DEVELOPMENT AND VALIDATION OF NOVEL EXTRACTION TECHNIQUES FOR THE DETERMINATION OF TOTAL AND BIOAVAILABLE POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN WASTEWATER AND WASTEWATER SLUDGE

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) belong to an important class of persistent organic pollutants commonly found at low concentrations in the environment. The sources of these compounds include entirely anthropogenic activities such as chemical industries, combustion and agricultural activities. Owing to their potential carcinogenicity, mutagenicity and teratogenicity, it is important to determine PAHs in several matrices, which include complex matrices such as waste water and waste water sludge. To date, very few local and international studies have examined the detailed presence and distribution of these compounds within environmental compartments such as waste water sludge. Although official methods approved by international bodies such as the United States Environmental Protection Agency are able to extract PAHs in waste water sludge, all of them involve two separate steps. The steps generally include extracting the target compounds along with other unwanted compounds followed by a cleaning step before analysis. The two-step approach increases the errors in measurements and its time-consuming. Further, these methods extract the total fraction of compounds in the sludge and not just the bioavailable fraction. In the context of the environmental impact of these compounds, especially when sludge contaminated with PAHs is used as an organic fertiliser, it is the bioavailable fraction that matters and not the total amount in the sludge.

Therefore, the primary goal of this study was to develop, optimise and validate novel extraction techniques for to selectively extract PAHs from waste water and waste water sludge. The novelty of these techniques is that they combine two or three extraction steps into a single step, thereby minimising errors and sample turnaround time. Further, one of the techniques measures the bioavailable fraction while the other technique measures the total concentration, thus offering needed complimentary results.

The first part of the work involved synthesising and characterising molecularly imprinted polymers (MIPs) that would enhance the specificity of the PAH extraction. Key in the synthesis was choosing the appropriate template that could selectively bind all 16 United States Environmental Protection Agency (US EPA) priority PAHs. The synthesised and characterised imprinted and non-imprinted polymers were found to have surface areas of 386.6 $m^2 \cdot g^{-1}$ and 360 $m^2 \cdot g^{-1}$ respectively. The high surface area of imprinted polymers is attributed to cavities from the imprinting process. The different PAHs consisting of low molecular weight (two and three rings), medium molecular weight (four rings) and high molecular weight (five and six rings) were investigated. The MIPs imprinted with medium molecular weight PAHs preferred high molecular weight PAHs. On the other hand, high molecular weight PAHs imprinted polymers performed better than all prepared polymers, and were selected for further investigations in this study.

Batch adsorption studies revealed that the Langmuir adsorption model best describes the nature of adsorption, suggesting a monolayer formation. The total adsorption capacity was found to be 5189 $ng \cdot mg^{-1}$, which is comparable to values reported in literature. Findings from batch-binding studies further suggested that the synthesised polymer was selective towards all 16 PAHs with an average binding capacity of 317.6 $ng \cdot mg^{-1}$ and a relative standard deviation of

14.2%. Heptane maintained at 40°C was selected as a suitable solvent for eluting the PAHs from the polymer cavities.

The prepared MIP particles were used to selectively extract the PAHs during the development of two novel techniques for quantitating these pollutants in waste water and waste water sludge. The polymer was combined with membrane-assisted solvent as part of the novel *in situ* two-way extraction from waste water samples. The membrane-assisted solvent extraction-polymer technique was further combined with Soxhlet extraction to form a three-way extraction technique designed for improved selectivity, specificity and extraction efficiency capable of handling complex solid samples like waste water sludge.

In this regard, the optimised conditions for the two-way extraction, the membrane-assisted solvent extraction and MIP combination were found to be: 25% dimethyl formamide as organic modifier in the aqueous sample, 80 mg of MIP particles in the membrane bag, 180-minute extraction time and 1000 rpm stirring rate. The extraction efficiency for this combination ranged between 61.2% and 96.8% with an average of 77.4% for all 16 PAHs. The method detection limit ranged between 0.01 $\mathrm{ng \cdot mL^{-1}}$ and 0.45 $\mathrm{ng \cdot mL^{-1}}$. This developed technique showed remarkable selectivity and extraction capability for the total PAHs in waste water. It was also applied successfully in the quantitation of the bioavailable fraction of PAHs in waste water sludge. This was achieved by firstly dissolving varying amounts of sludge containing known amounts of PAHs in deionised water and then using the membrane-assisted solvent extraction-imprinted polymer technique to quantify bioavailable fraction. The approach of quantifying the bioavailable fraction of PAHs found in sludge is the first of its kind and forms part of the innovation of this research expedition. To date, only the hollow fibre liquid-phase micro-extraction can be applied to extract samples with high dissolved solids but with limited selectivity compared to the developed technique.

The optimised conditions for the three-way extraction, the membrane-assisted solvent extraction and MIP in combination with Soxhlet extraction included using the hexane/ tetrahydrofuran mixture as an extraction solvent and refluxing for 16 hours. The procedure involved extracting PAHs from a 1 g sludge sample and transferring them across the membrane where they eventually bind into the polymer cavities. Hot heptane was then used to elute the PAHs and concentrate it down to 1 mL.

The extraction efficiency recorded for the transfer of PAHs from sludge to heptane using the developed three-way single-step technique ranged between 16.9% and 47.9%. It had a method detection limit ranging between 0.14 $\mathrm{ng \cdot mL^{-1}}$ and 12.86 $\mathrm{ng \cdot mL^{-1}}$. The impact of the developed extraction technique was demonstrated in the clean chromatographic patterns generated for both certified reference materials and real samples, thus indicating complete removal of all impurities (interferences) for final instrumental analysis.

To the best of our knowledge, no study has reported a three-way extraction technique or the bioavailable fraction of PAHs found in sludge. The developed techniques are therefore considered quite novel and innovative for handing complex matrices such as waste water sludge where matrix effects still affect current approved standard methods.

DISSEMINATION ACTIVITIES

Previous

S. Ncube, N.T. Tavengwa, E. Cukrowska, L. Chimuka. 2015. Development of novel and selective sample preparation technique based on smart polymers and membranes. 42nd National Convention of South Africa Chemical Institute. 29 November – 4 December, Durban, South Africa.

S. Ncube, N.T. Tavengwa, E. Cukrowska, L. Chimuka. 2016. Coming back of Soxhlet extraction: A novel three way extraction based on combination of Soxhlet, membrane and imprinted polymers in single format. Keynote lecture. EXTech 2016 and 22nd International symposium on separation sciences. 3-6th July, Torun, Poland.

Future

- International Conferences × 2
- Local conferences × 3
- Publications in international journals × 4

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LIST OF ABBREVIATIONS

POP	Persistent organic pollutant
WWTP	Waste water treatment plant
WRC	Water Research Commission
PAHs	Polycyclic aromatic hydrocarbons
EU	European Union
US EPA	United States Environmental Protection Agency
USA	United States of America
MIP	Molecularly imprinted polymer
SPE	Solid-phase extraction
HMW	High molecular weight
MASE-MIP	Membrane-assisted solvent extraction molecularly imprinted polymer
LMW	Low molecular weight
B[a]P	Benzo[a]pyrene
DBA	Dibenzo[a,h]anthracene
B[a]A	Benzo[a]anthracene
B[b]F	Benzo[b]fluoranthene
B[k]F	Benzo[k]fluoranthene
B[ghi]P	Benzo[ghi]perylene
Indeno	Indeno[1,2,3-cd]pyrene
USE	Ultra-sonication extraction
SFE	Supercritical fluid extraction
PLE	Pressurised liquid extraction
MAE	Microwave-assisted extraction
CXL	CO_2-expanded liquid
MASE	Membrane-assisted solvent extraction
HF-LPME	Hollow fibre liquid-phase micro-extraction
SPME	Solid-phase micro-extraction
GPC	Gel permeation chromatography
MI-SPE	Molecularly imprinted solid-phase extraction
EGDMA	Ethylene glycol dimethacrylate
MAA	Methacrylic acid
AIBN	Azobisisobutyronitrile
ACVA	4,4-azo(4-cyanovaleric acid)
4-VP	4-vinylpyridine
VB	Vinylbenzene
DVB	p-divinylbenzene

NIP	Non-imprinted polymer
EDS	Energy dispersive X-ray spectroscopy
SEM	Scanning electron microscopy
BET	Brunauer–Emmett–Teller
DCM	Dichloromethane
HPLC-UV	High-performance liquid chromatography
GC-TOF/MS	Gas chromatography – time-of-flight mass spectrometer
THF	Tetrahydrofuran
$\mathbf{r^2}$	Coefficient of determination
MDL	Method detection limit
MQL	Method quantitation limit
EF	Enrichment factor
SE-MASE-MIP	Soxhlet extraction with membrane-assisted solvent extraction with molecularly imprinted polymer
CRM	Certified reference material
MMW	Medium molecular weight
RSD	Relative standard deviation
ANOVA	Analysis of variance

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1 INTRODUCTION

South Africa generally lags in evaluating persistent organic pollutants (POPs) in sludge and/or waste water from municipal waste water treatment plants (WWTPs). The Water Research Commission (WRC) has previously supported research on sludge leading to five report volumes that deal with guidelines for using and disposing waste water sludge (WRC, 2006, 2009). However, none of these reports reviews POPs as toxic chemicals in sludge. Only metal elements, nutrients, odour and pathogenic components are mentioned in Volume 2 that deals with requirements for the agricultural use of waste water sludge (Snyman and Van der Waals, 2004). This implies that many of the dangers of agronomic sludge due to POPs are unassessed.

Currently, South Africa has no legislation concerning POPs in its sludge disposal guidelines. This is mainly due to a lack of data on the contamination of waste water sludge by POPs. Studies in the northern hemisphere have also observed a similar level of ignorance regarding the potential impacts of unmonitored sludge disposal among European Commission countries (Suciu et al., 2015; Wenzl et al., 2006). We believe that this stems from a lack of viable quantitation methods for POPs in sludge and, hence, limited insights on the detrimental effects of sludge used in agriculture or land-refill programmes. Developing viable quantitation approaches for POPs in sludge to provide scientific evidence can create awareness and contribute towards reconsidering sludge-monitoring principles in South Africa. Quantitative scientific evidence might serve as a baseline for developing improved waste water sludge treatment methods, disposal options and monitoring protocols. These initiatives will assist in protecting the environment and minimising human exposure to POPs.

Among trace organic pollutants is a group of compounds with two or more fused aromatic rings called polycyclic aromatic hydrocarbons (PAHs). They have received considerable attention because of their documented potential carcinogenic, teratogenic and mutagenic nature to human health and their persistence in the environment (Chimuka et al., 2015; Singh and Agrawal, 2008). The European Union (EU), for example, has set 6 $mg{\cdot}kg^{-1}$ as the maximum total concentration of the 16 United States Environmental Protection Agency (US EPA) priority PAHs in sewage sludge. Denmark and Sweden have set 3 $mg{\cdot}kg^{-1}$ while the United State of America (USA) has set 9.5 $mg{\cdot}kg^{-1}$ as the maximum concentration (Smith, 2009). Knowing the actual concentration of these pollutants and their bioavailable fraction is very important when planning future use of sludge, especially for agronomic purposes (Zuloaga et al., 2012). For South Africa, the quantitation of PAHs is critical because the capability of most WWTPs in the country to remove these compounds during treatment is unknown. It has been observed that 80% of WWTPs in South Africa dispose their sewage sludge on dedicated land disposal sites (Snyman and Van der Waals, 2004). Unmonitored introduction of PAHs in the environment, especially for agronomic purposes, without fully understanding the chemical composition of the waste water and sludge can result in adverse consequences in the near future. The long-term impact of PAHs in the environment cannot be underestimated; hence, the need for extensive scientific evidence on the PAH sludge content.

The WRC has continued to support research for developing analytical methods to assess the occurrence, fate and environmental risk of POPs in South African WWTPs, but not much has been reported on PAH sludge content. Selected polybrominated diphenyl ethers, phenols and phthalates esters have been reported in sludge and effluent samples of WWTPs and freshwater systems in Cape Town (Olujimi et al., 2012). The content of endocrine-disrupting compounds has been assessed at WWTPs in Pietermaritzburg (Manickum and John, 2014). The levels of ibuprofen, naproxen and triclosan in several Johannesburg and Durban WWTPs have also been reported (Amdany et al., 2014; Madikizela and Chimuka, 2016). This research has focused on quantitating PAHs in waste water and waste water sludge.

Although PAH method development and its application have been reported in South Africa, very little has been reported on waste water sludge. Most studies have reported only a portion of the priority PAHs, mainly in water and sediment or soil samples (Chimuka et al., 2015). Sludge is important as it acts as a sink for many pollutants such as PAHs that are not degraded during treatment processes. It is important to characterise these pollutants and their bioavailable fraction when planning future use of sludge – especially for agriculture purposes. However, sludge is one of the most difficult samples to analyse, which might explain the limited studies on quantitating POPs. Developing selective sample preparation techniques remains a priority for effective quantitation of POPs in these complex samples.

