance spectroscopy were used. Cyclic voltammetry (CV) was used to determine quantitative information about the electrochemical processes under various conditions, namely the reversibility of the reaction as well as the intermediates in the redox reactions. CV was used to determine the electron stoichiometry, diffusion coefficient (Randle-Sevcik plot) and surface concentration (Brown-Anson plot) of analyte species. CV was also used to electrochemically polymerise the Schiff base prepared zig zag monomer, phenazine-2,3-diimino(pyrrole-2-yl).

Square wave voltammetry (SWV) is a form of a linear potential sweep voltammetry which was used for quantitative measurement of electroactive species within the electrolyte solution. Various electrochemical parameters such as formal peak potential, peak separation, and finally, calibration curves were obtained using this technique, capitalising on the superior sensitivity of the SWV method for redox electrochemistry.

Fourier transform infrared spectroscopy (FTIR) can be used to determine the infrared absorption or emission spectra of solids, liquids and gases. In our research, FTIR was used to characterise the chemical bond vibrations in the starting materials and the monomer material, to confirm the desired synthesis product.

Nuclear magnetic resonance spectroscopy (NMR) is a technique that explores the magnetic properties of atomic nuclei. Proton (1H) NMR was used to study the magnetic resonance of the hydrogen nuclei within the starting materials and synthesised monomer material. 1H NMR revealed that the structure of the starting material was completely different to the product and that synthesised monomer material displayed properties similar to that of a Schiff base.

Scanning electron microscopy (SEM) provides three-dimensional images based on the principle of electron scattering and capturing. Heterogeneous organic and inorganic materials are scanned with a highly focused beam of electrons. The electrons interact with the samples and cause emission of secondary electrons which produce information about the surface composition and typography. Three-dimensional images for our materials were recorded in the nanometre (nm) to micrometre scale (µm). The information obtained from the materials under investigation was used to differentiate between the starting materials and newly synthesised Schiff base materials.

RESULTS AND DISCUSSION

Whilst electrochemical methods formed the mainstay of the project's investigations and are the basis of the outcomes and future recommendations, it is necessary to support materials preparation with relevant characterisation techniques. Therefore, in the results and discussion of data presented in the deliverable reports, evidence from spectroscopy and microscopy was incorporated to strengthen the presentation of new materials. The electrochemistry of individual PAH species was characterised in different aqueous analytical media and the effect of the PAH analyte species in mixed systems was evaluated. The effects of analysis in mixtures were evaluated using electrochemical methods (CV and SWV) as well as HPLC-UV/vis methods.

Aim 1

A detailed literature review of PAH detection using electrochemical methods of analysis was carried out and captured as a deliverable report. The report revealed that whilst there are many publications dealing with PAH detection, the application is limited to anthracene, naphthalene and, to a lesser extent, phenanthrene. Very few reports address mixed sample analysis and no reports were found on using the systematic approach for redox evaluation at simple chemical sensor interfaces that was reported on in this project.

Aim 2

The class of materials developed in this project, *viz*. zig zag polymers, are a completely novel class of compounds. Even though the original concept of zig zag polymers was first demonstrated as early as 1992, they were based on quarter thiophene (polymer) linkages. Thiophene is not a hydrophilic polymer and would present additional challenges for the purpose of this project. We prepared for the first time in our lab these type of compounds using a polypyrrole linkage. Two full-length peer reviewed research papers were published from this work.

Aim 3

The zig zag polymer principle was expanded to include three systems in a series of increasing ring size (analine, naphthalene and phenanthrene) as anchor molecules in the Schiff base monomer design (parallel research, not directly part of the scope of the current project). The WRC project focused on applying one of these materials in the single species redox characterisation, modified screen printed electrode quantification, and mixed sample analysis.

From the main results, the possibility of conclusively detecting and quantifying four PAHs was identified, i.e. anthracene, triphenylene, benzo[a]pyrene and fluoranthene, at simple chemical sensor interfaces.

Aim 4

HPLC-UV/vis analysis of the standards and mixed samples used in the electrochemical analytical protocol was done at a simple C18 column using a 75% CH_3CN mobile phase. Good performance was observed for single species analysis as well as for calibration curves determined within a mixed sample (cocktail). A demonstration model of the multichannel electrochemical approach was demonstrated and a critical evaluation of the cost effectiveness, portability and capability of the method was the basis of the final recommendations of the project.

GENERAL

The main objectives of the project were the demonstration of a one-pot electro-analytical protocol for on-site, real-time analysis of PAH hydrocarbons in a mixed sample. The progress of the research followed the original timeline and delivered on the development, capacity building and output expectations of the project. One MSc candidate has completed his MSc thesis and was promoted to a PhD (Xolani Ngema, 2015–2017). He has co-authored one peer-reviewed research article. One PhD candidate (Meryck Ward) has co-authored two full-length research articles and has completed his final PhD dissertation draft. He was earmarked for graduation in 2017. Both students have presented posters at international conferences, Xolani Ngema at the 3rd International Symposium on Electrochemistry, 26–28 May 2015, and Meryck Ward at the 67th Annual Meeting of the International Society of Electrochemistry, The Hague, Netherlands, 26–31 August, 2016.

CONCLUSIONS

A multichannel electro-analytical system has been developed for discrimination of PAH compounds as single species and in a mixed sample. The system employs simple chemical sensors which are robust and may be reasonably employed in real water samples, without fear of degradation. The individual electrocatalysts were able to quantitatively report on anthracene, 1-hydroxypyrene, benzo[a]pyrene and fluoranthene with good sensitivity. Evaluation of the PAH standards and mixed samples was carried out using a simplified HPLC-UV/vis method.

RECOMMENDATIONS FOR FUTURE RESEARCH

Optimisation and application of the multichannel system was addressed in a proof-of-principle manner; the system will require further validation and application to real sample mixtures for proper assessment of its value in routine on-site environmental analysis. However, we believe that the proof-of-principle demonstration has shown that all the elements are in place for effective implementation of the technology. The development of a compact one-stop system is highly feasible and will benefit from continued evaluation and testing.

The novel polymer electrocatalysts developed in this work and in parallel research provide for a wide range of new catalysts to be implemented in the multichannel demonstration model. The electrocatalysts are deposited *in situ* at commercial screen printed electrode arrays using the same multichannel system that, in subsequent steps, is employed for analysis of PAHs. The efficiency of polymerisation, washing steps and optimal flow conditions to benefit reliable and reproducible analytical performance still remains to be optimised.

It is envisaged that the transfer of the laboratory-scale technology, optimisation and validation as well as the final design of a compact portable electro-analytical system for PAH discrimination in aqueous mixtures will form the basis of a follow-up project focussing on technology transfer, application and seasonal monitoring of PAHs in water systems.

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Dr Esper Ncube	Rand Water

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LIST OF ABBREVIATIONS

1-OHP	1-hydroxypyrene
AFM	Atomic force microscopy
ANT	Anthracene BaP Benzo[a]pyrene
CHR	Chrysene
CV	Cyclic voltammetry
DE	Diffusion coefficient
EIS	Electrochemical impedance spectroscopy
ELISA	Enzyme linked immunoassay analysis
FLU	Fluoranthene
FTIR	Fourier transform infrared spectroscopy
HPLC	High performance liquid chromatography
ICP	Intrinsically conductive polymers
LOD	Limit of detection
NAP	Naphthalene
NMR	Nuclear magnetic resonance spectroscopy
PAH	Polyaromatic hydrocarbons/polycyclic aromatic hydrocarbons
PCR	Principal component recognition
PHEN	Phenanthrene
PYR	Pyrene
R^2	Regression coefficient
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SPE	Solid-phase extraction
SPCE	Screen printed carbon electrodes
SWV	Square wave voltammetry
TRIP	Triphenylene
U.S. EPA	United States of America Environmental Protection Agency
UV	Ultraviolet
UV/vis	UV/vis spectroscopy
WHO	World Health Organisation

CHAPTER 1

1.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are produced mainly through the pyrolysis process, usually through incomplete combustion of organic materials. Both natural and anthropogenic sources are major contributors to the release of PAHs into the environment. Natural sources include forest fires and volcanic eruptions; the main anthropogenic sources include vehicular emissions, residential wood burning, petroleum catalytic cracking and industrial combustion of fossil fuels. PAHs are widely distributed in the environment and were one of the first atmospheric pollutants to be classified as a potential carcinogen. Release of PAHs into the atmosphere allows for the possibility of PAHs to be transported over great distances, before deposition through atmospheric precipitation into waters, soils and vegetation. A wide range of sources contributes to total global atmospheric PAH emissions, including industrial emissions, agriculture, air, water, soil, foodstuffs and other sources such as medicines, dyes, plastics, pesticides and wood preservatives (1,2).

1.2 Polyaromatic hydrocarbons and health

Exposure to PAHs is detrimental to many ecosystems and to human health. PAHs are well known for their strong mutagenic, carcinogenic and toxic properties (3). Induced toxicity of PAHs is caused by their transformation to reactive products through chemical or biochemical processes, in particular by the incorporation of oxygen into their ring structures. Photo-oxidation produces reactive products with increased toxicity, which is enhanced when exposed to natural sunlight (4). The formation of adducts is the most common form of carcinogenesis as a result of direct interaction between PAHs and DNA helical structure. PAHs undergo metabolic activation to diol-epoxides which bind covalently to DNA. Adducts or induced oxidative stress results after metabolic activation, which, in turn, provokes mutation. The DNA repair mechanism which produces antibodies is inhibited by the rate at which these adducts are formed and, as a result, there is an increase in accumulation of mutations in the DNA which leads to the onset of carcinogenesis (5). An estimation of global atmospheric PAH emissions was made in 2004, wherein Africa was identified as the second highest contributor to the global burden of PAHs, and savannah fires were identified as the major source (6).

The U.S. EPA has classified several PAHs as probable carcinogens: benzo(a)pyrene (BaP), benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene (CHR), dibenz(a,h)anthracene (DB(a,h)A) and indeno(1,2,3-cd)pyrene (I(1,2,3-cd)P). These carcinogenic PAHs have been found in cigarette smoke, broiled foods and various polluted environments (7,8).

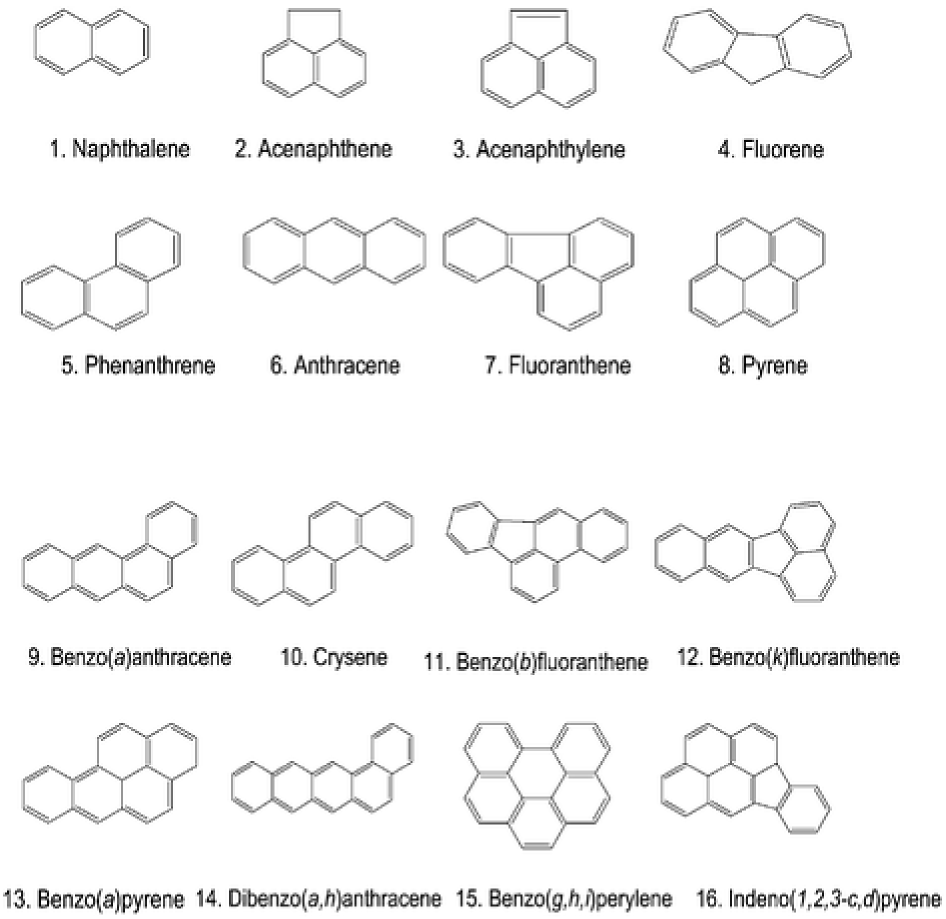


Figure 1.1: Chemical structure of 16 priority PAHs as identified by U.S.EPA (9)

The U.S. EPA prescribes liquid-liquid extraction and liquid-solid extraction, which is also called solid-phase extraction (SPE), as methods for pre-concentrating PAHs in drinking water samples. However, preparing individual samples is time consuming for both extraction methods, and a new SPE cartridge must be used for each sample when using the SPE method. The SPE method may be automated and coupled to high performance liquid chromatography (HPLC), which provides an efficient method for quantifying the 16 priority pollutants in water samples (Figure 1.1). However, these methods are still time consuming and costly.