In this project, two novel sample preparation techniques for quantitating POPs in waste water and waste water sludge samples using PAHs as a test case were developed. The approach was an attempt to minimise the number of analytical steps involved by integrating the extraction and clean-up steps into a single-step format for extracting PAHs in waste water sludge. This was achieved by combining the extraction efficiency of the Soxhlet extractor, the selectivity of a size exclusion membrane and the specificity of molecularly imprinted polymers (MIPs) for the sludge extraction. The set-up was combined into a single entity with the MIP-carrying membranes placed inside the Soxhlet apparatus. The membrane and the MIPs were also used in the second technique for extracting PAHs from waste water samples only. The MIP-membrane combination in our approach is the first of its kind for application in complex solid samples such as sludge. The developed techniques are unique, simple and selective. They allow for cheap extraction systems to be used to isolate both the total and bioavailable PAHs followed by chromatographic determination. It was important to assess the bioavailable fraction of PAHs in sludge samples to evaluate the toxicity effects of sludge when applied as a soil ameliorant.

The applicability of the developed techniques was tested on samples taken from the Northern Works and Goudkoppies WWTPs in Johannesburg. The research was an attempt to provide a scientific rationale for including PAHs and other POPs in legislature and quality assurance systems for sewage sludge disposal protocols. Johannesburg was targeted because it has a bulging population, the highest incidence of vehicles in streets, the busiest airport in Africa, complex restaurants and industrial activities – all of which are potential anthropogenic sources of PAHs; yet its PAH sludge content is unknown. The quantitative techniques developed during this study were used to provide scientific evidence of the presence of PAHs in waste water and waste water sludge.

2 LITERATURE REVIEW

2.1 Background

Sample preparation and clean-up steps are viewed as of paramount importance before analysing any chemical in aqua and biota samples (Chimuka et al., 2011a; Martín-Esteban, 2016; Ncube et al., 2016; Turiel and Martín-Esteban, 2010). Extracting trace organics from sediments and biota has many challenges such as low concentrations, complexity of biological samples, matrix interferences and the general similarity of organics. The need for efficient cost-effective sample preparation procedures requires the development of fast, simple and, if possible, solvent-free or solvent-minimised operations.

The approach before the advent of selective sorbents has been to extract the sample with a non-selective method such as solid-liquid extraction followed by a clean-up step of the extract using columns or solid-phase extraction (SPE) cartridges or disks packed with silica. This approach has been accepted in sample preparation of chemicals in complex samples and is still used widely. However, with the advent of more selective sorbents such as MIPs and immunosorbents, this approach is becoming less popular (Hennio, 1999; Turiel and Martín-Esteban, 2010). Using selective sorbents has the advantage that only target compounds are favoured during extraction. The effect of interfering compounds is thus reduced.

The use of adsorbents has been reported to be ineffective especially when analysing complex samples that consist of many interfering compounds present as both micro and macro molecules (Cacho et al., 2003; Chimuka et al., 2011b). For MIPs, the backbone of the polymer is non-specific and some hydrophobic high molecular weight (HMW) compounds get adsorbed on the backbone. Thus after extraction, the MIPs may need to be washed with an appropriate solvent to remove some of these interfering compounds (Gilart et al., 2014). The washing process is unfavourable because it also results in losing some of the target compounds (Cacho et al., 2003; Chimuka et al., 2011b). The loss of target compound is most pronounced if the target compound is also hydrophobic like PAHs. The adsorbed HMW matrix compounds also result in clogging of the MIP cavities, thus reducing efficiency.

In this research, a membrane was introduced to prevent the matrix components, especially HMW components, from coming into contact with the sorbent. The matrix compounds that manage to cross the membrane are either too small and easily washed out of the MIP cavities, or their functional groups and structural orientation do not permit their binding. Using membranes in combination with MIPs is generally described as the "membrane-assisted solvent extraction molecularly imprinted polymer" (MASE-MIP) technique (Chimuka et al., 2011b; Mhaka et al., 2009; Nemulenzi et al., 2009). The MASE-MIP approach can be likened to "prevention is better than cure", because matrix components are prevented from adsorbing on the sorbent altogether.

2.2 Polycyclic Aromatic Hydrocarbons

2.2.1 Physicochemical properties and fate of PAHs

PAHs are also referred to as polynuclear aromatic hydrocarbons or polyaromatic hydrocarbons. They comprise a large and diverse group of organic compounds characterised by at least two benzene rings fused in linear, cluster or angular arrangements. As hydrocarbons, these molecules are composed of only carbon and hydrogen atoms. Depending on the number of aromatic rings, the compounds can be classified either as low molecular weight (LMW) or HMW PAHs. The LMW PAHs have less than four aromatic rings; HMW PAHs have more than four rings.

Over a hundred individual and substituted PAHs have been documented, and they generally occur as complex mixtures rather than single compounds. PAHs are found in all environmental compartments and have a tendency to persist in the environment due to their hydrophobicity, inertness and limited biodegradability (Abdel-Shafy and Mansour, 2015). Where degradation occurs, the by-products are known to have elevated toxicity (Gaga and Ari, 2011). The toxicity of PAHs is generally enhanced by the presence of bay regions in their structural appearance as represented by the structure of benzo[a]pyrene (B[a]P) in Figure 1 (Cai et al., 2007; Nekhavhambe et al., 2014; Oleszczuk, 2007; Sun et al., 2009; Wenzl et al., 2006).

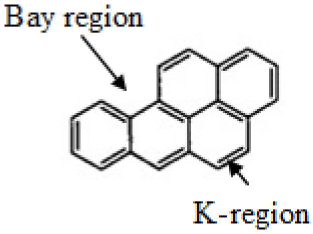
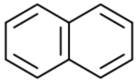
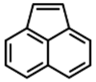
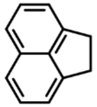
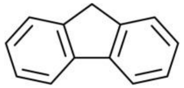
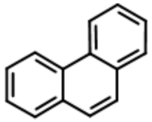
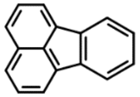
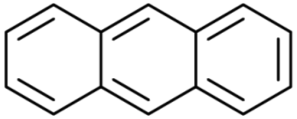


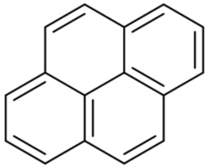
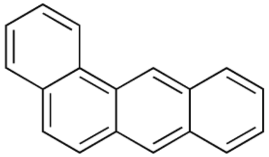
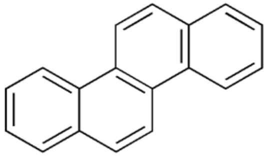
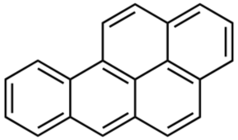
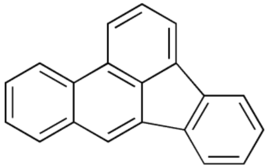
Figure 1: Structural features of PAHs contributing to toxicity

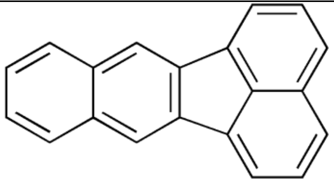
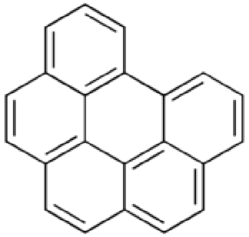
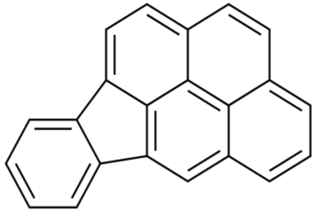
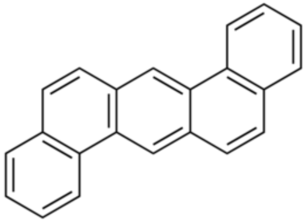
Environmental concerns of PAHs have necessitated that they are included in lists of priority pollutants by several regulatory agencies such as the US EPA, EU Environmental Protection Agency, and Environment Canada. The US EPA has identified 16 unsubstituted PAHs as priority pollutants, some of which are considered possible or probable human carcinogens (Shailaja and D’Silva, 2003). Their distribution in the environment and the potential risks to human health have received significant attention (Manoli and Samara, 1999).

The 16 US EPA priority PAHs and their relevant properties are given in Table 1. The observed physical characteristics such as boiling point and solubility in water (Table 1) are generally influenced by their conjugated π-electron systems, which in turn depends on the number and orientation of aromatic rings, and the molecular mass. HMW PAHs with linear conformations such as dibenzo[a,h]anthracene (DBA) are the least soluble in water.

Table 1: Physical and chemical properties of PAHs (Manoli and Samara, 1999)

Compound	**Structure**	**Molecular weight (g·mol^{-1})**	**Solubility (ng·mL^{-1})**	**Melting point (°C)**	**Boiling point (°C)**	**Log *K*ow**
Naphthalene		128	31.6	80.26	218	3.30
Acenaphthylene		152	1.93	92-93	265-275	4.07
Acenaphthene		154	3.93	95	279	3.98
Fluorene		166	1.68-1.98	116-117	295	4.18
Phenanthrene		178	1.20	100	340	4.45
Fluoranthene		202	0.20-0.26	11	375	4.90
Anthracene		178	0.076	218	342	4.45

Compound	**Structure**	**Molecular weight (g·mol^{-1})**	**Solubility (ng·mL^{-1})**	**Melting point (°C)**	**Boiling point (°C)**	**Log *K*ow**
Pyrene		202	0.077	156	393	4.88
Benzo[a]anthracene (B[a]A)		228	9.4E-03	162	437.6	5.61
Chrysene		228	2.8E-03	256	448	5.16
B[a]P		252	2.3E-03	179	495	6.06
Benzo[b]fluoranthene (B[b]F)		252	1.2E-03	168.3	481	6.04

Compound	Structure	Molecular weight (g·mol^{-1})	Solubility (ng·mL^{-1})	Melting point (°C)	Boiling point (°C)	Log *K*ow
Benzo[k]fluoranthene (B[k]F)		252	7.6E-04	215.7	480	6.60
Benzo[ghi]perylene (B[ghi]P)		276	2.6E-04	273	550	6.50
Indeno[1,2,3-cd]pyrene (indeno)		276	6.2E-02	163.6	536	6.58
Dibenzo[a,h]anthracene		278	2.49E-03	269.5	524	6.75

2.2.2 Sources of PAHs

PAHs are mainly introduced into the environment via natural and anthropogenic combustion of bituminous products (Amdany et al., 2014; Nielsen et al., 1983). Examples of natural sources include volcanic eruptions, degradation of biological materials and forest fires. Anthropogenic sources are numerous and result from industrial and human activities such as processing of coal and crude oil, combustion of natural gas, garbage incineration and vehicular exhausts. Such sources can be categorised into two broad groups: combustion of materials (such as coal, oil, gas and wood) for energy supply, and combustion for waste eradication (waste incineration) (Nielsen et al., 1983; Wild and Jones, 1995). Industrial and residential heating, power generation and mobile sources (vehicles, trains, airplanes and sea traffic) fall into the first group of sources, whereas the second category mainly covers incineration of municipal and industrial waste (Maliszewska-Kordybach, 1999). Other sources of PAHs include unregulated agricultural burning, smoke from crematoria, cigarette smoking, recreational fires and volatilisation from soils. A small number of PAHs are produced for commercial use, for example, as constituents in the production of plastics, dyes, pesticides and some drugs (Manoli and Samara, 1999).

In urban areas, the source of PAHs includes household human and chemical waste, automobile exhaust products, storm water run-off from both impervious and pervious areas such as roads, parking areas and construction sites, and industrial effluents from manufacturing chemicals. The PAHs enter WWTPs through sewerage. PAHs tend to accumulate in sludge during waste water treatment processes owing to their high affinity for carbonaceous particles. Sewage sludge is the semisolid material separated during waste water treatment processes (Cai et al., 2007; Singh and Agrawal, 2008; Zorpas et al., 2011).

A recent review on the status of PAHs in South Africa showed that these compounds are present in various South African environmental compartments (Chimuka et al., 2015). This is not surprising: South Africa is one of the largest economies in Africa and has several of the sources of PAHs mentioned above. South African energy relies mostly on coal combustion, which is one of the major sources of PAHs.

The review identified the Gauteng province as the major PAH pollution hotspot. Gauteng is the most industrialised province in South Africa. It also has the largest number of informal settlements without proper solid waste management. Gauteng has the largest number of vehicles on the road, which contributes to the PAH environmental burden. Most PAHs end up in sludge and waste water because of the open sewer system that takes storm water to WWTPs. The reported concentrations of PAHs in South African environments are more focused on air, water, soil and sediment samples. Very little has been reported in waste water and sludge despite being major routes for the fate of PAHs in the environment.

2.2.3 Exposure and health effects

2.2.3.1 Exposure

PAHs are strong lipid-soluble organic compounds, as indicated by their high log Kow values. Their entry routes into animal and human bodies are primarily through inhalation and absorption in the gastrointestinal tract, and partly through dermal adsorption (Boström et al., 2016; Chetiyanukornkul et al., 2006). PAHs deposited in the alveoli through inhalation are mostly absorbed unaltered into the bloodstream (Boström et al., 2016). Uptake through the gastrointestinal tract is by consuming contaminated dietary lipids. Their absorption from the intestinal tract is aided by the presence of bile salts. PAHs tend to concentrate mostly in the liver and fat-rich tissues of the body. Bioconcentration of PAHs in humans is not pronounced as most PAHs are metabolised quickly by the liver and excreted through faecal matter and urine.