In a case study in Delhi, India, a validation method was developed for the detection of priority PAHs in waste water and sediments, using HPLC. This validation method evaluated the limit of detection, limit of quantification, method recovery and reproducibility. Limit of detection ranged between 0.01–0.51 ppb and the limit of quantification ranged between 0.03–1.71 ppb.

Recoveries of the PAHs were in the region between 78(± 2.23%) to 100(± 14.68%) (10, 11). Benzo(a)pyrene and anthracene proved to be the most popular PAHS for detection in water, using both chemical sensor systems and biosensor systems.

1.3 Electrochemical methods for PAH detection

Intrinsically conducting polymers (ICPs) belong to a class of organic materials with unique electronic properties, i.e. electric conductivity up to 10^4 S/cm for doped polyacetylene, electrochromism, and electroactivity. Conjugated π electrons in the backbone of their macromolecules are responsible for these properties. Electrochemistry deals with the interaction between electrical energy and chemical change. Chemical reactions where electrons are transferred directly between molecules are called oxidation-reduction or (redox) reactions. The most studied conducting polymers are polypyrrole (PPy), polythiophene, polyfuran and other heterocyclic ICPs, polyaniline (PANI) and polyacetylene. Composites can be defined as materials that consist of two or more chemically and physically different phases separated by a distinct interface. The different systems are combined to achieve a system with more useful structural or functional properties which cannot be attained by any of the constituent alone. Composites have been employed in recent developments of polymer sensors for detection of PAHs.

Overoxidized-polypyrrole (PPy)/Silver-Gold(Ag-Au) Alloy nanoparticles composite, dendrimers, polyaniline-graphene composite and polyaniline (PANI) was used as the transducer material in the design of highly sensitive and selective sensors for anthracene. Overoxidized PPy was electrochemically prepared on a glassy carbon electrode as a thin film. A nanoalloy (Ag-Au) was drop coated onto the surface of the thin film to create the composite material, which was used as an electrochemical transducer. This new transducer was applied to the detection of anthracene (ANT) (prepared in acetonitrile) The dendrimer was prepared at a gold electrode surface and was used in the detection of phenanthrene in a mixture of acetonitrile (MeCN) and water. Various electrochemical techniques such as cyclic voltammetry (CV), square wave voltammetry (SWV) and alternating current voltammetry (ACV) were used in the development of these analytical protocols, and the limit of detection (LOD) was in the nmol/L range (Table 1.1).

Interference studies were also performed during detection using metal ions as well as organic species. A novel graphene-polyaniline composite was constructed by *in-situ* polymerisation of aniline in a suspension of graphene oxide. ANT was successfully detected using amperometry and SWV with an LOD in the micromolar range (12-15).

Table1.1: Selected examples of PAH detection by electrochemical methods

Material	**Electrochemical immunoassay**	**Polymer modified with metal nanoparticles**	**Immunosensor**	**Dendrimer**
Analytes	Benzo[a]pyrene Pyrenebutyric acid	Anthracene	Benzo[a]pyrene	Anthracene Phenanthrene
Methods	Cyclic voltammetry Electrospray Ionisation-mass spectrometry Surface plasma resonance	Cyclic voltammetry EIS	Amperometric detection	Cyclic voltammetry
LOD	2.4–10 ng/mL	1.69 x 10-7 M	1 –10 ng/mL	19 nM

1.4 Screening of selected PAHs at unmodified screen printed carbon electrodes (SPCEs)

Electrochemistry deals with the interaction between electrical energy and chemical change. Chemical reactions where electrons are transferred directly between molecules are called oxidation-reduction or (redox) reactions. In general, electrochemistry describes the overall reactions when individual redox reactions are separate but connected by an external electric circuit and an intervening electrolyte. CV measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode and measuring the resulting current. The redox wave obtained may be used for qualitative and quantitative analysis.

The initial screening of PAHs was done by CV at commercial screen printed carbon electrodes (SPCEs) without any modification to the SPCEs, supplied commercially by Dropsens® (Figure 1.2)

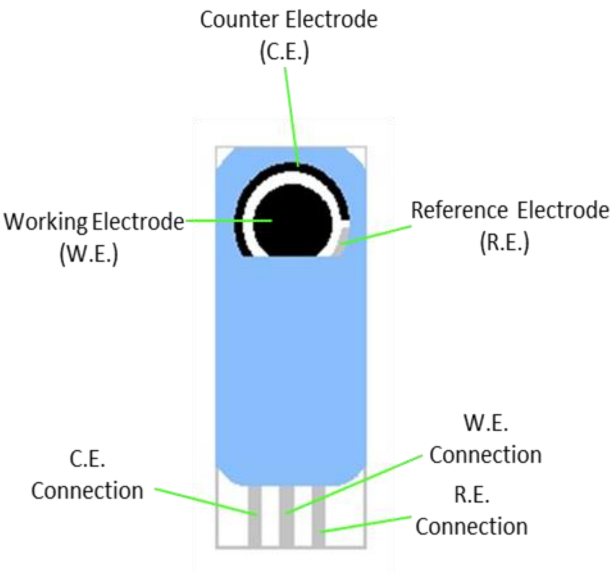


Figure 1.2: Schematic of a Dropsens screen printed carbon electrode (SPCE)

The novel polymer electrocatalysts developed in this work were coated onto the SPCEs in subsequent experiments and were employed for profiling and quantification of PAHs, particularly in aqueous media, i.e. HCl and $LiClO_4$. The use of organic solvents was avoided due to the implied risk to the environment. PAHs were determined as single species for which calibration curves were derived to assess the sensitivity and selectivity introduced by the polymer platforms. PAHs were also evaluated in mixed samples to test the ability of the electrochemical method to discriminate the identity and quantity of PAHs in a mixture. The analysis of individual and mixed samples was validated by a simple HPLC method with UV/vis detection, to verify the validity of single sample standards and mixed samples.

Finally, the best reporting transducers and experimental control that favoured the detection of selected PAHs reliably and reproducibly were incorporated into the design of a multichannel analytical system for the demonstration of a proof of concept electro-analytical system for the detection of PAHs. The validation of the system and its application to real sample analysis forms part of ongoing research to develop a fully portable system for on-site, real-time analysis of PAHs.

CHAPTER 2

2.1 Polymer design and electroanalysis of PAHs

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used as the main electrochemical tools in this investigation. CV is a linear sweep technique, whereas SWV is a frequency modulated voltammetric technique, with enhanced sensitivity. Various electrochemical parameters such as formal potential, potential peak separation, diffusion coefficient (Randle-Sevcik plot) and surface concentration (Brown-Anson plot) were obtained using these techniques. The sensitivity of the SWV technique was also useful for the analytical detection of the PAHs.

PalmSens ® provides a range of electrochemical instruments specifically designed for portable field experimentation and analysis. The standard configuration for an electrochemical cell includes a shielded cable with connections for a working, a counter and a reference electrode, all screen printed onto one platform, a screen printed carbon electrode (SPCE). Each of the polyaromatic hydrocarbons under investigation was dissolved in methanol to give a final concentration of 0.0005 M. Aliquots of these stock solutions were added to the SPCE and wetted with 0.1M HCl electrolyte. The initial redox chemistry evaluation showed that each PAH had its own distinctive electrochemical signature, but that in some cases the peaks were very closely positioned. Calibration curves, based on linear regression analysis, were applied in order to extract the linear region and sensitivity of the SCPE towards selected PAHs (Table 2.1)

Table 2.1: Analytical peak potential for each PAH determined from CV at SPCE, in 0.01 M HCL (vs Ag/AgCl).

Analyte	Peak Potential (mV)	Sensitivity (M)
Anthracene	-278	4.27 x 10-6
Naphthalene	84	7.10 x 10-6
Chrysene	65	4.46 x 10-6
Triphenylene	152	3.99 x 10-6
Fluoranthene	198	4.41 x 10-6
Acenaphthylene	166	5.59 x 10-6
Benzo[b]fluorathene	177	3.62 x 10-6

The 2,3, diaminophenazine was coupled to pyrole 2 carboxaldehyde in a typical aldol condensation reaction using acetic acid as catalyst. The Schiff base produced in this synthesis was fully characterised using nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared spectroscopy (FTIR) spectroscopy, scanning electron microscopy (SEM) and electrochemical methods (CV and SWV). The monomer, 0.5 mmol of phenazine-2,3-diimino (pyrrol-2-yl) was dissolved in 20 mL of a 1:1 mixture of dimethyl formamide (DMF) and hydrochloric acid (HCl), from which solution, the polymerised form was electrodeposited at the glassy SPCE using 25 cycles at 50 mV/s in the potential window -600 mV to +700 mV (vs Ag/AgCl) (Figure 2.1).

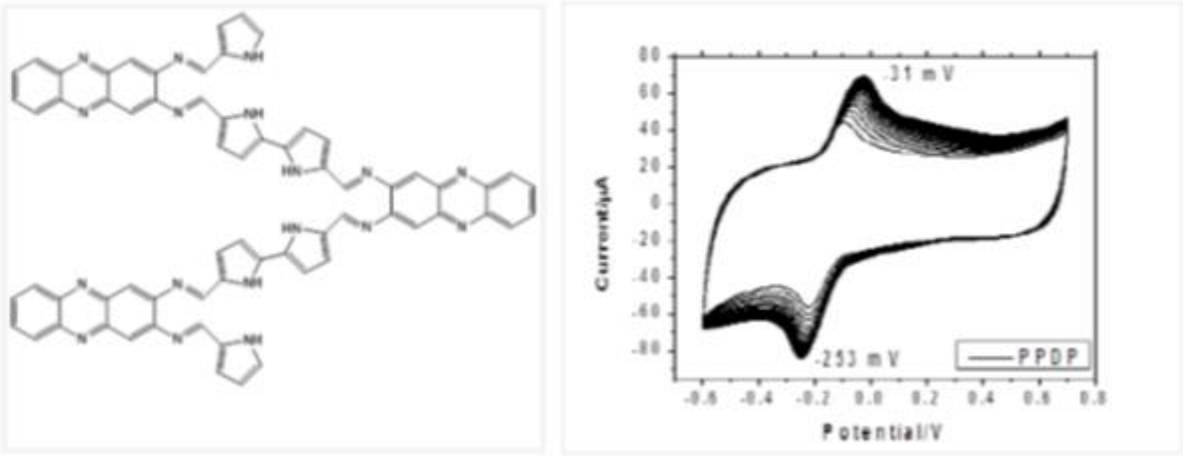


Figure 2.1: Structure and cyclic voltammogram of the *in-situ*-generated zig zag polymer

The initial synthesis was based on the 2,3 diamino phenazine to produce poly(phenazine-2,3-diimino(pyrrole-2-yl (PPDP) and a second polymer material was prepared starting with 2,3 aniline diamine to produce the polymer poly polymerised(N,N'-Bis-(1H-pyrrol-2-ylmethylene)-benzene-1,2-diamine) or in short PBPPD. Scan-rate-dependent CV evaluation of the *in-situ*-formed electrochemically deposited polymer film confirmed a diffusion coefficient consistent with thin layer diffusion i.e. $D_0 = 2.44 \times 10^{-14}$ cm^2/s. The polymer thin film was observed to exhibit strongly capacitive behaviour consistent with high surface area nanomaterials, and exhibited two distinct redox couples in the background signature in $LiClO_4$.

The eight selected PAH compounds were screened by SWV using the same experimental conditions as before, with the working electrode being the SPCE modified with PPDP polymer and all potentials measured vs Ag/AgCl reference electrode (Table 2.2).

Table 2.2: Summary of redox potentials determined by CV at PPDP

Analyte	Peak potential (mV)
Anthracene	-277
Naphthalene	81
Chrysene	63
Triphenylene	144
Fluoranthene	196
Acenaphthylene	162
Benzo[a]pyrene	178

Au nanoparticles were prepared in a separate wet chemical synthesis laboratory experiment. The nanoparticles were characterised by SEM and the dynamic light scattering method to confirm the morphology and size distribution of the produced Au nanoparticles. The Au nanoparticles were introduced into the electro-synthesis solution with the monomer, and incorporated into the polymer during the repeated cycling steps which attach the polymer and Au to the electrode surface to produce PPDP/Au. The evaluation of the PPDP/Au transducer in the presence of each individual PAH was done as for the unmodified platform (Table 2.3).

Table 2.3: Summary of redox potentials determined by CV at PPDP/Au.

Analyte	Peak potential (mV)
Anthracene	-278
Naphthalene	82
Chrysene	61
Triphenylene	140
Fluoranthene	190
Acenaphthylene	170
Benzo[a]pyrene	177

Small potential shifts to lower reduction potentials were observed for six out of the eight PAHs due to the enhanced electron transfer effect of the polymer and/or polymer with Au nanoparticles in the polymer matrix. However the major advantage of using a polymer modification is the reproducibility of the transducer.

From the concentration-dependent analytical response, it was observed that all of the calibration curves showed a very wide linear range in the ug/L to mg/L range. The LOD is reported in ug/L and shows quite a distribution, ranging from 23 ug/L for naphthalene to as high as 162 ug/L for Chrysene. LOD is a very important parameter in deciding which application the sensor system is most applicable for. A very low detection limit makes the sensors suitable for early signalling of PAH presence before it reaches contamination levels. This facilitates preventative intervention and contamination containment. The LOD measured for each of the PAHs is well within the measurement limits prescribed by the World Health Organisation (WHO) for contamination prevention or even pollution monitoring.

According to the WHO (1997), the concentration of individual PAHs in surface and coastal waters is generally = 50 ng/litre, excluding heavily polluted rivers. Concentrations above this level (sometimes into the 10 µg/litre range) indicate contamination by PAHs, mainly through industrial point sources and shipyards, atmospheric deposition and urban runoff. PAH levels in uncontaminated groundwater are usually in the range of 0–5 ng/litre.

2.2 Characterisation of polymer materials

The novel monomer (N,N'-Bis-(1H-pyrrol-2-ylmethylene)-benzene-1,2-diamine-BPPD) was derived from the condensation reaction between o-phenylenediamine and a pyrrole derivative. FTIR analysis was used to confirm that an imine (azomethine) function was present in the monomer material (Figure 2.2). The two important regions in the FTIR spectrum were used to confirm that the Schiff base had successfully been synthesised, the regions where (1°, 2°, 3°) amines stretching bands and the carbonyl stretching band were present (Figure 4). The sharp intense stretching bands at 3384.38 cm^{-1} and 3363.70 cm^{-1} are attributed to the primary (1°) amine associated with the structure of o-phenylenediamine (OPD). The stretching band of the secondary (2°) amine present in the pyrrole-2-carboxaldehyde (P2C) can be seen at 3146.63 cm^{-1}. The stretching band present at 3125.72 cm^{-1} is associated with the secondary (2°) amine of the polymers (not shown).

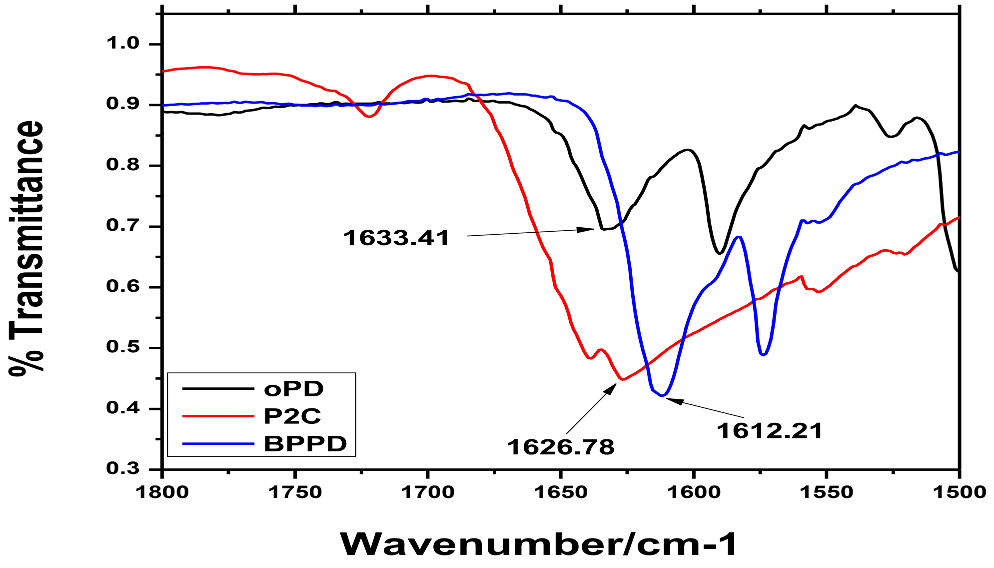


Figure 2.2: **FTIR spectra of starting materials (OPD and P2C) and the synthesised Schiff base monomer (blue line)**

Similarly, the characterisation of the starting materials and the polymers produced were also characterised using H1NMR. By identifying the proton environment in the starting materials, the imine linkage in the monomer product could be distinguished from starting material spectra and served to confirm the synthesis product in each case (Figure 2.3).

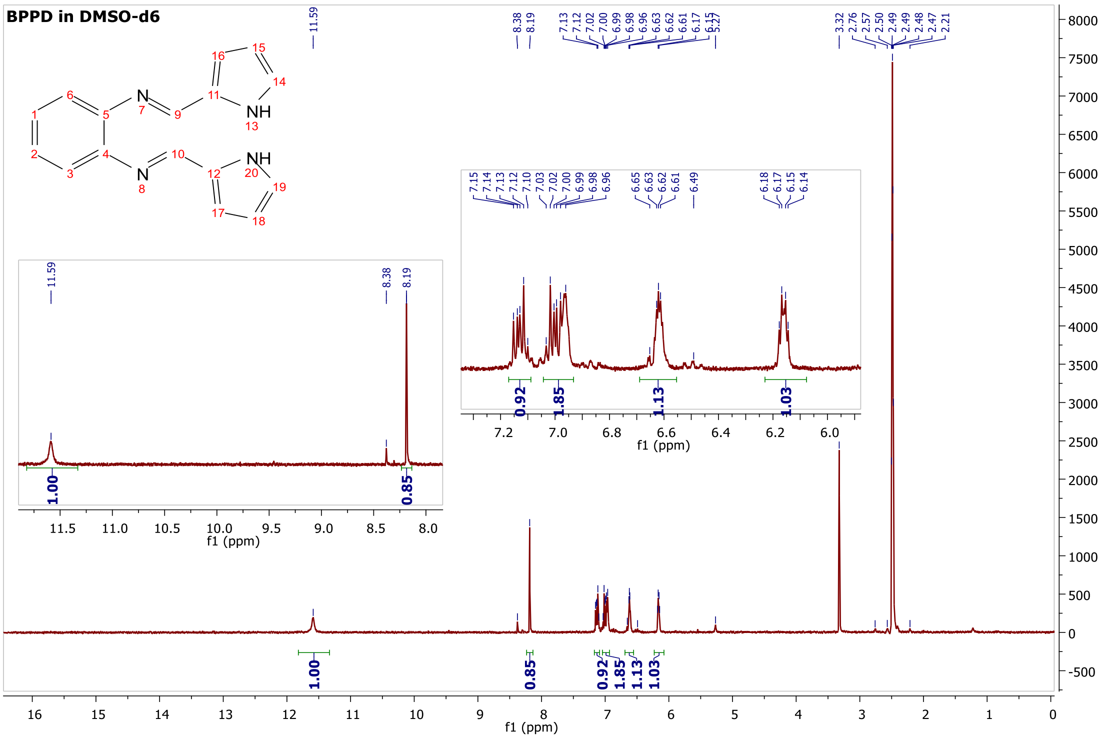


Figure 2.3: H1 NMR spectrum of the cross-linked Schiff base monomer

2.3 Analysis of PAH – single species and mixtures

A 20 mM solution of BPPD was prepared in dimethylformamide and 0.1 M hydrochloric acid. This was then electrochemically deposited using CV technique, onto the surface of a SPCE, creating an electrochemical transducer. The polymer (PBPPD) or poly N,N’-Bis-(1H-pyrrol-2-ylmethylene)-benzene-1,2-diamine) was electrodeposited at the surface of a SPCE by repeated potential cycling between – 0.6 V to 0.7 V for 15 cycles, at a scan rate of 50 mV/s (Figure 2.4).

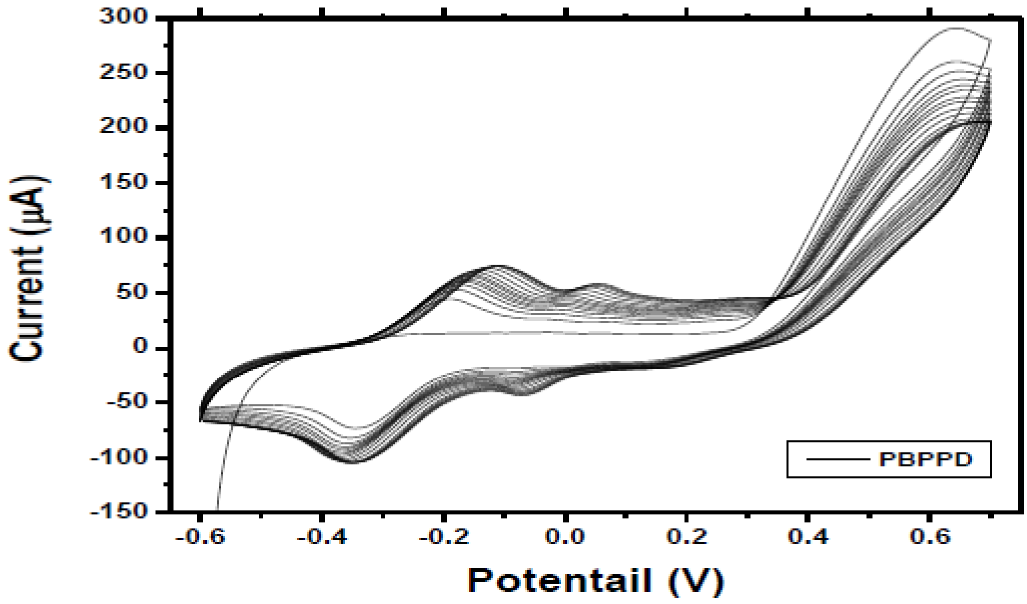
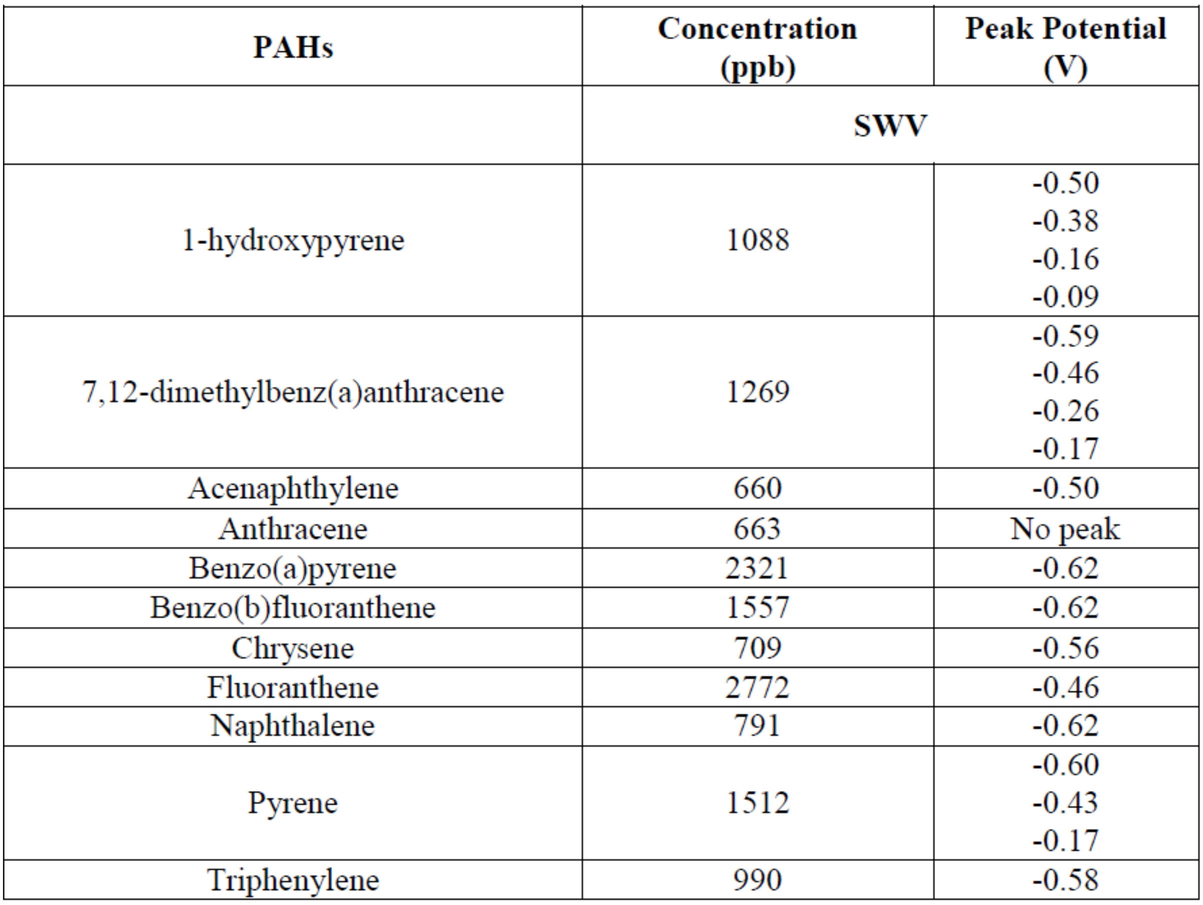