2.2.3.2 Health effects

The general adverse health effects of PAHs to humans, animals and a variety of flora and fauna species are widely documented (Abdel-Shafy and Mansour, 2015; Dean and Suess, 1985; Maliszewska-Kordybach, 1999). Generally, acute toxicity of PAHs in human health is not well established while chronic effects related to how they interfere with organism biochemistry within the cell functioning, which is a major concern. The major health and ecological concerns of PAHs are because of their carcinogenic potential (Baird et al., 2005; Boström et al., 2016). Animal studies have revealed the potential of PAHs to cause gene mutations, developmental defects and tumours. Many individual PAHs have been tested for tumorigenicity. It has been observed that PAHs with bay regions were likely to be potent carcinogens (Boström et al., 2016).

B[a]P is the most potent carcinogen of the 16 US EPA priority PAHs. It is considered as a marker for environmental pollution by PAHs (Baird et al., 2005; Cai et al., 2007). Six other PAHs are known to have carcinogenic effects on humans. These are:

- B[a]A
- B[b]F
- B[k]F
- Chrysene
- DBA
- Indeno

2.3 Various Techniques for Extracting PAHs

A series of critical steps are involved when analysing organics in biological and environmental samples. These procedures start by collecting, treating and storing the sample. This is followed by extraction, isolation and identification of the target compound(s). The final step is quantitation and data handling. The choice of extraction method is viewed as a crucial step for good chemical analysis since it could influence the quality and credibility of the analytical results obtained. Sample preparation is the most delicate task involved in an analytical scheme.

This step contributes over 30% of potential sources of error due to contamination or loss of analyte (Rawa-Adkonis et al., 2006).

Analysis of PAHs generally consists of a solvent extraction step followed by clean-up and concentration using an adsorbent. For solid samples, conventional solid-liquid extraction techniques such as Soxhlet extraction and ultra-sonication extraction (USE) are still used extensively in present analytical procedures (Lau et al., 2010; Luque et al., 2015; Manoli and Samara, 1999; Shu et al., 2003; Zuloaga et al., 2012). These classical techniques require large volumes of organic solvents and extraction takes long to complete. New modern sample preparation techniques such as supercritical fluid extraction (SFE), pressurised liquid extraction (PLE) and microwave-assisted extraction (MAE) have been developed as an alternative to these classical techniques for analysing PAHs (Carabias-Martínez et al., 2005; Sibiya et al., 2013; Zhang et al., 2012; Zuloaga et al., 2012).

2.3.1 Classical techniques

Of the classical techniques, the USE technique is one of the most used techniques for extracting organic pollutants like PAHs from solids (Banjoo and Nelson, 2005; Liu et al., 2007). Its application in sludge samples to extract all US EPA PAHs has been reported (Abad et al., 2005; Busetti et al., 2006). Another technique, which is a modification of the USE technique, is the vortex-assisted extraction technique combined with dispersive liquid-liquid micro-extraction (Leng et al., 2012).

The Soxhlet extraction technique is generally a popular technique for extracting POPs from soils and sediments mainly because of its high extraction efficiency compared to modern techniques (Banjoo and Nelson, 2005; Martinez et al., 2004). Soxhlet extraction has therefore been vastly used as a benchmark technique to extract organics from soils and sediments. It is still the reference technique for almost all new extractions. It is still the recommended method by the US EPA (US EPA 3540C Soxhlet extraction) for extracting semi-volatile and non-volatile organics from solid matrices (Banjoo and Nelson, 2005). Strategies aimed at improving the classical Soxhlet extractor have led to the advent of Soxtec, an automated Soxhlet.

Several studies have used the Soxhlet system to extract PAHs from sludge samples (Moreda et al., 1998; Suciu et al., 2015; Zhang et al., 2012). The US EPA 3540C Soxhlet extraction (US EPA, 1996a) is followed by the silica gel clean-up method. The SW-846 US EPA 3630C has been reported in the extraction of PAHs from complex solid samples (Cai et al., 2007; Pakpahan et al., 2011). The automated Soxtec systems have been used to monitor PAHs (Jaouen-Madoulet et al., 2000; Maliszewska-Kordybach et al., 2008).

2.3.2 Modern pressurised extraction techniques

Pressurised extraction techniques form part of green analytical extraction techniques because they either use less organic solvents or use green solvents such as water and CO_2. Examples of these modern sample extraction techniques for solid samples include SFE, PLE, MAE and pressurised hot water extraction.

The PLE technique has been used to extract the 16 US EPA PAHs in sludge samples (Aemig et al., 2016; Zielińska and Oleszczuk, 2016). The technique has also been applied in the analysis of the 16 PAHs in soils and sediments (Saim et al., 1998; Sprovieri et al., 2007; Waqas et al., 2015). Several articles have also used the MAE technique to extract the US EPA priority PAHs from solid samples including sludge samples (Magi et al., 2014; Shu et al., 2003; Tomaniová et al., 1998).

The SFE technique has been successfully applied to extract PAHs in soil samples and in vegetable oils using pure CO_2 as the supercritical fluid (Berset et al., 1999). Modified CO_2 in which an organic solvent is mixed with CO_2 has also been introduced in the analysis of PAHs (Librando et al., 2004; Wenclawiak et al., 1997; Yusty and Davina, 2005). The resultant supercritical CO_2-solvent mixture is referred to as a CO_2-expanded liquid (CXL) (Al-Hamimi et al., 2016; Yusty and Davina, 2005). These CXLs have performed better than using only CO_2. Better efficiencies have been recorded than with the classical Soxhlet extraction when extracting especially HMW PAHs.

2.3.3 Membrane-based extraction techniques

Membrane-based extraction techniques include membrane-assisted solvent extraction (MASE) and the miniaturised hollow fibre liquid-phase micro-extraction (HF-LPME) techniques. We could only find one article that reported the application of HF-LPME in the extraction, isolation and preconcentration of PAHs from liquid samples (Charalabaki et al., 2005). The MASE technique is not a favourable approach when analysing PAHs with extraction efficiencies below 20% reported in the extraction of seven PAHs from seawater (March et al., 2011).

Modern approaches have aimed at combining these membranes with solid sorbents. When the analytes cross the membrane, they are selectively isolated by adsorption onto the adsorbent and finally preconcentrated by elution using an appropriate solvent. MIPs are the preferred adsorbent because of their specificity towards target analytes. The MASE-MIP method has been proven to greatly increase the selectivity of the membrane approach (Chimuka et al., 2011b; Mhaka et al., 2009; Nemulenzi et al., 2009). As far as this research is concerned, the combination of membranes and adsorbents has not been reported in the extraction of PAHs. One of the targets of this report was therefore to develop and evaluate a MASE-MIP method for extracting PAHs from waste water samples.

2.3.4 Other extraction methods

Other methods that have been reported to extract PAHs include Quick Easy Cheap Effective Rugged Safe, known as QUECHERS, (Salem et al., 2016) and the alkaline saponification technique in an alcoholic and non-alcoholic media (Mohammad et al., 2016; Olatunji et al., 2014; Rose et al., 2016; Viegas et al., 2012). Passive sampling technology using semipermeable membrane devices has also been reported in the monitoring of PAHs in aqueous samples (Chimuka et al., 2015; Miege et al., 2003) and in sludge ameliorated soil samples (Zielińska and Oleszczuk, 2016).

Low-cost natural adsorbents such as biocharred green coconut and goose eggshells have been applied in isolating PAHs in aqueous samples (Crisafully et al., 2008; Nuhu et al., 2012). Commercialised techniques such as the fexIKA 200-control series extractor have been reported (Gfrerer et al., 2002). Solid-phase micro-extraction (SPME) with mainly polydimethylsiloxane fibres for extracting PAHs from aqueous samples has also been reported (Bagheri et al., 2007; Coelho et al., 2008; Doong et al., 2000; King et al., 2004; Martin and Ruiz, 2007; Negrão and Alpendurada, 1998; Popp et al., 2000; Purcaro et al., 2007).

2.4 Comparative Studies of PAH Extraction Techniques

Several articles have done comparative studies of various techniques used to extract PAHs from aqueous and solid samples. A review on the techniques used to extract PAHs in soil samples has been done (Lau et al., 2010). Most studies have reported comparable performances for both the classical and modernised techniques for extracting PAHs from solid samples (Berset et al., 1999; Heemken et al., 1997; Miege et al., 2003; Song et al., 2002). On the other hand, several studies have demonstrated that modern pressurised techniques perform better than Soxhlet extraction, USE and shaking (Hollender et al., 2003; Houessou et al., 2006; Wenclawiak et al., 1997). The MAE technique has also proven to be a viable alternative for Soxhlet extraction and USE techniques (Shu et al., 2003; Tomaniová et al., 1998).

Conversely, classical techniques, especially the Soxhlet extraction and USE techniques, have in some instances gave better recoveries than modern PLEs (Drabova et al., 2012; Saim et al., 1997; Shu and Lai, 2001). Other PAH extraction techniques that have been compared with results showing similar recoveries include HF-LPME and SPME (Charalabaki et al., 2005), the matrix solid-phase dispersion method and the MAE technique (Pena et al., 2008).

2.5 Isolation and Preconcentration of PAHs

Almost all techniques used to extract PAHs are followed by a clean-up step where PAHs are isolated from the matrix effects and finally preconcentrated for detectability in chromatographic determination (Rawa-Adkonis et al., 2006). This step almost always uses SPE or gel permeation chromatography (GPC) with various adsorbent phases while membrane-based extractions like membrane-assisted solid extraction and liquid-phase micro extractions and of late MIPs have been introduced (Cacho et al., 2003; Chimuka et al., 2011a; Ferrer and Barceló, 1999; Hussen et al., 2007).

2.5.1 Silica gel, alumina and florisil adsorbents for SPE and GPC

Using silica gel, alumina and florisil as potential adsorbents to isolate the 16 US EPA priority PAHs from matrix effects has been investigated with results showing that better selectivity and reproducibility are achieved with activated silica gel (Ahad et al., 2015; Berset et al., 1999; Buczyńska et al., 2015; Gaga and Ari, 2011; Pastor et al., 1997). Other studies have reported using US EPA approved and also commercially available adsorbents such as StrataE, StrataM and Supelclean Envi-18 (Busetti et al., 2006; Cai et al., 2007; Drabova et al., 2012; Tomaniová et al., 1998).

Other SPE-based adsorbents used during isolation of PAHs include low-density polyethylene (Chen and Chen, 2005) and octadecyl (C_{18}) particles (Crisafully et al., 2008; Hajisamoh, 2013; Kiss et al., 1996; Moja et al., 2013; Oleszczuk, 2006; Pakpahan et al., 2011; Sibiya, 2012). Carbon nanotubes as SPE sorbents have shown great promise in isolating PAHs from matrix effects in water samples (Augusto et al., 2010; Ma et al., 2010). Titanium nanotubes modified with n-octadecanethiol as an SPE adsorbent have been reported by Pan et al. (2013).

2.5.2 Molecularly imprinted solid-phase extraction

The technique of molecular imprinting involves fabricating polymer material with specific recognition sites for the target analyte. A detailed overview of the synthesis and properties of MIPs is given in Section 2.6. MIPs have already been used in a broad range of applications from structural studies of ligand-receptor interactions to selective binding matrices in detection, separation and purification (Andersson, 2000; Augusto et al., 2010; Martín-Esteban, 2016; Mayes and Mosbach, 1997; Sarafraz-Yazdi and Razavi, 2015). The ability of MIPs to selectively bind the target analyte is based on the theory that after removing the template molecule, the interactions between complementary functionalities present in the imprint molecule and the monomer(s) prior to the initiation of polymerisation are conserved in the product polymer.

The technique of using these polymers as adsorbents for analyte isolation is referred to as “molecularly imprinted solid-phase extraction” (MI-SPE). The first fabrication of MI-SPE was presented by Sellergren in 1994 in which an ethylene glycol dimethacrylate (EGDMA)/ methacrylic acid (MAA) copolymer molecularly imprinted with pentamidine was prepared and applied to isolate this antibiotic drug from urine (Augusto et al., 2010; Sellergren, 1994).

2.6 MIPs for PAHs

2.6.1 The MIP concept

In molecular imprinting, the imprinting process occurs by polymerising a functional monomer and a cross-linker in the presence of a template. The functional monomer interacts with the template molecule while the cross-linker controls the morphology of the polymer by imparting mechanical stability to the polymer formed. During polymerisation, the functional monomer and cross-linker molecules form a complementary three-dimensional shape around template molecules (Sarafraz-Yazdi and Razavi, 2015; Vasapollo et al., 2011). The template molecules are then removed and cavities with recognition-binding sites remain in the polymer matrix where a specific target analyte or group of structurally related analytes can then rebind during extraction from real environmental samples. The principle of MIP synthesis is illustrated in Figure 2.

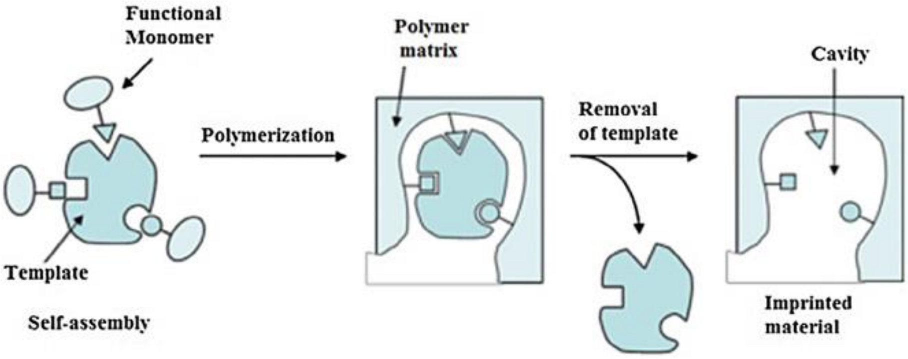


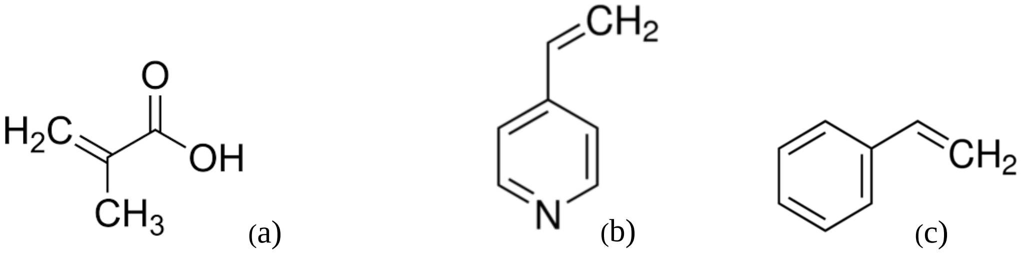
Figure 2: Synthesis of a MIP (Sarafraz-Yazdi and Razavi, 2015)

The three basic synthetic approaches to MIP fabrications are covalent, semi-covalent and non-covalent. In covalent imprinting, the analyte is covalently bound on the MIP cavities. In non-covalent imprinting, the analyte and the template are bound via weak interactions such as Van der Waals and hydrogen bonding. Semi-covalent imprinting involves preparing the MIP by covalently binding the template to the functional monomer. During application, the analytes however bind to the cavities through non-covalent interactions (Sarafraz-Yazdi and Razavi, 2015; Turiel and Martín-Esteban, 2010).

While covalent bonding is more stable and selective, non-covalent imprinting is preferred because of the weak interactions that are easy to cleave during washing and elution of the analytes from the cavities. It also allows for application in extraction of multiple analytes of similar topology (Augusto et al., 2010; Sarafraz-Yazdi and Razavi, 2015; Spivak, 2005). In non-covalent imprinting, the recognition of analytes is controlled by the shape and size of the cavities through hydrophobic and π–π interactions between template and monomer molecules (Baggiani et al., 2007).

The most common non-covalent imprinting approach is bulk thermal polymerisation. The entire process involves interaction of the template and the functional monomer followed by polymerisation in the presence of a cross-linker. Free radicals are used to initiate polymerisation; hence, the process is sometimes called free radical polymerisation (Braunecker and Matyjaszewski, 2007; Wang et al., 2012). Azo-based initiators such as azobisisobutyronitrile (AIBN) and 4,4-azo(4-cyanovaleric acid) (ACVA) are used as the source of free radicals (Cormack and Elorza, 2004; Guo et al., 2011). The AIBN undergoes thermolysis at 60°C forming two 2-cyanoprop-2-yl radicals. The main disadvantage of bulk polymerisation is that the product is a solid mass that needs to be crushed into a fine powder. During grinding, most imprinted cavities are broken and MIP granules have a random shape and size distribution (Carabias-Martínez et al., 2005).

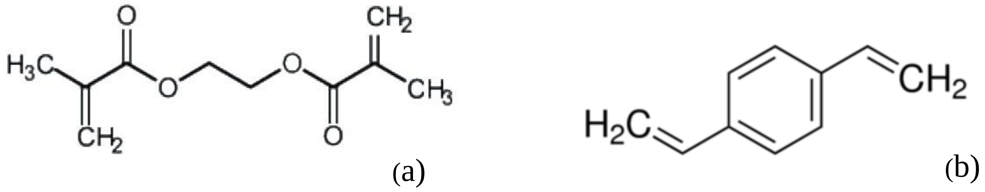
Synthesising MIPs for PAHs offers a challenge because PAHs are lipophilic with no pronounced functional groups. Thus, steric cavities are created through hydrophobic and π–π interactions. As shown in Figure 3, common monomers used in MIP synthesis are acidic MAA, basic 4-vinylpyridine (4-VP) and neutral vinylbenzene (VB). These compounds interact with PAHs through hydrophobic interactions. The presence of aromatic rings in 4-VP and VB results in introduction of π–π interactions. Some studies have confirmed that a 4-VP-based MIP has a better selectivity towards B[a]P than an MMA-based MIP (Lai et al., 2004). In non-covalent imprinting, complimentary intermolecular interactions between the functional monomer and the template are critical (Carabias-Martínez et al., 2005; Lai et al., 2004), thus VB as a monomer is expected to offer a better three-dimensional interaction with the PAH ring structure. The presence of the nitrogen atom in 4-VP may result in poorer retention of PAHs than when using VB as the functional monomer. The C=C bond found in all three monomers is involved in addition polymerisation with the C=C bond of the cross-linker.



(a) (b) (c)

Figure 3: (a) MAA, (b) 4-VP, and (c) VB

The most commonly used cross-linkers are EGDMA and p-divinylbenzene (DVB). Their structures are shown in Figure 4. A cross-linker serves as a stabiliser for the imprinted cavities and also imparts mechanical stability to the polymer matrix to retain its molecular recognition capability (Cormack and Elorza, 2004; Vasapollo et al., 2011). DVB gives MIPs that are rigid and selective towards a target analyte. This is important in MIP imprinting technology where the target is a single analyte. The long chain in EGDMA results in cavities that are not so selective due to flexibility of the cross-linker backbone (Gilart et al., 2014; Lai et al., 2004; Spivak, 2005). When analysing several target analytes, cross-selectivity becomes advantageous because more sterically related analytes can be sequestrated.



(a) (b)

Figure 4: (a) EGDMA and (b) DVB

Molecular modelling software has recently been introduced to select the best monomer for a particular template by calculating the binding energies and spatial configuration of several template-monomer complexes (Augusto et al., 2010; Dai et al., 2016; Madikizela et al., 2016). This allows different monomers and cross-linkers to be screened to produce a MIP with high selectivity for a particular analyte. Where several analytes are targeted, molecular modelling becomes undesirable and the experimental approach is the only solution.

2.6.2 Synthesis of MIPs for PAHs

Pichon and Chapuis-Hugon (2008) reviewed using MIPs to extract PAHs in plant and environmental samples. Their application as SPE sorbents has greatly increased selectivity of the SPE clean-up step after a liquid/solid-liquid extraction from solid samples or directly from aqueous samples. Table 2 summarises PAHs used as templates.

Table 2: List of MIPs for PAHs

PAH Template	Target PAHs	Monomer	Cross-linker	Application	Reference
Pyrene	23 PAHs in spiked water	4-VP	DVB	GPC	(Baggiani et al., 2007)
B[a]P	B[a]P only in water samples	MAPA	EGDMA	MI-SPE	(Çorman et al., 2017)
Anthracene	Five PAHs in aqueous solution	Tetraiodobisphenol A	Phloroglucinol	QMB optosensor	(Dickert et al., 1999)
Commercial MIP (SupelMIP^{TM}-SPE)	16 US EPA PAHs in tea leaves and vegetable oils	VB	DVB	MI-SPE	(Drabova et al., 2012, 2013)
Toluene as a pseudo-template	Naphthalene, fluoranthene, phenanthrene and pyrene in waste water and seawater	4-VP	EGDMA	Thin films	(Egli et al., 2015)
Non-imprinted polymer (NIP)	Fluoranthene, B[b]F and B[a]P in soil, sludge and sediment	VB	DVB	MI-SPE	(Flotron et al., 2005)
b-naphthol	Naphthalene in spiked water	TOPC	TEOS	MI-SPE	(Guo et al., 2011)
Anthracene	Anthracene in spiked aqueous samples	MAA	EGDMA	Direct	(Hassan et al., 2016)
Methylbenz[a]pyrene	B[a]P in cigarette smoke	4-VP	DVB	MI-SPE	(Ho et al., 2010)
B[a]P	B[a]P in cigarette smoke	MAA	TRIM	MI-SPE	(Ho et al., 2011)
1-hydroxypyrene	1-hydroxypyrene in aqueous solution	VB	DVB	direct	(Kirsch et al., 2001)

PAH Template	Target PAHs	Monomer	Cross-linker	Application	Reference
Multi-template (16 PAHs)	Eight fluorescent active PAHs in sediments	MAA	EGDMA	MI-SPE	(Krupadam et al., 2010b)
Multi-template (Five PAHs)	Five PAHs used as templates in dust	4-VP	EGDMA	MI-SPE	(Krupadam et al., 2010a)
Multi-template (6 PAHs)	Six PAHs used as templates in water	MAA	EGDMA	MI-SPE	(Krupadam et al., 2010a)
B[a]P	B[a]P in aqueous samples	4-VP	DVB	MI-SPE	(Lai et al., 2004)
Phenanthrene	Anthracene, phenanthrene, pyrene, and Pery in milk samples	VB-co-MAA copolymer on magnetic nanoparticles		Direct	(Li and Wang, 2013)
Pyrene and naphthalene (binary imprinting)	Pyrene and chrysene	4,4′-diisocyanatodiphenylmethane	Poly(ethylene glycol)	Sensors	(Lieberzeit et al., 2008)
Fluoranthene	Fluoranthene	Tetraiodobisphenol A	Phloroglucinol	RTP optosensor	(Sánchez-Barragán et al., 2005)
16 US EPA PAHs	16 US EPA PAHs in sea water	PTMS	TEOS	MI-SPE	(Song et al., 2012)
Pyrene	Pyrene, chrysene and B[a]P in aquatic and air samples	Pyrene and terthiophene monomer electropolymerized on gold surface of a QCM crystal		Sensors	(Tiu et al., 2016)
B[a]P	B[a]P in spiked water samples	Tetrabromobisphenol A	Phloroglucinol	Optosensor	(Traviesa-Alvarez et al., 2007)
Anthracene	Anthracene in aqueous samples	VB	DVB	MI-SPE	(Yatim and Ariffin, 2011)

Common PAHs are pyrene and B[a]P. Using these two PAHs is rather contrasting because B[a]P is highly carcinogenic and serves as a marker for PAH pollution while pyrene is suitable as an imprinting molecule because it is not considered carcinogenic or mutagenic.

Krupadam et al. (2010a) and Song et al. (2012) used a mix of different PAHs as templates for one MIP, which is called multi-template MIP imprinting. Ho et al. (2010) and Kirsch et al. (2001) reported dummy template imprinting where a compound with structural orientation close to the target analyte(s) is used. It eliminates template bleeding and imprinting effect.

To the best of our knowledge, no article has reported using MIPs to analyse PAHs from waste water and waste water sludge. Our study was aimed at searching for a PAH that can be used as a template in the synthesis of a MIP with an effective cross-selectivity for PAHs from an aqueous solution. The premise of the study was that there is PAHs that can create cavities with high affinity for all 16 US EPA priority PAHs. The PAHs investigated as potential molecular imprinters were selected based on the number of rings and steric orientation of the rings. The functional monomer used to interact with PAHs was VB as it has a more complete benzene ring than 4-VP and was expected to form stronger interactions with PAH rings. EGDMA was chosen as a cross-linker because it has shown better selectivity towards a group of structurally related compounds (Ho et al., 2010). The target was to create cavities that are flexible yet class-selective. The selected MIP was then used during the development of two novel extraction techniques to determine total and bioavailable PAHs in waste water and waste water sludge.

2.6.3 Characterisation of MIPs

MIPs are commonly characterised for their morphology, chemical composition and molecular recognition behaviour. For chemical composition, several techniques for solid samples are used. These include Fourier transform infra-red spectroscopy to probe some diagnostic signals due to both polymerisation and analyte adsorption in the cavities (Cormack and Elorza, 2004; Vasapollo et al., 2011).