Figure 2.4: Electrochemical polymerisation of PBPPD polymer at SPCE

SWV was applied in an oxidative sweep for the detection of selected PAHs in HCl and $LiClO_4$. Various experimental parameters were optimised to improve the detection and quantification of the selected PAHs (Table 2.4).

The concentration dependent behaviour of the PAH species at the PBPPD polymer electrode was observed to have a sigmoidal response typical of competitive binding events at the electrode interface (Figure 2.5 and 2.6). The sigmoidal curves were evaluated by fitting a Boltzman curve based on the non-iterative least squares method. The two PAHs that were efficiently discriminated at the PBPPD interface were BaP and fluoranthene (FLA).

Table 2.4: Electrochemical evaluation of PAHs at PBPPD polymer electrodes



PAHs	Concentration (ppb)	Peak Potential (V)
	SWV	
1-hydroxypyrene	1088	-0.50 -0.38 -0.16 -0.09
7,12-dimethylbenz(a)anthracene	1269	-0.59 -0.46 -0.26 -0.17
Acenaphthylene	660	-0.50
Anthracene	663	No peak
Benzo(a)pyrene	2321	-0.62
Benzo(b)fluoranthene	1557	-0.62
Chrysene	709	-0.56
Fluoranthene	2772	-0.46
Naphthalene	791	-0.62
Pyrene	1512	-0.60 -0.43 -0.17
Triphenylene	990	-0.58

The analytical peak for BaP was observed at -0.6 V vs Ag/AgCl and at -0.54 V for the FLA reference electrode. Calibration data for individual PAH species using the Boltzmann plot gave rise to a LOD (n = 3) for BaP and FLA, found to be 9.46x10^{-7} and 1.07x10^{-9} ppb respectively. The Boltzmann provided high sensitivity (slope of the linear region in the curve) of 712.04 μA/ppb and 627.21 μA/ppb respectively. The linearity coefficient (R^2) was determined to be 0.9975 and 0.9941 for BaP and FLA. When the BaP and FLA were prepared as a mixed sample containing both PAH species, the sensitivity towards each analyte was calculated to be 627 and 781 μA/ppb respectively.

The WHO guideline for BaP in water is 0.7 ppb which means that the sensitivity of this method will produce a current response of 498 μA associated with detection at the prescribed detection limit.

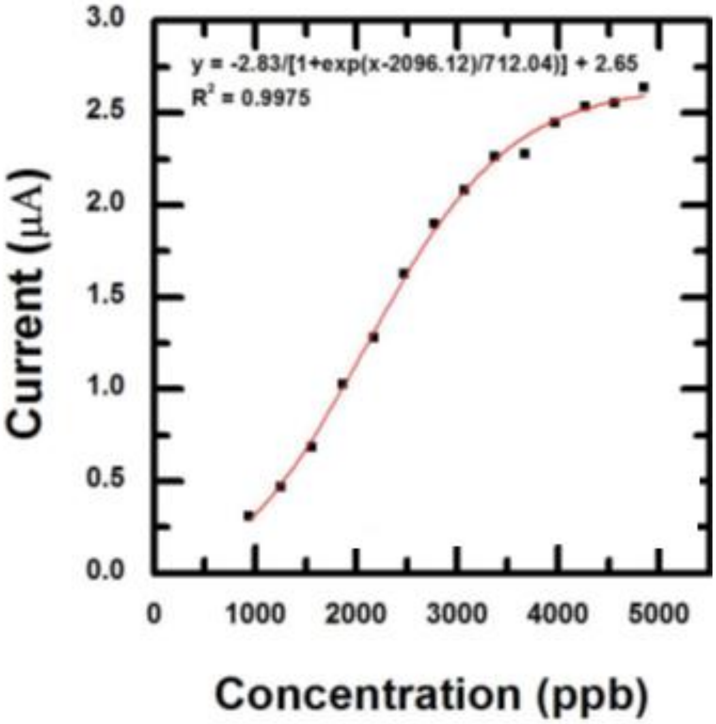


Figure 2.5 **Calibration curve for detection of BaP at PBPPD transducer**

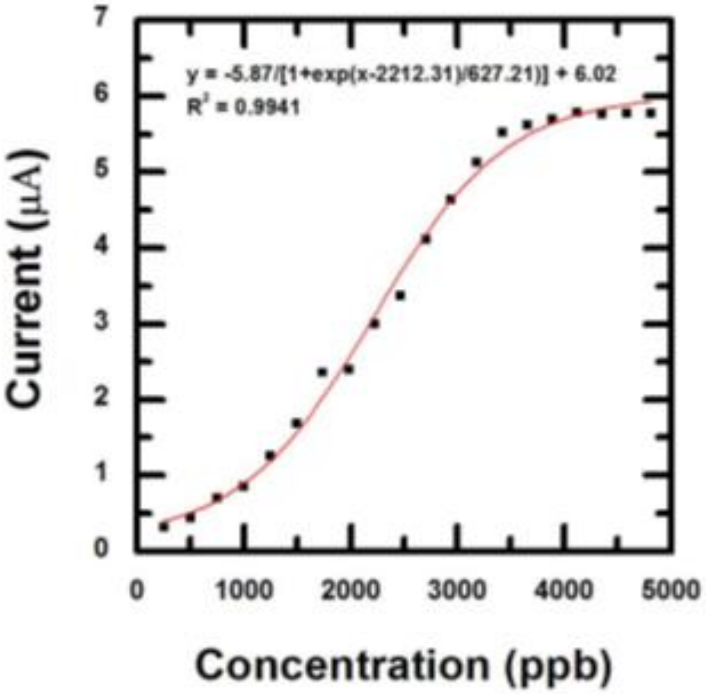


Figure 2.6 **Calibration curve for detection of FLA at PBPPD transducer**

The concentration-dependent behaviour of BaP and FLA at the PPDP polymer electrode was observed to follow a linear regression (Figures 2.7 and 2.8). The calibration curves were evaluated by fitting a linear regression line, based on the method of least squares. The analytical peak was observed at -0.60 V vs Ag/AgCl for BaP, and at -0.50 V for FLA.

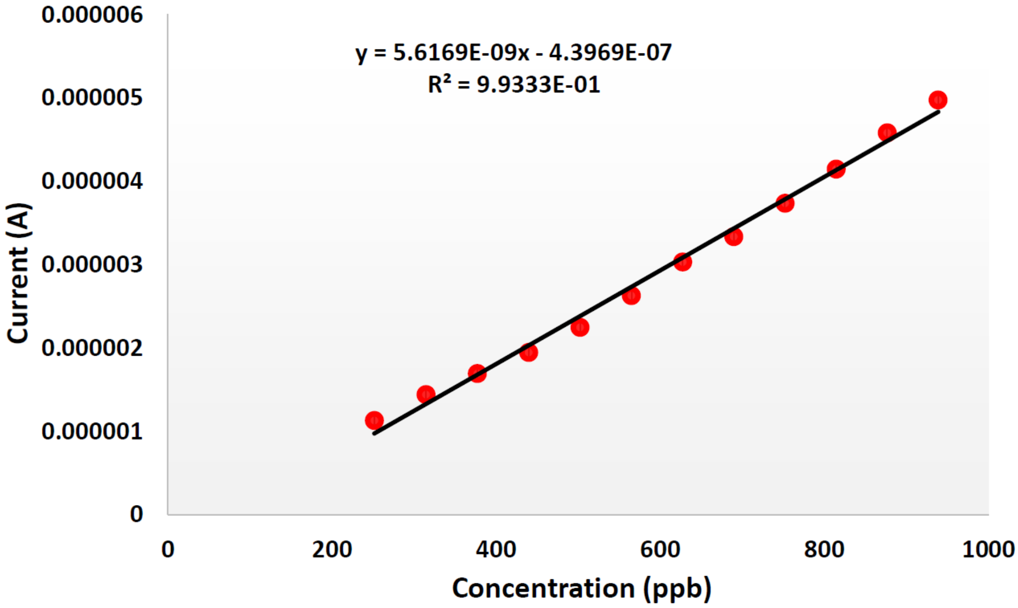


Figure 2.7: **Calibration for benzo[a]pyrene at the PPDP electrocatalyst in LiClO4**

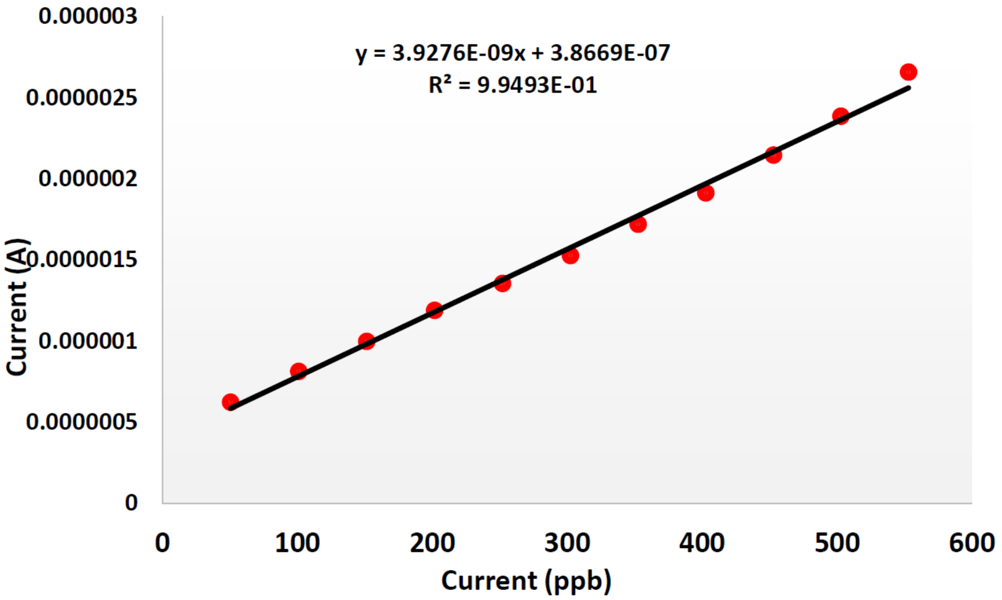


Figure 2.8: **Calibration for fluoranthene at the PPDP electrocatalyst in $LiClO_4$**

The sensitivity towards BaP detection was determined from the slope of the straight line to be 5.6169 x 10^{-3} μA/ppb. The analytical peak for fluoranthene was observed at -498 mV vs

Ag/AgCl reference electrode. The sensitivity of the sensor to standard additions of FLA was determined from the slope of the straight line to be 3.927 x 10^{-3} µA/ppb.

Upon combining the two PAHs into one mixture, a small shift was observed in the analytical peak position of the two analyte species. The analytical peak for BaP and FLA moved towards more negative of approximately 100 mV, i.e. BaP was measured at -565 mV and FLA at -386 mV. As the concentration of species in the mixture was increased by standard additions, the analytical peaks showed a linear response over a similar concentration range as before (Figure 2.9).

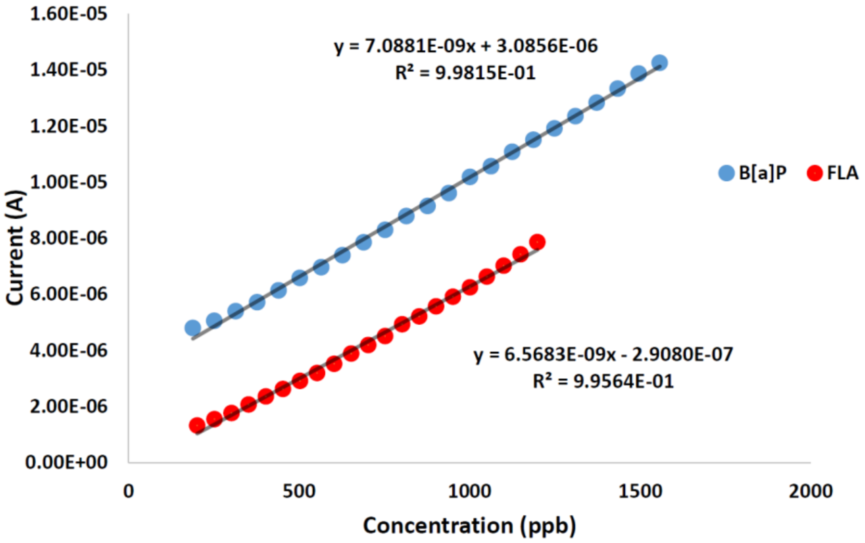


Figure 2.9: Calibration for BaP and FLA in a mixed sample at the PPDP transducer

The sensitivity of the sensor to the detection of the individual species, as well as the LOD, was not adversely affected by combining the two analyte species into the same analytical matrix.

In order to evaluate the effect of interferences due to cross-reactivity of PAHs in the same mixture, a cocktail containing all eight species of PAHs was prepared in the laboratory from standards supplied by Sigma Aldrich. The concentration of the desired PAH (previously optimised as an individual standard) was then evaluated in the presence of the other PAHs which acted as interfering species. In nature (real samples), it is highly unlikely that PAHs will occur as individual species and hence evaluation of their cross-reactivity within the same class is a high priority. Two separate PAH cocktails were prepared for evaluating high and low concentration effects in interference and detection (Tables 2.5 and 2.6).

Table 2.5: Concentration of PAHs in cocktail 1 (high concentration)

	PAHs	**Concentration in working solution (ppb)**
PAHs Cocktail 1	Anthracene	222
	Acenaphthylene	190
	Benzo[a]pyrene	315
	Benzo[b]fluoranthene	315
	Chrysene	285
	Fluoranthene	252
	Naphthalene	160
	Triphenylene	285

Table 2.6: Concentration of PAHs in cocktail 2 (low concentrations)

	PAHs	**Concentration in working solution (ppb)**
PAHs Cocktail 2	Anthracene	22
	Acenaphthylene	19
	Benzo[a]pyrene	32
	Benzo[b]fluoranthene	32
	Chrysene	29
	Fluoranthene	25
	Naphthalene	16
	Triphenylene	29

Individual PAH species were prepared as analyte solutions and these were added to the cocktail mixture to elicit a concentration-dependent response. The effect of two different electrolytes, i.e. HCl and LiClO4, was evaluated, in order to propose optimal analytical solutions for PAH detection and quantification in simulated real samples. Anthracene and triphenylene were evaluated at the SPCE, whereas BaP, naphthalene and FLA were evaluated at the N,N'-Bis-(1H-pyrrol-2-ylmethylene)-benzene-1,2-diamine (BPPD) polymer electrode. The same clear differentiation in analytical peak was observed for anthracene in the presence of cocktails 1 and 2 (Figure 2.10).

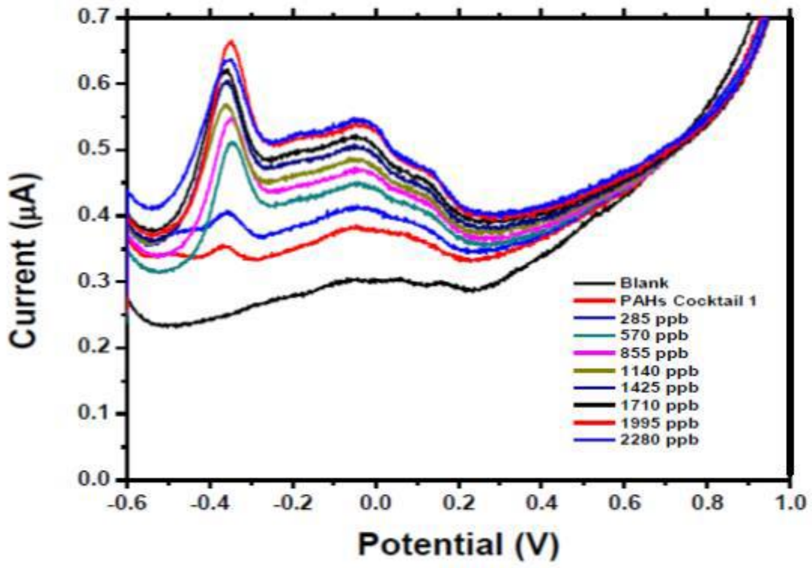


Figure 2.10: Detection of increasing anthracene concentration at a bare screen printed carbon electrode in the presence of cocktail 1

The electrochemical signature of benzo[a]pyrene displayed an oxidative peak at – 601 mV (Figure 2.11). A detection limit of 120 ppb was determined from the linear range of the calibration curves.

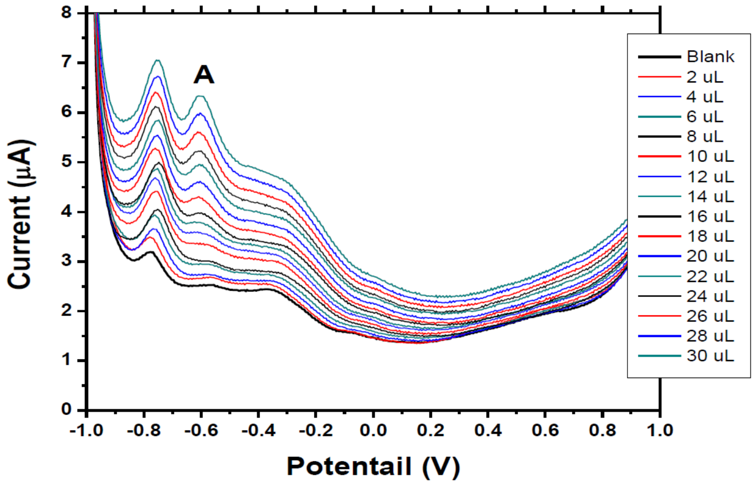


Figure 2.11: Electrochemical signature of BaP, analytical peak at BPPD polymer modified SPCE

The polymer systems, originally designed, were expanded to include a 1,2 diamino benzene and a 1,2 diamino naphthalene anchor in the Schiff base design of ligands. Also, the incorporation of metal nanoparticles into the polymer matrices was explored. The effect of electrolyte medium (both aqueous and low pH) was also investigated and a summary of some of the parallel results is provided as a reference point for what is possible in the exploration in the multichannel electrochemical evaluation system. (Table 2.7).

Table 2.7: Electrocatalysts proposed for multichannel analysis of PAHs in mixed sample

Electrochemical platform	Potential window (V)	Proposed PAH	Electrolyte	Electrochemical Technique
Bare SPCE	-0.5 to 0.8	Chrysene	0.1 M HCl	SWV
PPDP	-0.5 to 0.4	Anthracene Naphthalene	0.1 M HCl	SWV
PPDP/Au	-0.3 to 0.4	Phenanthrene	0.1 M HCl	CV
PBPPD	-1.0 to -0.2	Benzo[a]pyrene Fluoranthene	0.1 M $LiClO_4$	SWV

CHAPTER 3

3.1 HPLC analysis of PAHs

HPLC methods are useful for PAH analysis, as ultraviolet (UV) and fluorescence detection may be readily coupled for quantitative analysis with enhanced selectivity (16,17). GC methods tend to be problematic when analysing chemicals with high molecular weights (greater than 300 amu) due to their low volatilities(18). HPLC methods overcome these problems as HPLC can perform suitable analysis of compounds with higher boiling points and molecular weights. HPLC can be equipped with different detectors which include UV, fluorescence and diode array detectors. Fluorescence detectors are probably the most sensitive among the existing modern HPLC detectors, capable of sensitivity 10–1000 times higher than that of the UV detector for strong UV-absorbing materials. Fluorescence detectors are very specific and selective among other optical detectors, specifically for fluorescent species in samples (19). HPLC methods were developed for simultaneous determination of PAHs; however, there are alternative methods for PAH detection such as fluorescence detection due to the fact that most PAH compounds have high natural fluorescence (20, 21).

HPLC analysis of single standards and PAH cocktails was performed using an Agilent 1200 Series HPLC coupled to a UV detector, set at a wavelength of 254 nm, and a capillary pump. The analytical protocol was adapted from literature using the generic HPLC/UV system available in out laboratory in isocratic mode (17-20). All data obtained was processed using HPLC 3D ChemStation Software and at the flow conditions specified here (Table 3.1).

Standard solutions of each PAH species were prepared by dissolving 10 mg of the desired PAH in 20 mL CH_3CN (stock solution). Sonication was used to assist the dissolving of the PAHs, and when completely dissolved, the stock solutions were further diluted to 50 mL with CH_3CN, to produce a final concentration of 0.2 g/L of stock solution for each individual PAH (Table 3.2). The retention time of each PAH single standard was recorded, after injection as an individual sample.

Table 3.1: Experimental conditions for HPLC analysis of PAHs

Column	Agilent Eclipse XDB C18 5 µm 4.6 x 150 mm
Flow Rate	1 ml/min
Column Temperature	Room temperature
Injection Volume	20 µl
Run Time	20 min
Mobile Phase (Isocratic)	75% CH_3CN
Detection	UV at 254 nm

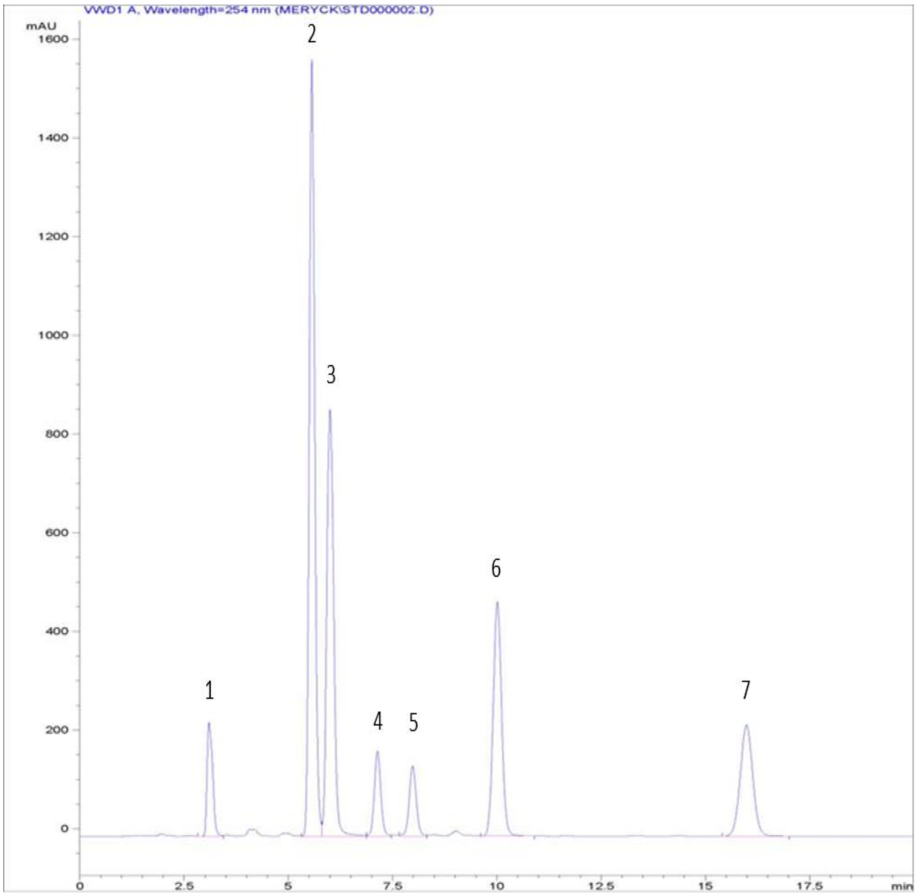


Figure 3.1. Combined stock standard containing: 1. 1-OHP, 2. PHEN/NAP, 3. ANT, 4. FLU, 5. PYR, 6. CHR, 7. BaP.