Elemental composition of the polymer can be assessed using various techniques such as powder X-Ray diffraction and energy dispersive X-ray spectroscopy (EDS). Unfortunately, these elementary analysis techniques cannot identify the trace presence of analytes in the cavities. Solid nuclear magnetic resonance can be used to gain insights in any changes in the chemical environments during polymerisation and analyte binding. However, this technique is still underutilised in MIP characterisation studies (Cormack and Elorza, 2004; Karim et al., 2005; Vasapollo et al., 2011). Morphological studies are done using mainly scanning electron microscopy (SEM) while pore-related parameters are investigated using nitrogen sorption porosimetric techniques such as Brunauer–Emmett–Teller (BET) analysis. Molecular recognition behaviour is explained by doing batch-rebinding studies and calculating adsorption (kinetic and isotherm) models (Chrzanowska et al., 2015; Gao et al., 2016; García-Calzón and Díaz-García, 2007; Lagha et al., 2011).

2.6.4 Batch studies

Batch adsorption studies are essential to understand the performance of an adsorbent. Knowing the mechanism of adsorption and the extent of the MIP-binding capacity becomes important so that the amount of MIPs to be used in a system to extract PAHs from environmental samples can be defined. Parameters assessed during batch studies include solution pH, initial concentration, contact time and temperature depending on the type of analyte and the extraction technique used. The solution pH is important for analysing compounds with functional groups that exist in different charged states at different pH values. Initial concentration is used for calculating adsorption isotherm models. Thermodynamic studies are needed to calculate the Gibbs free energy, a quantity that explains the spontaneity and feasibility of the adsorption process. The contact time batch results are used in calculating Lagergren kinetic rate equations to explain the mechanism of adsorption.

2.6.4.1 Adsorption kinetic modelling

Kinetic rates of adsorption reactions are important in determining the mechanism of adsorption and the time it will take for the MIP to reach its equilibrium (maximum) binding capacity. The dependence of the adsorbate-adsorbent interaction on the contact time can be explained using the Lagergren kinetic equations. The Lagergren kinetics equation has been most widely used for the adsorption of an adsorbate from an aqueous solution.

2.6.4.2 Adsorption isotherm studies

The Langmuir and the Freundlich isotherms are the two commonly compared models when explaining adsorbate-adsorbent interactions. The Langmuir model assumes that the adsorbates form a monolayer on the adsorbent active sites while the Freundlich model predicts a multilayer formation on the surface of the adsorbent.

3 RESEARCH METHODOLOGY

3.1 Chemicals and Reagents

A 2000 mg·L^{-1} PAH mixture with 99.9% purity was obtained from Supelco (Bellefonte, PA, USA). Hexane, heptane, methanol, dichloromethane (DCM), tetrahydrofuran (THF), acetone and cyclohexane were purchased from Sigma-Aldrich (Johannesburg, South Africa). Empty 3 mL cartridges with 10 µm frits were obtained from Sorbent AB (Frölunda, Sweden). Membrane bags and accessories were supplied by Gerstel (Mülheim, Germany).

3.2 Instrumentation

The solutions were stirred using a WiseStir MS-MP8 stirrer. A WiseCube WIG-105 Precise Shaking Incubator was used for temperature-controlled shaking. Both pieces of equipment were obtained from Wisd Laboratory Instruments, Wertheim, Germany. The high-performance liquid chromatography (HPLC-UV) system used during preliminaries was from Bischoff Chromatography, Leonberg, Germany.

A Pegasus 4D one-dimensional gas chromatograph and a time-of-flight mass spectrometer (GC-TOF/MS) (LECO Corporation, St. Joseph, MI, USA) were used for quantitating PAHs. The chromatograms were obtained on a 7890B gas chromatography system with a 7683B Series Injector (Agilent Technologies, DE, USA). Analytes were separated on a 30 m × 0.32 mm × 25 µm Rxi-5Sil MS capillary column (Restek Corporation, Bellefonte, PA, USA). Its active functional group is 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane. Helium (>99.99%) was used as carrier gas at a flow rate of 1 mL·min^{-1}. The sample injection volume was 1 µL in the splitless mode at an injection temperature of 300°C. The interface temperature was set at 250°C, the temperature of the source was 300°C, and the detector voltage was at 200 mV. The temperature program is summarised in Table 3. The ionisation mode was electron impact (E +70 eV). The total runtime was 32 minutes.

Table 3: Temperature program

Rate	Temperature (°C)	Hold time (min)
Initial	80	0.5
20	180	2
20	200	2
20	240	1
10	250	3
10	260	3
20	285	2
5	290	1
20	300	2
15	310	0.5

Data was acquired in the full scan mode from 45 amu to 500 amu at a rate of 10 scan·s^{-1}. Data processing was done using ChromaTOF software version 4.5.1(LECO Corp, St. Joseph, MI, USA). Signal-to-noise minima were set at 3 S/N, and each chromatogram was processed over a complete ion mass range after a 3.6-minute solvent delay. Positive identification of all the PAHs was based on retention times of standards and quantitation ions obtained prior to analysing waste water extracts. Where necessary, confirmation ions were used to identify the analytes in addition to quantitation ions. A four-minute acquisition delay was set to avoid solvent front into the MS system. A four-second peak width was selected with 700 set as the minimum similarity match before assigning analyte names to peaks at specific retention times. The MainLib and Replib libraries were used to assign analytes to quantitation ions.

3.3 Selecting an Efficient PAH-imprinted MIP

The initial approach was to investigate and select a PAH that could be used as a template to synthesise a MIP that would quantitatively extract all 16 US EPA priority PAHs. Six different PAHs were chosen for the investigation based on their steric properties and/or structural orientation of their rings. Of the six PAHs, three were pyrene-based and the other three fluoranthene-based with a five-membered ring in their structure. The pyrene-based PAHs were pyrene (four rings), B[a]P (five rings) and indeno (six rings). The fluoranthene-based PAHs were fluoranthene (four rings), B[k]F (five rings) and B[ghi]P (six rings). In all studies, styrene (also called p-vinylbenzene) was used as the functional monomer. Styrene is more complementary to PAHs than vinylpyridine and methymethacrylate monomers. EGDMA was used as a cross-linker because of its flexibility compared to DVB (Spivak, 2005).

Some of the MIPs synthesised from individual imprints were physically combined at 1:1 (w/w) and investigated. The investigated combinations are shown in Table 4. For example, a pyrene-imprinted MIP was combined with a fluoranthene-imprinted MIP (both are LMW and have four rings). The pyrene-imprinted MIP was also combined with a B[a]P-imprinted MIP (sterically related four- and five-ringed imprints) and with a B[ghi]P-imprinted MIP (sterically related four- and six-ringed imprints). Eventually, the B[a]P-imprinted MIP would also be combined with a B[ghi]P-imprinted MIP (sterically related five- and six-ringed imprints). The same reasoning was used in combining the fluoranthene-based imprinted MIPs.

Table 4: MIP combinations

🟩 Fluoranthene-based MIP
🟦 Pyrene-based MIP combinations
🟫 Ring-based MIP combinations

Indeno						
B[ghi]P	🟫					
B[k]F	🟩					
B[a]P		🟦	🟫			
Fluoranthene	🟩		🟩			
Pyrene		🟦		🟦	🟫	
	Indeno	B[ghi]P	B[k]F	B[a]P	Fluor-anthene	Pyrene

3.3.1 Synthesis of the MIPs

A mixture of 3 mL of DMF and 0.03618 mmol (equivalent to 7-10 mg) of the PAH template was added in a round bottom flask. It was then heated to 60°C while stirring at 500 rpm for five minutes to completely dissolve the template. An amount of 20 µL of VB functional monomer was added into the dissolved mixture while stirring and heating for a further 30 minutes. Thereafter, 400 µL of the EGDMA cross-linker was added to the mixture. Heating and stirring continued for another 60 minutes. The system was purged with nitrogen gas for five minutes to remove oxygen from the reactor. Then, 10 mg of the ACHCN initiator was added and the temperature was slowly increased to 80°C. Polymerisation was achieved within an hour. The system was allowed to cool down to room temperature.

The MIPs were transferred into a petri dish and dried in an oven at 70°C. The dry MIP was ground into a fine powder and transferred into a 50 mL vial. Six MIPs imprinted with the PAHs chosen for investigation as potential imprints were synthesised. A 50 mL solution of hexane/ DCM (4:1 v/v) was then added into the six vials. The DCM was used to remove the porogen molecules trapped within the MIP three-dimensional structure. While removing imprint molecule creates cavities, removing porogen molecules creates some diffusion pathways that become essential during re-adsorption of analytes and their subsequent elution from the cavities.

The vials were placed on a WiseCube shaking incubator. Shaking was done at 150 rpm for four hours while the temperature was maintained at 45°C. The supernatants were analysed for the imprint molecule using GC-TOF/MS. This was repeated every two hours until the imprint PAH was no longer detected. A NIP was also synthesised in a similar way as the MIP but in the absence of a template. Synthesis and washing of NIPs and MIPs were done simultaneously.

3.3.2 MIP screening studies

A 5 mL toluene solvent was spiked at 2 $\mu g \cdot mL^{-1}$ PAH mixture. Thereafter, 20 mg of the MIP was dropped into the solution and allowed to adsorb PAHs for three hours. The mixture was filtered through 0.45 µm frits. The filtrate was analysed using a GC-TOF/MS. The same procedure was done simultaneously for all synthesised MIPs, both individually and as combinations. A PAH favoured by a particular MIP or MIP combination would have a low peak area in the supernatant. The MIP favoured by most PAHs was chosen as the best one for extracting the 16 US EPA priority PAHs and was used in subsequent studies.

3.3.3 Batch studies

Batch sorption studies were done to understand the extent of the adsorption of PAHs on the surface of the synthesised MIP. Adsorption isotherms were used to explain the extent of adsorption on the MIP while kinetic studies were done to understand the mechanism of adsorption. To explain the extent of adsorption, two adsorption isotherm models were applied on the data obtained from the effect of varying the concentration of PAHs in the donor solution. The effect of contact time was investigated and the Lagergren pseudo-first and second order models applied to understand the mechanism of adsorption.

3.3.3.1 Effect of initial concentration

The effect of the initial concentration of PAHs in solution was investigated by placing 10 mg of the selected MIP directly into six 2 mL hexane solutions spiked in the 0.1-5 µg·mL^{-1} range with the 16 PAHs. Binding was allowed to occur at ambient conditions for 60 minutes. The amount of each PAH extracted by the MIP particles was calculated as the difference between the spiking amount and the amount remaining in solution after extraction. The maximum amount of each PAH adsorbed by a unit mass of the MIP (also called the adsorption capacity) was calculated using Equation 1. The total adsorption capacity at each spiking concentration was then calculated as the sum of the individual capacity values using Equation 2. The imprinting factor was calculated as the ratio of equilibrium adsorption capacity values of the MIP and the NIP using Equation 3.

$$q_{PAH} = \frac{(C_i - C_e)V}{m} \tag{1}$$

Where q_{PAH} is the adsorbed amount of each PAH per unit mass of the MIP (ng·mg^{-1}), C_i is the spiking concentration of each PAH (ng·mL^{-1}), C_e is the equilibrium concentration of each PAH after adsorption (ng·mL^{-1}), V is the volume of the spiked solution (mL), and m is the mass of the MIP (mg).

$$q_e = \sum q_{PAH} \tag{2}$$

Where q_e is the total equilibrium adsorption capacity (ng·g^{-1}).

$$IF = \frac{q_{MIP}}{q_{NIP}} \tag{3}$$

Where IF is the imprinting factor, and q_{MIP} and q_{NIP} are the adsorption capacities of the MIP and the NIP respectively.

The equilibrium adsorption capacity at each spiking concentration was then used in Langmuir and Freundlich isotherm models to identify whether the adsorption was monolayer or multilayer. The Langmuir isotherm predicts formation of a monolayer with adsorption reaching a maximum with increase in analyte concentration. The Freundlich model predicts formation of a multilayer and assumes that adsorption continues linearly with an increase in concentration of analytes.

The linearised Langmuir adsorption isotherm model is described using Equation 4. A straight-line graph of C_e/q_e versus C_e was then plotted and the linearity of the graph calculated as the coefficient of determination (r^2). The Freundlich isotherm model was evaluated using Equation 5. The values of $\log q_e$ were plotted against C_e. The linearity of the plot was presented as an r^2 value. The r^2 values from the Langmuir and the Freundlich linear graphs were then compared. The acceptable model was the one with the highest r^2 value.

$$\frac{C_e}{q_e} = \frac{1}{Qb} + \frac{C_e}{Q} \tag{4}$$

Where C_e is equilibrium concentration (µg·L^{-1}), q_e is the amount adsorbed at equilibrium (µg·g^{-1}), Q is maximum adsorption capacity (µg·g^{-1}), and b is a constant related to energy of adsorption (L µg^{-1}).

$$log q_e = \ log K_f + \frac{1}{n} log C_e \tag{5}$$

Where K_f is the equilibrium constant (ng·mL^{-1})$^{1/n}$ and n is the adsorption intensity.