Table 3.2: Concentration and retention time of each PAH injected as an individual standard

PAH	Concentration, ppm	Retention time, (minutes)
ANTHRACENE	202	6.18
CHRYSENE	212	10.02
NAPHTHALENE	206	5.71
PHENANTHRENE	202	5.70
FLUORANTHENE	204	7.14
BENZO[A]PYRENE	210	16.02
PYRENE	202	8.07
1-HYDROXYPYRENE	206	3.09

All individually injected PAH standards produced peaks at retention times that were well separated from each other, and all standards eluted in around 16 minutes, with the 1-hydroxypyrene peak appearing at 3.09 minutes and the benzo(a)pyrene peak appearing at 16.01 minutes (22). The only PAH species that showed overlapping peaks were naphthalene and phenanthrene.

Calibration curves for BaP, FLA, 1-OHP were constructed using the same concentration range as for the electrochemical method, i.e. lower ppm. Even though this concentration range was very high for proper analytical assessment of the method and potential real samples, we worked with these concentrations as a direct comparison to the electrochemical protocol developed. Sensitivity of the HPLC/UV method to the detection of 1-OHP, FLA and BaP ranged from 3.61×10^{-2} to 1.21×10^{-1}.

3.2 Mixed sample analysis

A mixed sample containing anthracene, chrysene, naphthalene, phenanthrene, fluoranthene, benzo(a)pyrene, pyrene and 1-hydroxypyrene was prepared by suitable dilution to produce a working concentration of 25 ppm for each individual analyte in the mixture. HPLC analysis was conducted by using 20 µL injections of the mixed sample with detection by UV at 254 nm. The mobile phase (75% CH3CN) required thorough degassing prior to use. System suitability was assessed based on the reproducibility of the BaP peak (17.21 minutes in the cocktail) in order to verify that the system was performing reliably for the particular analysis. The relative standard deviation (2.4% RSD) was determined from the reproducibility of the BaP peak in consecutive injections of the mixed sample (n=5)

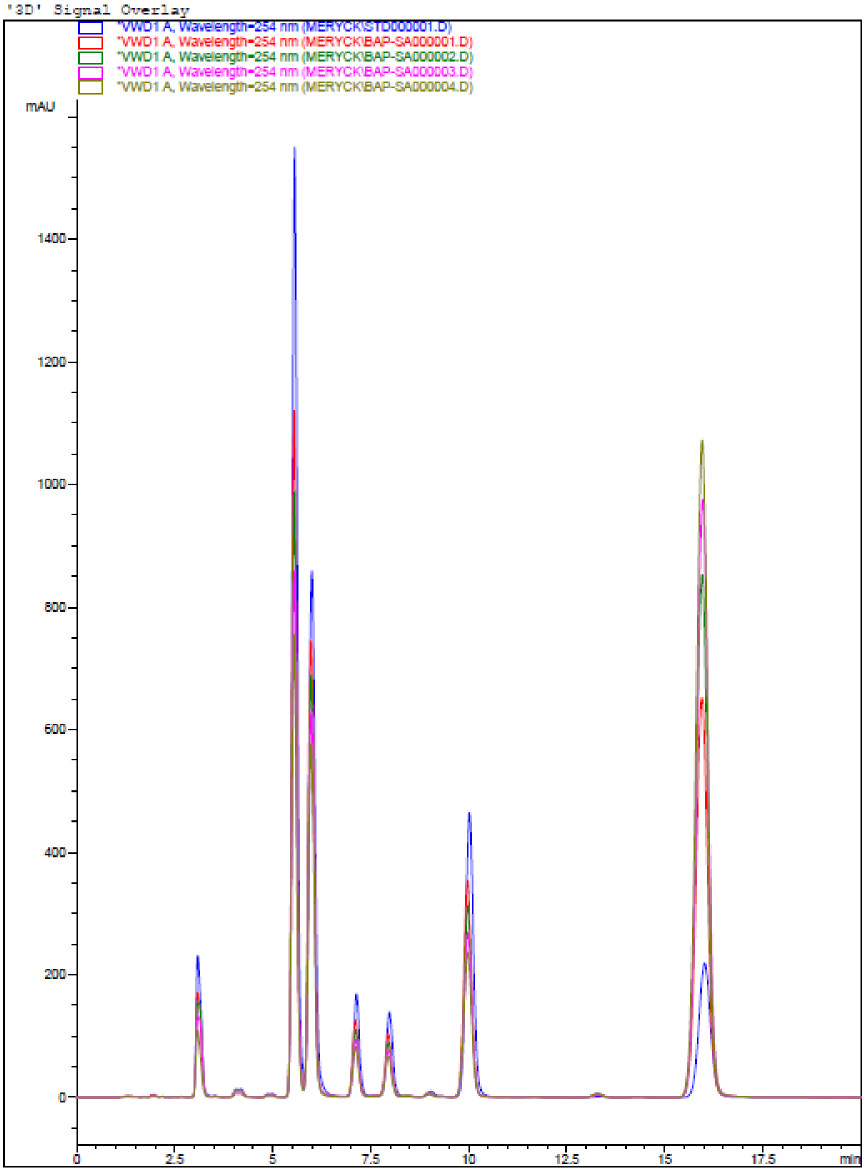


Figure 3.2: Overlay of mixed PAH sample (blue line) with increasing concentrations

An increase in BaP was observed as the concentration of BaP was increased, whereas all other PAH peaks decreased due to the dilution effect of consecutive injections. However, all peaks remained well resolved and, in principle, any of the PAH peaks could be used for quantification in this mixture. However quantitative analysis of PAHs with the HPLC/UV method was not the aim because many other columns and/or detectors would yield much better sensitivity and LOD for PAH analysis. However, it was necessary to see that the electrochemical method and the very basic HPLC/UV method available to us in house, both produced positive response profiles for the selected PAHs. The HPLC evaluation provided evidence that the PAHs supplied to the project were stable and reliable for the duration of the project. Initially, stability was evaluated by measuring UV/vis absorption profiles directly over time (the 3 years of the project) and the HPLC/UV evaluation at the end corroborated the validity of the samples even after prolonged storage. This could have been achieved by continued direct UV/vis analysis, but the HPLC provided additional information about peak shape, retention time and mixed sample analysis profiles which was not as clearly evident from electrochemical analysis only. In addition, electrochemistry is a specialised technique which is often not well understood by the general public and in doing the validation of the sample integrity and identity by HPLC, we have reinforced the validity of electroanalysis, based on the same samples as those used in the HPLC/UV experiments.

As a consequence of its physical properties, BaP is predominantly particle phase, rather than gas phase, and in the environment it is associated with soils and sediments. In the atmosphere, PAHs may undergo direct photolysis, react with pollutants such as ozone, hydroxyl radicals, nitrogen dioxide and sulfur dioxide, yielding diones, nitro- and dinitro-PAHs, and sulfonic acids, respectively. They may be degraded by some fungi and microorganisms in the soil and are expected to bio-accumulate in organisms (aquatic and terrestrial) that cannot metabolise them (25). In developing countries where biom the contribution of inhalation to BaP exposure could be as high as 138–3320 ng/day and therefore inhalation would be the main contributor to the total daily intake.

It is clear therefore that the ppm concentration range used in the HPLC/UV method was too high for direct application to real samples (typically lower ppb). However, the HPLC/UV proof-of-principle was developed for the same concentrations for which reliable signals were possible in the electrochemical evaluation. However, in electrochemistry, much smaller sample volumes are used for the bulk electrolyte and standard injections, automatically providing a diluted analytical environment, with greater sensitivity towards the target analyte species.

CHAPTER 4

4.1 Multichannel analytical protocol

Multi-array sensor systems are made up of a group of sensors usually aligned in a preferred geometrical design. Many commercial array sensors have been produced over the years, some of which incorporate the more common gold (Au), platinum (Pt), carbon (C) and silver (Ag) platforms, while others incorporate modified platforms (nanoalloys). Sensors may be prepared on different substrates (ceramic, alumina ceramic and glass) which house these working electrodes in various geometrical designs (Table 4.1). These multi-array analytical interfaces require multichannel potentiostats to selectively control electrochemical activation of the working electrodes of the array. Techniques such as amperometry and voltammetry may be individually or simultaneously controlled (analysis dependent) at each working electrode, to effect deposition of material, characterisation of materials and solution species as well as quantification using very dilute solutions.

Table 4.1: Commercially available array sensor systems

Company	No. of working electrodes	Electrode Materials	Substrate	Working Electrode Diameter (mm)
Dropsens	4	C (4W110) Au, Ag, Pt and C modified with nanoparticles	Ceramic	2.95
	8	Au (8X220) C (8X110) Pt	Ceramic	2.56
	96	C (96X110) Au, Ag, Pt and C modified with nanoparticles	Ceramic	3.00
BVT Technologies	8	Au, Pt, Ag, Au-Py Alloy	Alumina Ceramic	1.00
Gwent Sensors Ltd	16	Au, C, Pt	Alumina Ceramic	0.84
MicruX	2	Pt, Au	Glass	1.01
	3	Pt, Au	Glass	0.62
	8	Pt, Au	Glass	1.01
	1	Pt, Au	Glass	1.01

Technol	2	Pt, Au	Glass	0.01

A wide variety of multichannel potentiostats have already been released to the market, with related software for data analysis. The Palmsens multipotentiostat (µStat 8000P) was used for all electrochemical measurements, which consists of eight channels that can respond simultaneously to drive eight individual experiments. The electrochemical cell comes in two possible designs – the wall-jet flow cell (FLWCL8X) and the flow-through cell (FLWCL8X1C). The wall-jet flow cell is designed to allow constant flow of solution across individual working electrodes without making contact with the rest of the working electrodes in the array sensor, and was employed in the analytical protocol developed here. A flow chart showing the experimental setup and the various features of multichannel analysis was used as a guideline for the hands-on demonstration for the final steering committee (Figure 4.1)

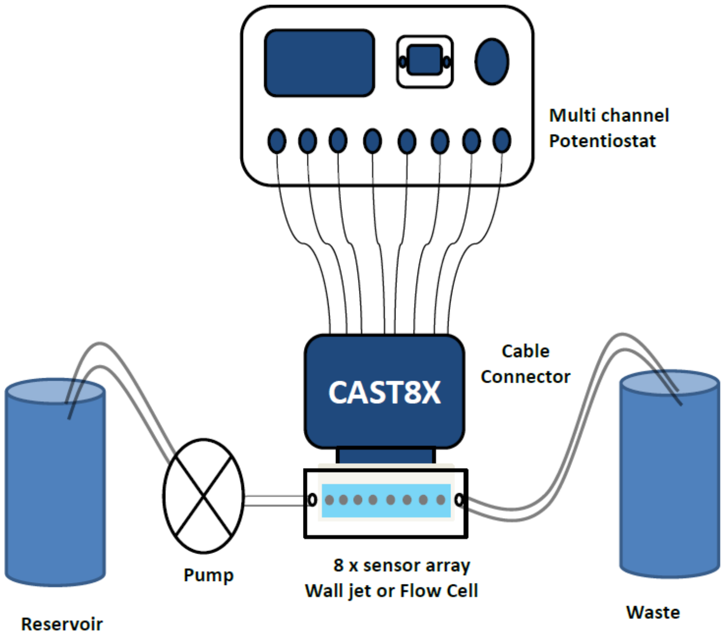


Figure 4.1: **Flow chart showing practical setup for multichannel analysis of PAHs**

The eight-channel SPCE is the same electrode material used throughout in this study and the multichannel will be evaluated using the unmodified and polymer-modified electrodes in the wall-jet design.