3.3.3.2 Effect of contact time

The effect of contact time on attaining equilibrium-binding capacity was investigated from 20-300 minutes. An amount of 10 mg of MIPs was placed in a 5 mL hexane solution containing 2 µg·mL^{-1} of PAHs. Thereafter, 10 µL aliquots of the solution were collected at 20, 40, 60, 80, 100, 120, 150, 200, 250 and 300 minutes. The aliquots were injected manually into the gas chromatography system. The data obtained was used in plotting the linearised Lagergren pseudo-first and second order models using Equations 6 and 7 respectively.

$$log(q_e - q_t) = log q_e - \frac{k_1}{2.303} t \tag{6}$$

Where q_e *and* q_t are adsorption capacity parameters (µg·g^{-1}) at equilibrium and at time, t (min), respectively, and k_1 is the Lagergren rate constant for first order adsorption (min^{-1}).

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{t}{q_e} \tag{7}$$

Where k_2 is the Lagergren rate constant for the second order adsorption.

3.3.4 MIP-PAH elution solvent

The type of elution solvent is important to ensure that minimal solvent is used to avoid dilution but at the same time can elute the target analytes with ease. PAHs are non-polar compounds and interact with MIP cavities through Van der Waals and π–π interactions. Thus, heptane, hexane, toluene and DCM were investigated. Polar solvents such as methanol and acetonitrile are used in MIP template elution where the target is to break hydrogen bonding (Martín-Esteban, 2001; Martín-Esteban, 2016). These solvents were therefore not investigated since the template-monomer interactions are hydrophobic and π–π interactions. In addition, polar solvents like methanol are not compatible with gas chromatography analysis and their use as eluent would require reconstitution with a non-polar solvent for injection into the gas chromatography system. The order of ascending polarity of the chosen solvents is heptane < hexane < toluene < DCM.

Commercial MIPs (120 mg) were directly placed in 10 mL of hexane spiked at 500 µg·mL^{-1} with the 16 PAHs. Extraction was done over three hours at 40°C. The MIPs were then divided into 10 mg portions. Thereafter, 3 mL of each of the four solvents were used to elute the PAHs from the MIP cavities. The eluting solvents were added in three 1 mL aliquots. Hot solvents maintained at 40°C were also tested to investigate the effect of temperature on breaking the PAH-MIP interactions. The extractant would then be concentrated down to 1 mL using a slow flow of nitrogen gas and then injected directly into the gas chromatography system. Peak areas were used to compare the eluting ability of the solvents. Elution efficiency was confirmed by comparing the amount eluted as a ratio of the expected amount that was adsorbed.

3.3.5 Characterisation

Several characterisation techniques were used. The surfaces of imprinted and non-imprinted materials were studied using SEM and EDS. The samples were either gold/palladium or carbon sputter-coated for SEM or EDS analysis respectively. The specific surface area, pore volume and size of particles of both the NIP and the MIP were determined by BET analysis using the adsorption isotherm of N_2 at 77 K. The stability of the polymer was evaluated using TGA.

3.3.6 Swelling of MIPs in various solvents

The swelling behaviour of the imprinted polymer was investigated in six solvents by immersing 1.2 g of the MIP in 12 mL of distilled water at 25°C for 12 hours. The sorbent weight increase allowed the calculation of the swelling percentage using Equation 8.

$$\text{Swelling ratio (\%)} = \frac{(W_s - W_d)}{W_d} \times 100 \qquad (8)$$

Where W_s and W_d are the weights of the swollen and dry sorbent samples (in g) respectively.

3.4 Life Cycle Inventory Assessment

An inventory analysis phase of the life cycle assessment was done with the aim of providing insights into the feasibility of synthesising these MIPs and then applying them in the quantitation of PAHs from complex environmental samples. The potential for environmental impact was considered by comparing the amount of the imprint PAH and the amount of PAHs that a unit mass of the synthesised MIP could adsorb.

3.4.1 Harvesting of MIPs

The amount of imprint used and the capability of the imprinted polymer were compared to explain and validate the feasibility of synthesising the MIP and applying it as a viable alternative when quantifying PAHs from environmental samples. The imprinting ability was calculated using Equation 9 as the amount of the imprinting PAHs needed to synthesise a unit mass of the MIP.

$$q_i = \frac{m_i}{m_{MIP}} \qquad (9)$$

Where q_i is the imprinting ability in mg·g^{-1}, m_i is the mass of the imprinting PAH in mg and m_{MIP} is the mass of the synthesised MIP in g.

An imprinting efficiency was evaluated to understand the relationship between the amount of the imprinting PAHs used and the maximum amount of the PAHs that could be adsorbed by the synthesised MIP. The imprinting efficiency was calculated using Equation 10 as the ratio of the imprinting ability versus the adsorption capacity.

$$IE = \frac{q_e}{q_i} \times 100 \tag{10}$$

Where IE is the imprinting efficiency, q_e is the maximum adsorption capacity per unit mass of the MIP (mg·g^{-1}), and q_i is the imprinting capacity per unit mass of the MIP (mg·g^{-1}).

3.4.2 Imprinted cavity efficiency

The theoretical number of imprinted cavities per unit mass of the MIP was calculated using Equation 11. The assumption was that each imprinting particle results in the formation of a single cavity. This value was compared with the experimental number of cavities, which was determined using Equation 12. The experimental value gives the exact number of cavities that are available for binding PAHs. Finally, the cavity efficiency was calculated using Equation 13 as the fraction of MIP cavities that are available for sequestrating PAHs.

$$n_{PC} = \frac{q_i N_A}{M} \tag{11}$$

Where n_{PC} is the predicted number of cavities per unit mass of the MIP (g^{-1}), q_i is the imprinting capacity per unit mass of the MIP (g·g^{-1}), N_A is the Avogadro constant (mol^{-1}), and M is the relative molar mass of the imprinting molecule (g·mol^{-1}).

$$n_{EC} = \sum_{i=1}^{16} \frac{q_e N_A}{M} \tag{12}$$

Where n_{EC} is the number of experimental cavities per unit mass of the MIP (g^{-1}), q_e is the maximum adsorption capacity per unit mass of the MIP (g·g^{-1}), N_A is the Avogadro constant (mol^{-1}), and M is the relative molecular weight of the adsorbed PAH (g·mol^{-1}).

$$CE = \frac{n_{EC}}{n_{PC}} \times 100 \tag{13}$$

Where CE is the cavity efficiency.

3.4.3 Regeneration of the MIP

The prospect of engineering a polymer that can be reused in its original state is part of green chemistry technology. The efficiency of the synthesised MIP after repeated application in adsorbing PAHs from an aqueous solution was investigated. An amount of 10 mg of MIPs was placed in a 5 mL hexane solution spiked at 1000 µg·mL^{-1}. After a two-hour extraction time, the MIPs were removed and the final concentration of the solution used in calculating the equilibrium adsorption capacity using Equations 1 and 2. The MIPs were then washed at least five times with hexane or until no PAH was identified in the eluent. The same MIPs were then reused on a fresh spiked solution of the same concentration and volume. The procedure was repeated seven times. The reusability of the MIP was evaluated by comparing the equilibrium adsorption capacity at each extraction cycle.

3.5 Development of the MASE-MIP for PAHs in Waste Water

3.5.1 Theoretical basis

The design of the developed MASE-MIP techniques involves suspending MIPs in a water immiscible organic solvent inside a membrane bag. Target organic compounds diffuse from the aqueous sample through the membrane into the bulk of the organic acceptor solution because of the concentration gradient. The transferred target compounds are in turn adsorbed into the cavities of the MIP particles. This is essential as it shifts the equilibrium towards transfer of analytes from the aqueous sample solution to the organic acceptor phase. The semipermeable membrane is used to minimise matrix effects by preventing the compounds with larger molecular weights from reaching the acceptor phase. Including MIPs with cavities specific for binding target analytes eliminates the need for a further clean-up step as is the norm with all other liquid-based extraction techniques. The analytes can then be eluted from the MIP cavities using an appropriate organic solvent. The main advantage lies in the formation of a single-step extraction and clean-up procedure. The technique is also highly selective and specific towards target analytes. This technique was therefore designed, optimised and evaluated for quantitating total PAHs from waste water samples.

3.5.2 MASE-MIP extraction procedure

The MASE-MIP system was set up as shown in Figure 5. The membrane extraction cell consisted of a 20 mL headspace vial filled with 15 mL of spiked donor phase solution. The membrane bag was attached to a metal funnel and fixed with a polytetrafluoroethylene ring. The membrane bag with MIP particles and 1 mL organic solvent was placed inside the extraction cell containing the donor solution and stirred for preset times. After extraction, the acceptor content (MIP and the acceptor solvent) was transferred into a 3 mL empty cartridge with a 10 µm frit at the bottom and mounted onto an SPE unit. The acceptor solvent was separated from MIP particles by opening the SPE valve slowly and allowing it to flow out by gravity at about 1 mL·min^{-1}. A full vacuum was applied for five minutes to completely dry the MIP particles. The trapped PAHs were eluted with three 1000 µL fractions of methanol before being analysed directly by HPLC-UV. Further optimisations were done followed by GC-TOF/MS during which hot hexane was used as the eluting solvent.

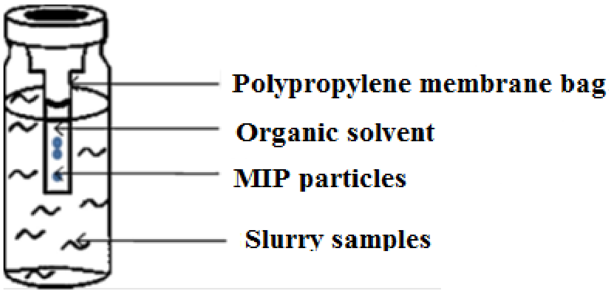


Figure 5: Experimental set-up showing MASE combination with MASE-MIP combination

3.5.3 Optimisation of the MASE-MIP

Parameters adopted from the MIP optimisation experiments were using heptane as the acceptor phase in the membrane bag and as the eluting solvent for GC-TOF/MS analysis. For quantitation on an HPLC-UV system, methanol was used as the eluting solvent. The HPLC-UV was used mainly to optimise the organic content of the donor phase.

3.5.3.1 Addition of an organic modifier

Investigating the addition of an organic modifier in an aqueous donor phase was necessary because PAHs are hydrophobic and tend to adsorb on the walls of the container. Polar solvents that dissolve in water are used to minimise this effect (Benanou et al., 2003). Ethanol, acetonitrile, ethanol and DMF were investigated as potential organic modifiers. Each organic modifier was added at 15% of the aqueous donor solution containing 0.5 $\mu g \cdot mL^{-1}$ of individual PAHs. A total of 50 mg of MIPs was dispersed in a toluene acceptor phase and stirred at 420 rpm for 60 minutes. Once the best modifier was identified, its composition in the donor phase was also optimised. During this phase, the PAHs were quantified using an HPLC-UV system. Thereafter, the optimised amount of the organic modifier was used in subsequent experiments.

3.5.3.2 Effect of stirring rate

The effect of stirring was investigated from 420-1000 rpm on a multipoint magnetic stirrer. The stirrer had a maximum limit of 1200 rpm. Stirring is expected to enhance extraction by increasing the kinetic energy of the analytes and their chance to be at the donor phase-membrane interface. Extraction was done for 60 minutes from a 0.5 $\mu g \cdot mL^{-1}$ donor phase using 50 mg of MIPs placed in a membrane bag. An HPLC-UV instrument was used to quantify the PAHs. The optimum stirring rate was used in subsequent optimisation studies.

3.5.4 Kinetic and adsorption studies

Batch studies involving the effect of contact time and initial concentration were done to understand the extent of transfer of PAHs from an aqueous media across the membrane and their eventual adsorption on to the cavities of the synthesised MIP. Contact time was investigated up to 360 minutes. The concentration of spiked aqueous solution was 5 $\mu g \cdot mL^{-1}$ and 1000 rpm stirring rate.

The effect of initial concentration on the transfer of PAHs across the membrane into the cavities of the MIP particle was investigated in the 600-1000 $ng \cdot mL^{-1}$ range. At that stage, quantitation was done on a GC-TOF/MS system. Heptane was used as the acceptor phase in the membrane bag. Elution was done using 3 mL of heptane maintained at 40°C.

3.5.4.1 Transfer kinetics

Transfer efficiency of PAHs from a spiked aqueous solution across the membrane and their eventual binding on the MIP cavities was investigated. Water containing 25% DMF was spiked with a mixture of 16 PAHs in the 600-1000 $ng \cdot mL^{-1}$ range. 50 mg of MIPs and 1.5 mL of heptane were placed inside the membrane. The membrane bag was then submerged inside 10 mL of the spiked donor phase. Extraction was done on a magnetic stirrer set at 1000 rpm stirring rate for three hours. The PAHs were transferred across the membrane and adsorbed into the MIP cavities. The absorbed PAHs were then eluted with 5 mL of heptane maintained at 40°C. The extraction efficiency and enrichment factor values were calculated using Equations 14 and 15.

$$E = \frac{C_i - C_f}{C_i} \times 100 \tag{14}$$

Where E is the extraction efficiency, C_i and C_f are the PAH concentrations in the donor phase before and after extraction respectively.

$$EF = E \times \frac{V_D}{V_A} \tag{15}$$

Where *EF* is the enrichment factor, and V_D and V_A are the volumes of the donor and acceptor phases.