The experiments that were evaluated in the multichannel analysis included polymerisation of the monomer BPPD, single standard concentration profiling and a mixed sample of BaP and FLA (Table 4.2)

Table 4.2: Electrochemical experiments and parameters for the evaluation of multichannel performance

Analyte	**Electrode**	**Potential (vs Ag/AgCl)**	**Electrolyte**	**Technique**
BPPD (monomer)	SPCE	-0.7 to 0.7 V	HCl	CV(polymerisation)
PBPPD (polymer)	SPCE	-0.7 to 0.7 V	HCl	CV(characterisation)
1-OHP	SPCE		HCl	SWV
FLA	PBPPD	-0.54 V	$LiClO_4$	SWV/Amperometry
BaP	PBPPD	-0.7 V	$LiClO_4$	SWV/Amperometry

A simple flow diagram is provided here to understand the sequence of experiments conducted on the multichannel system (Figure 4.2)

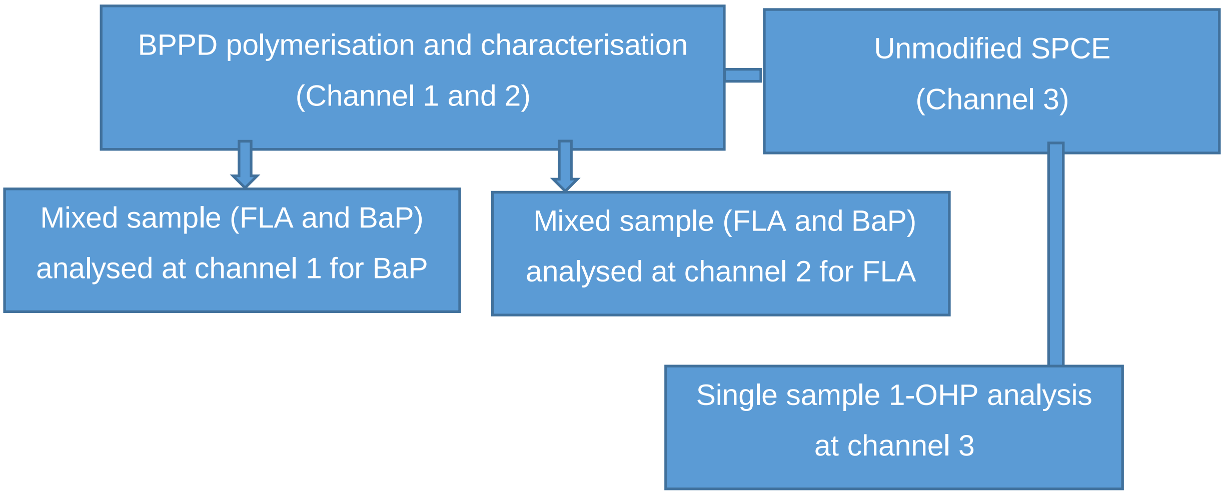


Figure 4.2: Flow chart for experiments performed on the multichannel system

4.2 Electropolymerisation of BPPD and polymer characterisation

In the multichannel experiment, the same monomer starting solution was used to effect the polymerisation, at two different electrodes using the same starting solution. The polymerisation was done by repeated potential cycling from -0.7 to +0.7 V at a scan rate of 50 mV/s for 15 cycles to produce a thin film of the polymer (poly(N,N'-Bis-(1H-pyrrol-2-ylmethylene)-benzene-1,2-diamine) at the SCPE in channel 1 and 2 (Figure 4.2). The monomer feed solution was then replaced with a 0.1 M HCl electrolyte solution and, using the same CV techniques, the diffusion coefficient (De) was evaluated. The De for the polymerised thin film was calculated from the scan-rate-dependent CV performed at the PBPPD-modified SCPE electrode, over a wide range of scan rates. A Randles Sevcik plot may be obtained as the linear relationship between peak current and the square of the scan rate (Figure 4.3)

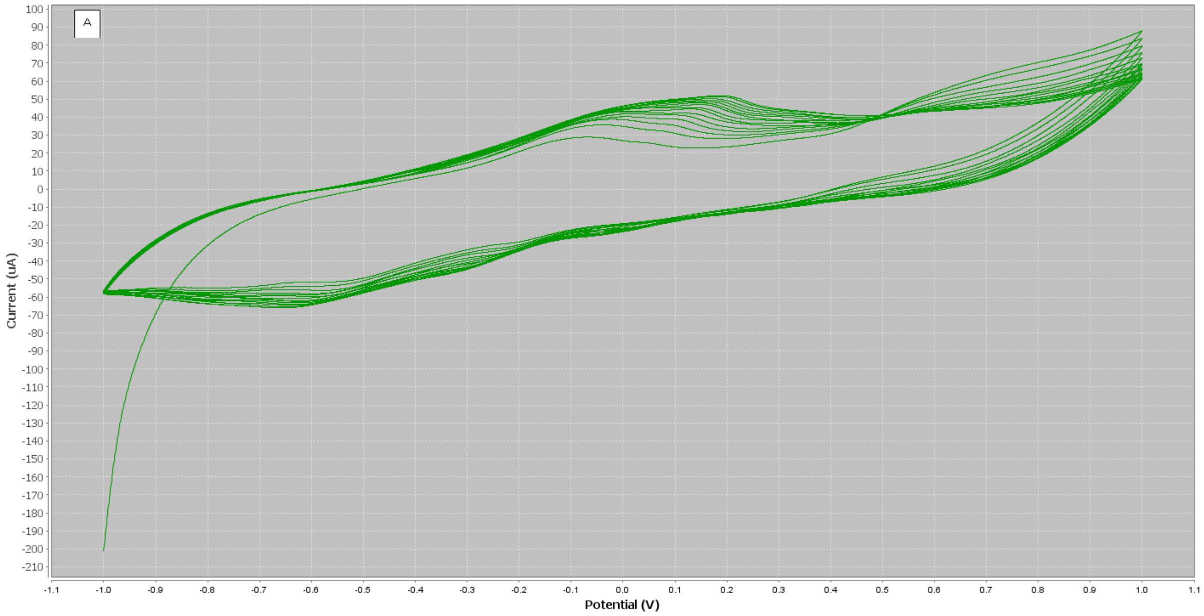


Figure 4.3: Electrochemical polymerisation of PBPPD at SPCE electrode in channel 1.

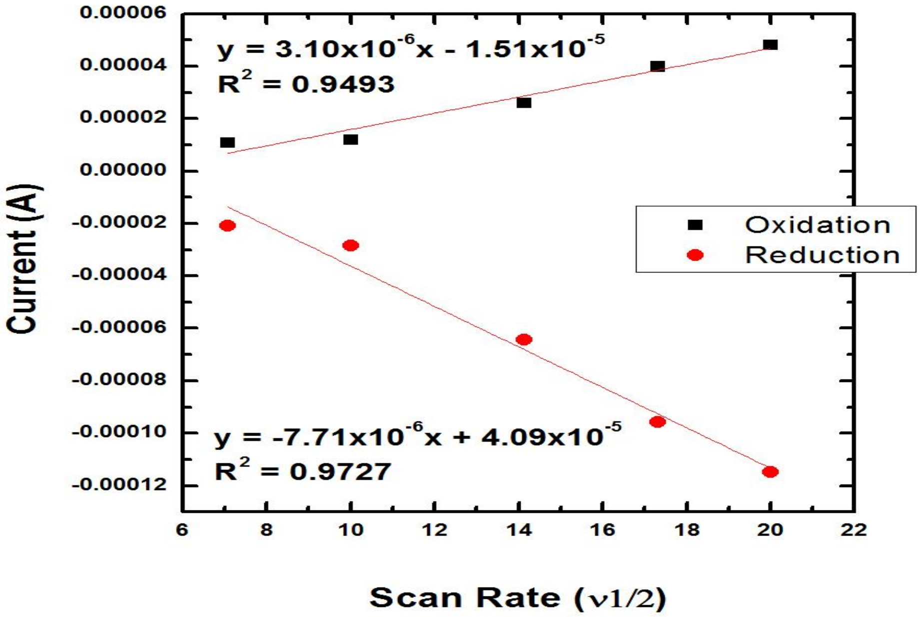


Figure 4.4: Randles Sevcik plot for the scan-rate-dependent CV reported at channel 1 and 2 of multichannel experiment

The De may be calculated from the slope of the plot, according to the Randles Sevcik equation. The De's calculated for the two channels were 2.08x10^{-12} cm^2/s, 1.28x10^{-11} cm^2/s respectively. These values for De are in good agreement with diffusion-controlled behaviour in thin film materials (26, 27).

4.3 Mixed-sample analysis using split feed from single sample reservoir

A mixed sample of fluoranthene and benzo[a]pyrene was prepared as a single sample constituted by equal proportions of the two analyte species. The single sample was delivered to the two channels (1 and 2) where the PBPPD polymer was polymerised previously. The PBPPD polymer was used as the electrocatalyst, whilst the voltammetric parameters in each cell were controlled to effect the detection under different experimental conditions. The peak potential applied for the detection of fluoranthene was -540 mV vs Ag/AgCl and the scan rate was kept constant at 50 mV/s. The applied potential for benzo[a]pyrene was -700 mV vs Ag/AgCl and the scan rate used was 10 mV/s. Each channel was evaluated by SWV (a potential sweep technique) as well as by amperometry (a fixed potential technique) at four different concentrations.

Amperometry is a simple, non-destructive technique that measures the proportional rise in redox current as a function of time. It is a technique used to report the direct Faradaic current associated with the oxidation or reduction of a species, and amperometry is not affected by the charging current of any other species, since it is not a sweep technique.

At the single potential applied for the oxidation of BaP (channel 1), the current due to BaP oxidation was reported as a decreasing current due to the adsorption of BaP onto the polymer electrode. At the single potential applied for the oxidation of FLA (channel 2), the current due to FLA oxidation was reported as an increasing current due to the oxidation of FLA at the polymer electrode (Figure 4.5 a and b). The standard concentrations were prepared to be in the lower ppm range, but it was not possible to calculate the dilution factor due to uncertainty in the flow control of the experimental system. Hence, calibration plots are based on relative concentration trends rather than actual values. The calibration plot for BaP was normalised with respect to the first measurement in order to represent both concentration profiles as increasing plots (Inset to graphs).

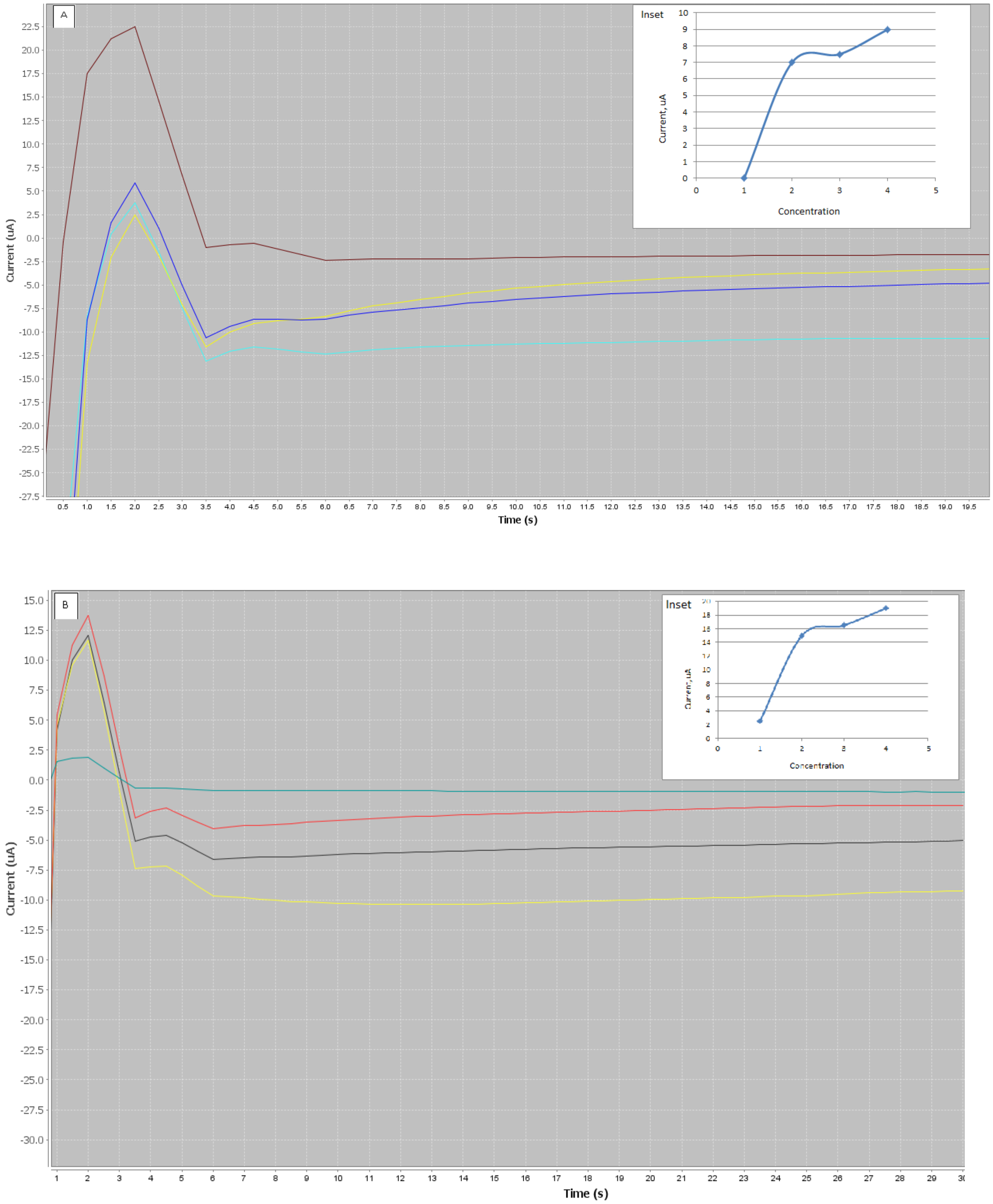


Figure 4.5: Amperometric analysis of (a) benzo[a] pyrene and (b) fluoranthene at PBPPD in 0.1 M LiClO4, with the respective calibration curve (Inset).

Clear, sharp peaks were observed for BaP and FLA at their respective electrodes. The rise in peak current was due to the concentration spike delivered to each cell. By fixing the analytical potential in each cell, we have ensured that only the selected species, i.e. BaP in channel 1 and FLA in channel 2, was quantified.

4.4 Conclusions

A multichannel electroanalytical system has been developed for the discrimination of PAH compounds as single species and in a mixed sample. The system employs simple chemical sensors which are robust and may be reasonably employed in real water samples, without fear of degradation. The individual electrocatalysts were able to quantitatively report on benzo[a]pyrene and fluoranthene with good sensitivity. The electrochemical method developed has shown promising results in laboratory experiments for the selective detection of benzo[a]pyrene and fluoranthene at polymer-modified SPCE, in the wall-jet arrangement of the multichannel analytical assembly.

CHAPTER 5

RECOMMENDATIONS AND FUTURE WORK

PAHs are highly hydrophobic species that are readily absorbed from the gastrointestinal tract of humans and other mammals, where they particularly deposit in the fatty tissue. Cytochrome P450-mediated mixed function oxidase systems are primarily responsible for oxidation or hydroxylation of these PAHs. Whilst there are numerous well-known, natural and anthropogenic sources of PAHs, it is perhaps less well known that PAHs can be produced biologically by certain plants and bacteria or formed during the degradation of plant material.

Polyaromatic hydrocarbons are not the easiest analyte species to detect based on redox chemistry. Natural degradation processes of PAHs include biodegradation, photo-oxidation, and chemical oxidation adsorption to soil particles, leaching, bioaccumulation (28). However the end product of each of these processes varies widely, based on the nature of the PAH species involved, since they have a unique structure, physical, chemical and biological properties. Chemical oxidation appears to be a minor PAH degradation mechanism under most environmental conditions, directly affected by the molecular weight and structure of the compound, the physical state and temperature of the compound, and the strength of the oxidising agent all impact the outcome. Fenton's reagent (using H_2O_2 for degradation) is the most commonly used oxidising agent which provides very aggressive oxidising conditions that commonly achieve the highest total PAH degradation for low molecular weight PAHs compared to high molecular weight PAHs.

Therefore, it should be emphasised that any method proposed for analysis of PAHs based on chemical or electrochemical oxidation is fraught with many challenges. The novel polymer electrocatalysts developed in this work, and in parallel research, provide for a wide range of new catalysts to be implemented in a controlled electrocatalytic approach. Unlike total degradation that may be achieved by high voltage electrolysis, electrocatalysis seeks to identify unique signals for identification of analyte species and intermediates that may be used for quantification and the understanding of degradation mechanisms The electrocatalysts were developed from Schiff base monomers synthesised by aldol condensation chemistry to produce reliable product in good yield.

These polymers were then deposited *in situ* at commercial screen printed electrode arrays to evaluate and quantify the oxidation current generated by electrochemical oxidation of selected PAH species. For the first time in this project we have reported on the electrochemical redox signals, reported at commercial SPCEs and novel polymer-coated electrodes, of a wide range of the most significant PAHs, in a comprehensive manner. Both the materials aspects of the actuator type polymers and the electrochemical signal reporting of PAHs are completely novel aspects of the research concluded. The PAHs studied are listed by the U.S. EPA as priority pollutants.

The novel electrocatalysts were then assembled in a multi-array configuration as a pre-text to the development of a fully independent electroanalytical tool for discrimination and quantification of PAHs in mixed samples. However, due to severe constraints in terms of time and finances, only a proof-of-concept was demonstrated in this project. Further validation and mixed sample analysis and, in particular, low-level detection of real samples was not performed and will be incorporated in future development of the method. Equally, the efficiency of polymerisation, washing steps and optimal flow conditions to benefit reliable and reproducible analytical performance still remains to be optimised.

It is envisaged that the transfer of the laboratory-scale technology, optimisation and validation, as well as the final design of a compact, portable electroanalytical system for PAH discrimination in aqueous mixtures, will form the basis of a follow-up project focussing on technology transfer, application and seasonal monitoring of PAH in municipal water systems. However, we believe that the proof-of-principle demonstration has shown that all the elements are in place for effective implementation of the technology.

One of the major challenges identified is that of insufficiently low detection levels of the electrocatalysts for the trace amounts of PAHs expected in water sampling. The actuator electrocatalysts were evaluated to have large contribution of capacitive current in the total sample current that suppressed the effect of the Faradaic current in analytical signals. An enhancement of electrochemical transduction may be effected by incorporating a suitable electron wiring effect, which may be achieved by doping the polymers with metal nanoparticles or other high-surface-area nanomaterials. Any development to improve the electrochemistry of the materials will directly benefit the overall analytical protocol developed here. However, the systematic approach demonstrated in this work is ground-breaking and paves the way for all future developments.

REFERENCES

1. Pashin, Y.V. and Bakhitova, L.M. Mutagenic and carcinogenic properties of polycyclic aromatic hydrocarbons. *Environmental Health Perspectives*, 1979. 30: p. 185-189.
2. Gan, S., Lau, E.V. and Ng, H.K. Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). *Journal of Hazardous Materials*, 2009. 172(2–3): p. 532-549.
3. Zhang, L., Bai, Z., You, Y., Wu, J., Feng, Y., Zhu, T. Chemical and stable carbon isotopic characterization of PAHs in aerosol emitted from two indoor sources. *Chemosphere,* 2009. 75: p.453-461.
4. Ahangar, A.G. Sorption of PAHs in the soil environment with emphasis on the role of soil organic matter: a review. *World Applied Sciences Journal*, 2010, 11(7), p.59 -765.
5. Borm, P.J., Cakmak, G., Jermann, E., Weishaupt, C., Kempers, van Schooten, P.F.J., Oberdoster, G., Schins, R.P. Formation of PAH-DNA adducts after in vivo and vitro exposure of rats and lung cells to different commercial carbon blacks. *Toxicology and Applied Pharmacology*, 2005 (2), p.157-167.
6. Zhang, Y. and S. Tao, Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. *Atmospheric Environment*, 2009. 43(4): p. 812-819
7. Silva, T., Azevedo, D.d.A. and. Neto, F.R.d.A. Distribution of polycyclic aromatic hydrocarbons in surface sediments and waters from Guanabara Bay, Rio de Janeiro, Brazil. *Journal of the Brazilian Chemical Society*, 2007. 18: p. 628-637.
8. Hecht, S., Carcinogen derived biomarkers: applications in studies of human exposure to second hand tobacco smoke. *Tobacco Control,* 2004. 13(Suppl 1): p. i48-i56.
9. Keith, L.H., The Source of U.S. EPA's sixteen PAH priority pollutants. *Polycyclic Aromatic Compounds,* 2015. 35(2-4): p. 147-160.
10. Bruzzoniti, M.C., Funqi, M. and Sarzanini, C. Determination of EPA’s priority pollutant polycyclic aromatic hydrocarbons in drinking waters by solid phase extraction-HPLC. *Analytical Methods*, 2010. 2: 739-745.
11. Kumar, B., Verma, V.K., Gaur, R., S. Kumar, S., C.S. Sharma, C.S. and A.B. Akolkar, A.B., Validation of HPLC method for the determination of polycyclic aromatic hydrocarbons (PAHs) in waste water and sediments. *Advances in Applied Science Research,* 2014. 5(1) 201-209.
12. Makelane, H.R. Tovide, O., Sunday, E.C., Waryo, T. and Iwuoha, E.I., Electrochemical interrogation of G3-Poly(propylenethiophenoimine) Dendritic Star Polymer in phenanthrene sensing. *Sensors*, 2015, 15 p. 22343-22363.

13. Rassie, C., Olowu, R.A., Waryo, T.T., Wilson, L., Williams, A., Baker, P.G., Iwuoha, E.I., Dendritic 7T-polythiophene electro-catalytic sensor system for the determination of polycyclic aromatic hydrocarbons. *International Journal of Electrochemical Science*, 2011. 6: p. 1949-1967.
14. Mailu, S.N., Waryo, T.T., Ndangili, P.M., Ngece, F.R., Baleg, A.A., Baker, P.G. and Iwuoha, E.I. Determination of anthracene on Ag-Au alloy nanoparticles/overoxidized-polypyrrole composite modified glassy carbon electrodes. *Sensors*, 2010. 10: p. 9449-9465.
15. Tovide, O., Jahed, N., Sunday, C.E., Pokpas, K., Ajayi, R.F. Makelane, H.R., Molapo, K.M., Johnb, S.V., Baker, P.G., Iwuoha, E.I. Electro-oxidation of anthracene on polyanilino-graphene composite electrode. *Sensors and Actuators B: Chemical,* 2014. 205: p. 184-192.
16. Kim, K.-H., Jahan, S.A., Kabir, E., Brown, R.G., A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environment International*, 2013. 60: p. 71-80.
17. Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B.,Rajendran, P. Nishigaki, I., Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, 2015. 5(3): p. 182-189.
18. Bressan, L.P., Cicero do Nascimento, P., Schmidt, ME.P., Faccin, H, Bohrer, D., Salting-out assisted liquid-liquid extraction and partial least squares regression to assay low molecular weight polycyclic aromatic hydrocarbons leached from soils and sediments. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2017. 173: p. 749-756.
19. Oliveira, M., Slezakova, K., Alves, M.J., Fernandes, A., Teixeira, J.P., Delerue-Matos, C., Pereira, M.D.C., Morais, S., Polycyclic aromatic hydrocarbons at fire stations: firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. *Journal of Hazardous Materials*, 2017. 323, Part A: p. 184-194.
20. Pushparajah, D., D.F.V. Lewis, and C. Ioannides, Up-regulation of CYP1A1 and phase II enzymes by 5-ring isomeric polycyclic aromatic hydrocarbons in precision-cut rat hepatic slices: Importance of molecular shape. *Toxicology in Vitro*, 2017. 40: p. 203-213.
21. Pashin, Y.V. and Bakhitova, L.M., Mutagenic and carcinogenic properties of polycyclic aromatic hydrocarbons. *Environmental Health Perspectives,* 1979. 30: p. 185-189.
22. Habibi, M.H; Hadjmohammadi, M.R. Determination of some polycyclic aromatic hydrocarbons in the Caspian seawater by HPLC following preconcentration with solid-phase extraction. *Iran. J. Chem. Chem. Eng.*, 2008, 27(4), p91-95.

23. Organization, W.H., Air Quality Guidelines for Europe. 2nd ed. European Series, ed. W.R. Publications. 2000, Copenhagen: World Health Organization Regional Office for Europe.

24. Jongeneelen, F.J., Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *Ann Occup Hyg*, 2001. 45 (1): p. 3-13.

25. Polycyclic aromatic hydrocarbons. H Choi, R Harrison, H Komulainen, J. M. Delgado Saborit. Chapter 6, WHO Guidelines for Indoor Air Quality: Selected Pollutants.

26. Atta, N.F.,Galal, A., Shimaa M. A., Determination of the diffusion coefficients for charge transfer through homo-, bilayered- and co-polymers of 3-methylthiophene and N-methylpyrrole. *Int. J. Electrochem. Sci.*, 2012, 7: 785 – 805.

27. Hlamulo R. Makelane, Oluwakemi Tovide, Christopher E. Sunday, Tesfaye Waryo Emmanuel I. Iwuoha. Electrochemical interrogation of G3-Poly(propylenethiophenoimine) dendritic star polymer in phenanthrene sensing. Sensors 2015, 15, 22343-22363.

28. Abdel-Shafy, H.I. Mansour, M.S.M. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 2016, 25 (1), 107–123