3.5.4.2 Method detection and quantitation limits of the MASE-MIP method

Defining the method detection limit (MDL) and method quantitation limit (MQL) was important to determine the smallest amount of each PAH that could be reliably measured with a degree of precision using the developed method. The two quantities are related to the enrichment factor (EF) and the instrumental limits using Equations 16 and 17 respectively. Generally, the instrument limit of detection and quantitation was set at 3 S/N and 10 S/N respectively using the mass spectrometry method of the GC-TOF/MS instrument.

$$MDL = \frac{LOD}{EF} \tag{16}$$

Where *MDL* is the limit of detection of the extraction method (ng·mL^{-1}), *LOD* is the instrument limit of detection (ng·mL^{-1}) and *EF* is the enrichment factor of the analyte from the donor phase to the acceptor (elution) phase.

$$MQL = \frac{LOQ}{EF} \tag{17}$$

Where *MQL* is the limit of quantitation of the extraction method (ng·mL^{-1}) and *LOQ* is the instrument limit of quantitation (ng·mL^{-1}).

3.6 Development of the SE-MASE-MIP for PAHs in Waste Water Sludge

3.6.1 Theoretical basis

The combination of Soxhlet extraction with the MASE-MIP (SE-MASE-MIP) technique involves placing MIPs inside a membrane bag. The bag is then suspended inside the extraction solvent in the bottom flask of the Soxhlet apparatus as shown in Figure 6. The theoretical basis of the SE-MASE-MIP technique is that as the analytes are extracted from a solid sample using Soxhlet extraction, they are immediately transferred across the membrane into the cavities of the MIPs. This eliminates the need for the further clean-up step, which is common with Soxhlet extraction and all other solid-liquid extraction techniques, by introducing an *in situ* clean-up procedure. The new approach cannot be applied on other techniques such as SFE and USE. In these techniques, the MIPs will have to be in contact with the sample. In the conventional

Soxhlet system, the MIPs will only be in contact with the extraction solvent. The SE-MASE-MIP technique was developed, optimised and evaluated for extracting total PAHs in sludge samples followed by gas chromatographic determination.

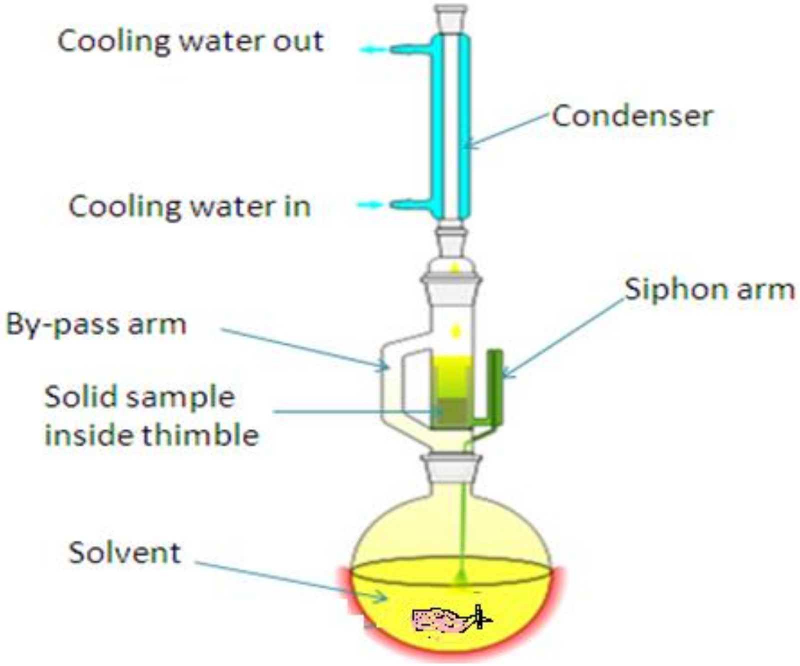


Figure 6: Soxhlet apparatus with a membrane sachet containing MIPs

The bioavailable amount of PAHs was determined by dissolving sludge samples in deionised water followed by MASE-MIP extraction. The bioavailable PAHs can be viewed as the fraction of the total sludge PAHs that easily dissolve in water and become available to the environment. Determining the bioavailable fraction of PAHs against the total amount of PAHs in sludge samples is critical because the total concentration may not give a good picture of the toxicity of the sludge due to these pollutants. It is the bioavailable fraction that defines the toxicity of sludge especially when applied as a soil ameliorant in agricultural land or landfill sites.

3.6.2 General procedure

The general procedure involved pouring 20 mL of an extracting solvent into a 50 mL Soxhlet round-bottomed flask. A specific amount of MIPs would then be weighed into a membrane. An acceptor phase (1 mL) was transferred into the membrane bag. The membrane bag was tightened with a string. The MIP-membrane sachet was dropped into the flask containing the solvent. A sewage sludge sample was then placed into the extraction thimble. The set-up was heated to the boiling point of the extracting solvent and the solvent allowed refluxing for a preset period of time. When the siphon arm filled with the solvent, the solvent and extracted PAHs were drained back into the flask. The solvent was allowed to reflux every time when extracting the PAHs and depositing them into the flask. The PAHs then crossed the membrane and eventually adsorbed into the MIP cavities. Each extraction was done in triplicate. Figure 7 shows the set-up in the lab in which six simultaneous extractions were done.

Figure 7: Soxhlet extraction set up with six simultaneous extractions of PAHs from sludge samples

After extraction, the MIP-membrane sachet was removed from the flask and the MIPs were transferred into a 3 mL empty cartridge with a 10 µm frit at the bottom. The cartridges were mounted onto an SPE unit. A full vacuum was applied for two minutes to completely dry the MIP particles. The trapped PAHs were eluted gravitationally with three 1 mL fractions of organic solvent. This was then concentrated to 1 mL before analysis using gas chromatography.

3.6.3 Optimisation of the SE-MASE-MIP

The two important parameters in Soxhlet extraction are the type of extraction solvent and extraction time. For an SE-MASE-MIP approach, the effect of the boiling temperature of the extraction solvent on the MIP-binding ability also becomes important. The effect of temperature on both the transfer of PAHs across the membrane and their subsequent binding in the MIP cavities was therefore included in the optimisation experiments. The investigated temperature range (20-80°C) was based on the boiling temperatures of the extractions solvents. The accepting solvent placed inside the membrane was also investigated.

During preliminary studies, PAHs were extracted from loam soil of 100 µm particle size spiked with 400 µL of 100 $\mu g \cdot mL^{-1}$ PAH standard solutions. The 400 µL of 100 $\mu g \cdot mL^{-1}$ was first diluted with 20 mL of methanol and then poured into the 40 g soil sample, which resulted in a spiked soil sample of 1 $\mu g \cdot g^{-1}$ mixed PAH concentration. This was to allow for homogenous distribution of PAHs. The mixture was stirred with a stirring rod and methanol was allowed to evaporate at ambient conditions for one day in darkness. The preliminary results were confirmed by extracting the PAHs from 1 g of the sludge certified reference material (CRM) of 100 µm particle size and analysis on a GC-TOF/MS.

3.6.3.1 Effect of temperature on extraction of PAHs

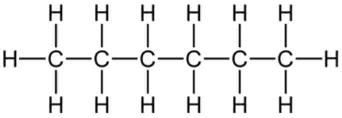
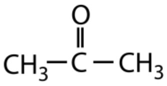
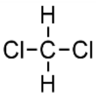
The initial step in this study was to check the effect of temperature on the transfer kinetics of PAHs across the membrane and the subsequent binding in the cavities of the MIPs. A volume of 30 mL of the 500 $\mu g\ L^{-1}$ mixture containing 16 PAHs in cyclohexane solution was transferred into the flask of the Soxhlet apparatus. 20 mg of MIPs was placed inside the membrane bag. This was dropped into the flask containing the 30 mL PAH-solvent solution. The set-up of the Soxhlet apparatus was completed but without the thimble and sludge sample. The MIP-solvent solution was heated and its temperature maintained at 40°C, 60°C and 80°C for three hours. A control was also set up and maintained at room temperature (±20°C).

After a three-hour heating period, the membrane containing MIPs was separated from the solvent and the PAHs eluted from the MIPs by gravity elution with 3 mL of hexane. The 3 mL extractant solution was concentrated to 1 mL and injected directly into the gas chromatography system. The binding temperature was also investigated when the MIPs were placed directly in the cyclohexane-PAH solution.

3.6.3.2 Choice of extracting solvent

The aim was to identify a solvent that would effectively extract PAHs from sludge contained in the thimble and allow for their consequent sequestration by MIPs placed inside a membrane. Each solvent was heated to its boiling point and allowed to reflux. When choosing the potential extraction solvents, several physicochemical properties were considered. Boiling point, polarity and water solubility were used as reference properties. The premise for this approach was to relate the properties of the potential solvents to the physical properties of the PAHs and the sludge, and the type of bonds involved in MIP-PAH binding. Generally, the polarity of the extraction solvent should be closely related to that of the target compounds (Pena et al., 2010). Thus, solvents with minimal polarity and boiling points less than water were selected. Water solubility ranged from non-soluble to totally miscible in water. Table 5 shows the chosen solvents and their relevant properties.

Table 5: Physical properties of potential extraction solvents

Solvent	Structure	Molar mass (g·mol^{-1})	Boiling point (°C)	Polarity	Solubility in water (%w/w)
Hexane	 $H-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-H$	86.18	69	0.1	0.001
Acetone	 $CH_3-\overset{\overset{O}{\|}}{C}-CH_3$	58.08	56	5.1	100
DCM	 $Cl-\overset{H}{\underset{H}{C}}-Cl$	84.93	40	3.1	1.6
THF		72.11	66	4.0	100
Cyclohexane		84.16	81	0.2	0.01

In addition to the five chosen solvents, a combination of hexane and polar solvents was investigated. Acetone and THF were the two polar solvents tried in combination with hexane. The preliminary studies involved extraction from spiked soil samples. The preliminary results were further confirmed by extracting the PAHs from 1 g of the sludge CRM with a particle size of 100 µm. Each solvent was allowed to reflux at its boiling point. After an eight-hour extraction period, heating was stopped and the extraction solvent was allowed to cool to ambient temperature. The MIP-membrane bag was removed from the flask and the PAHs eluted on an SPE elution system. The supernatant was finally analysed by GC-FID.

3.6.3.3 Acceptor phase organic solvent

After identifying hexane and the hexane/THF mixture as the best extracting solvents, the next step was to investigate the acceptor phase to be placed in the membrane bag. Hexane, heptane and toluene were chosen. Hexane is also the donor phase. Heptane was investigated as a solvent less polar than hexane. Toluene is more polar than hexane and is the common acceptor phase in MASE-MIP extractions (Chimuka et al., 2011b).

A membrane bag containing the MIPs suspended in the acceptor phase was submerged into the Soxhlet flask containing a 30 mL hexane solution spiked at 2 µg·mL^{-1}. The donor phase was heated at 40°C for three hours. The peak areas corresponding to the amount of PAHs sequestrated were calculated and compared for the three acceptor phases.

3.6.4 Transfer kinetics

Transfer efficiency was evaluated as the extent of quantitative extraction of PAHs from the sludge sample using the Soxhlet extractor, followed by adsorption by the MIPs enclosed in a membrane bag and their eventual enrichment into the elution solvent. The kinetic transfer efficiency was evaluated as both the EF and the extraction efficiency.

3.6.4.1 Transfer efficiency across the membrane (Effect of initial concentration)

Transfer kinetics across the membrane was investigated by extracting PAHs from a 20 mL hexane solution spiked in the 600-1000 ng·mL^{-1} range. Considering the binding capacity and the volume of the membrane, 50 mg of the synthesised MIP was used to investigate the efficiency of the PAH transfer. Extraction was performed over an optimised equilibrium time of 180 minutes. The purpose of this procedure was to determine the efficiency of transfer of PAHs from the hexane solution across the membrane into the cavities of the MIP. For the 1000 ng·mL^{-1} spiked solution, 10 µL aliquots of the hexane donor solution were collected at 150, 180, 200, 250, 300 and 300 minutes. Thus, a total of 60 µL was collected. This volume is 0.3% of the 20 mL hexane donor solution. Therefore, its effect on the equilibrium concentration was insignificant. The difference in concentration of the PAHs in solution before and after extraction was used to calculate the transfer efficiency using Equation 18.

$$E_m = \frac{C_i - C_f}{C_i} \times 100 \qquad (18)$$

Where E_m is the transfer efficiency across the membrane, and C_i and C_f are the PAH concentrations in solution before and after extraction.

3.6.4.2 SE-MASE-MIP extraction efficiency

The efficiency of the optimised SE-MASE-MIP method was tested as the extent of PAH extraction from the solid sample using a Soxhlet system followed by transfer across the membrane into the cavities of the MIP particles and their eventual elution from the cavities into the eluting solvent. This was done by placing 1 g of sludge CRM containing the 16 PAHs in the thimble of the Soxhlet system and extracting PAHs using a hexane/THF solution mixture. A total of 80 mg of the MIP was placed in a membrane bag that was dropped into the flask containing the hexane/THF mixture. The system was allowed to reflux at 60°C for 16 hours. The MIPs were then transferred into the SPE system and eluted with 5 mL of heptane, which was maintained at 40°C. The eluent was then concentrated to 1 mL and injected directly into the GC-TOF/MS.

The CRM sludge material had a total PAH content of 10.76 $\mu g{\cdot}g^{-1}$. This implies that if 1 g of the sludge CRM was placed in the Soxhlet extractor and 100% extraction efficiency was assumed, 10.76 µg of PAHs would be transferred into the 20 mL hexane solution in the flask, and cross the membrane bag into the cavities of the MIP. The theoretical concentration of PAHs after eluting and concentrating the eluent to 1 mL would be 10.76 $\mu g{\cdot}mL^{-1}$. The efficiency of the SE-MASE-MIP system was then calculated as the ratio of the experimental concentration versus the expected PAH concentration in the eluting solvent using Equation 19. The EF was calculated as the ratio of the concentration of PAHs in the eluent after extraction versus the concentration in the sample before extraction using Equation 20.

$$E_S = \frac{C_e}{C_p} \times 100 \tag{19}$$

Where E_s is the Soxhlet-membrane-MIP extraction efficiency, C_p is the predicted concentration of PAHs in the eluting solvent, and C_e is the experimental concentration of PAHs in the eluting solvent.

$$EF = \frac{C_H}{\rho V C_s} \tag{20}$$

Where *EF* is the enrichment factor, C_H is the individual PAH concentration in the heptane eluent after extraction ($\mu g{\cdot}mL^{-1}$), C_s is the individual PAH concentration in the solid sample before extraction ($\mu g{\cdot}g^{-1}$), ρ is the density of heptane ($g{\cdot}mL^{-1}$), and *V* is the volume of the heptane eluent after concentrating (mL).

3.6.4.3 MDL and MQL of the SE-MASE-MIP method

The same approach used for calculating the MDL and MQL for the MASE-MIP technique in Section 3.5.4.2 was used in calculating the MDL and MQL for the SE-MASE-MIP method.

3.7 Quantitation of PAHs in Goudkoppies and Northern Works WWTPs

The developed MASE-MIP and SE-MASE-MIP methods were used to determine the concentrations of the 16 US EPA priority PAHs in waste water and waste water sludge samples respectively. The samples were obtained from two WWTPs that differed in the type and capacity of waste water received. The WWTPs chosen were the Northern Works in the north of the city of Johannesburg and the Goudkoppies in the south.

The Northern Works WWTP is the largest in the Gauteng region, which treats 400 million litres of waste water every day. This plant serves the area north of Hillbrow Ridge including Alexandra, Edenvale, Randburg, Sandton and parts of Midrand and Roodepoort. It is situated in the Diepsloot area. The Northern Works WWTP receives mainly domestic waste water from residential areas and the food industry. Goudkoppies is a much smaller plant with a capacity of 139 million litres. Goudkoppies WWTP collects and treats sewage from the city centre and the south-eastern areas of Johannesburg where most industries and mine dumps are situated. The waste water flows into the two treatment plants by gravity. Waste water treated in the Northern Works WWTP is discharged into the Jukskei/Crocodile River catchment in the north while Goudkoppies discharges into the Klip/Vaal River catchment in the south. Both catchments are classed as sensitive to the discharge of waterborne pollutants.

The two WWTPs use anaerobic digestion to stabilise the sewage sludge. The Northern Works plant uses the methane rich biogas produced from anaerobic digestion to produce about 1600 $\mathrm{MWh \cdot year^{-1}}$ of electricity to power the plant itself. The digestate from the anaerobic digesters is sun-dried and composted. The resultant compost is used as a fertiliser in agricultural farms. It is this agricultural use of sludge that is of concern when it comes to POPs such as PAHs.

3.7.1 Sample collection and storage

Anaerobically digested and sun-dried sludge, and waste water samples were collected in 250 mL brown bottles with Teflon caps. The samples were collected during the same season with permission from the Goudkoppies and Northern Works WWTPs. The samples were loaded in cooler boxes and transported by road to the Chemistry Department, University of the Witwatersrand, Johannesburg, South Africa. The waste water samples were instantly stored at 4°C. The samples were monitored constantly to prevent accumulation of biogas during storage. Before analysis, the samples were removed from the refrigerator and placed in darkness at ambient conditions for at least three hours. The sludge samples were then oven-dried at 70°C for two days in petri dishes. The dried sludge was crushed using a pestle and mortar. A powder of particles of 100 μm was collected through a sieve.

3.7.2 Extraction of PAHs from samples

The developed techniques were used to extract the PAHs from real samples. B[k]F and indeno-imprinted MIPs, which were combined at 1:1 (w/w) and suspended in 1 mL heptane inside a membrane bag, were used as adsorbents. The optimised parameters of the developed MASE-MIP technique were applied to quantify total PAHs in waste water sample. The optimised SE-MASE-MIP technique was used to quantitate the total PAHs in waste water sludge samples from the same WWTPs. The bioavailable fraction of sludge PAHs was determined using the MASE-MIP technique after dissolving sludge in deionised water.

For both waste water and waste water sludge samples, surrogate standards were added before extraction to monitor the efficiency of the extraction methods. The surrogate standard was more important in the MASE-MIP extraction of PAHs from waste water considering that the optimised efficiencies were determined from spiked water samples in the absence of matrix effects. Internal standards were added in the vials containing the heptane solution after eluting the PAHs from the MIP cavities. In both cases, deuterated PAHs were used.

3.7.3 Source of PAHs in sludge samples

Anthropogenic sources of PAHs can be classified as either petrogenic or pyrogenic sources. Petrogenic sludge sources have higher levels of LMW PAHs while pyrogenic sources are characterised by an abundance of HMW PAHs. The petrogenic sources emanate from burning of liquid petroleum fuels while the burning of coal, wood and grass is described as a pyrogenic source. Concentration ratios of various PAHs can be used to declare these sources. In this study, the following ratios were used to identify the sources of PAHs in the two WWTPs under investigation:

- Anthracene to anthracene plus phenanthrene
- Fluoranthene to fluoranthene plus pyrene
- B[a]A to B[a]A plus chrysene
- Indeno to indeno plus B[ghi]P

Each ratio consists of a pair of PAHs with the same molecular mass.

Using a molecular weight of 178, a ratio of the anthracene to anthracene plus phenanthrene (Ant/178) above 0.10 indicates pyrogenic sources due to combustion. Inversely, petrogenic sources result in a ratio less than 0.10. For the pyrene to pyrene plus fluoranthene (Pyr/202) ratio, the petrogenic-pyrogenic transition occurs at the ratio of 0.50. Considering the molecular weight of 228, the B[a]A to B[a]A plus chrysene (B[a]A/228) ratio should be lower than 0.20 for petrogenic sources and above 0.35 for pyrogenic sources. A ratio between 0.20 and 0.35 is an indication that both sources exist. The indeno to indeno plus B[ghi]P (indeno/276) ratio should be below 0.20 for petrogenic sources and above 0.50 if the source is pyrogenic. If the ratio is in the 0.20-0.50 range, it implies that both sources contribute to the PAH levels.

3.7.4 Quantitation of the bioavailable PAHs in sludge

Dried sludge with a 100 μm particle size was dissolved in 50 mL deionised water. The sludge was added at 1% w/v (0.5 g sludge in 50 mL water). The mixture was spiked at 200 ng·mL^{-1} with deuterated PAHs (naphthalene-D8, acenaphthene-D10 and anthracene-D10). The dissolution was done in triplicate. The mixtures were shaken at 150 rpm for 16 hours. The mixtures were centrifuged to separate the aqueous phase from the sludge particles. Thereafter, 12 mL of the solution was decanted into the three MASE-MIP vials. These were extracted using the developed MASE-MIP technique. The MIP eluents were spiked at 300 ng·mL^{-1}, 600 ng·mL^{-1} and 900 ng·mL^{-1} with a standard solution of the 16 PAHs. The procedure was repeated for 2% w/v (1 g sludge) and 4% w/v (2 g sludge). The bioavailable PAHs were calculated as a fraction of the total PAH content in sludge.

4 RESULTS

4.1 Calibration Results

Table 6 shows the calibration results and instrument limits of detection and quantification. Good coefficients of determination for the calibration curves ranging from 0.9972-0.9998 were obtained. The limit of detection value set ranged from 0.07-7.50 ng·mL^{-1}.

4.2 Synthesis and Selection of MIPs

4.2.1 Selecting the best PAH-imprinted MIP

To ensure that a MIP, which maximises the extraction of PAHs, was selected, the three top performing imprinted polymers in the extraction of each of the 16 US EPA priority PAHs were selected. A summary of the preferred MIPs in the extraction of individual PAHs is given in Table 7. Naphthalene, acenaphthylene, acenaphthene and fluorene were excluded from the results analysis because they have large peak areas that made it almost impossible to analyse the results of other PAHs. It was observed that almost all PAHs did not favour themselves as imprints. Fluoranthene was an exception. Previous studies have reported that a MIP imprinted by a particular PAH would favour the sequestration of that PAH and other PAHs closely related to it (Baggiani et al., 2007). Figure 8 shows how the top three of each of the pyrene- and fluoranthene-based PAHs and the combinations compared with each other when extracting HMW PAHs.

Our observation might be related to the possibility of competition for binding sites considering that MIPs were selected by extracting all 16 PAHs simultaneously. The MIPs synthesised from the five-ringed PAH templates appear prominently in their binding preference for six-ringed PAHs. For example, the B[k]F and B[a]P-imprinted MIPs were most effective in the sequestration of the three six-ringed PAHs. This scenario has also been reported where the anthracene-imprinted MIP was observed to enhance the binding of chrysene instead of itself (Dickert et al., 1999). The flexibility of the EGDMA as a cross-linker, the steric cross-sectional similarity (length and size) of five- and six-ringed PAHs, and the higher hydrophobicity of the six-ringed PAHs (Hafidi et al., 2008) allow for a stabilised interaction between an HMW PAH and the cavities of a MIP imprinted with a five-ringed PAH.

Table 6: Identification parameters and calibration results of the 16 US EPA priority PAHs (n = 3, RSD < 10%)

PAH	**t_R (min)**	**Quantitation ion (m/z)**	**Confirmation ions (m/z)**	**Regression line**	**r^2**	**Limit of detection ($ng\cdot mL^{-1}$)**	**Limit of quantitation ($\mu g\ L^{-1}$)**
Naphthalene	04:54	128	127; 129	y = 39056x − 251 599	0.9998	0.07	0.21
Acenaphthylene	07:07	152	153; 151	y = 15131x − 53 720	0.9992	0.26	0.85
Acenaphthene	07:27	154	153; 152	y = 21181x − 35 434	0.9982	0.39	1.30
Fluorene	08:31	166	165; 167	y = 14348x − 192 786	0.9973	0.49	1.62
Phenanthrene	11:01	178	176; 179	y = 12405x − 250 688	0.9996	0.61	2.02
Anthracene	11:10	178	89; 179	y = 9 582.1x − 146 650	0.9983	0.79	2.61
Fluoranthene	13:56	202	203; 101	y = 7 714.2x − 2 763.7	0.9990	3.33	11.0
Pyrene	14:32	202	203; 101	y = 8831x − 230 360	0.9972	0.88	2.91
B[a]A	18:43	228	229; 114	y = 4 580.3x − 124 614	0.9981	2.05	6.78
Chrysene	18:52	228	229; 114	y = 5 083.9x − 39 609	0.9996	2.03	6.69
B[b]F	23:32	252	253; 126	y = 3 139.7x − 29 909	0.9975	2.46	8.11
B[k]F	23:38	252	253; 125	y = 3 432.3x − 173 292	0.9979	2.50	8.25
B[a]P	24:51	252	253; 126	y = 2 814.8x − 2 434.4	0.9998	7.50	24.8
Indeno	29:24	276	138; 227	y = 2 647.4x – 64 254	0.9996	3.75	12.4
DBA	30:26	278	139; 279	y = 3 019.7x − 251 319	0.9979	5.36	17.7
B[ghi]P	29:32	276	138; 277	y = 2 678.3x − 28 191	0.9997	4.55	15.0

Table 7: Summary of PAHs and their MIP template preference

PAH	Rings	Preferred imprinting PAH and/or PAH combinations [a]
Phenanthrene	3	Flu/Pyr > B[k]F/B[a]P > Indeno
Anthracene	3	Flu > Flu/Pyr > Pyr
Fluoranthene	4	Flu > B[a]P/B[ghi]P ≈ B[k]F/B[a]P ≈ Flu/B[k]F ≈ Flu/Pyr
Pyrene	4	Flu/Pyr > Pyr/B[a]P ≈ Fluoranthene ≈ Pyrene > B[k]F/Indeno
Benz[a]anthracene	4	B[k]F/Indeno > Indeno > B(a)P/B[ghi]P
Chrysene	4	B[k]F/Indeno > Indeno ≈ Flu/Pyr ≈ B(k)F > Pyr/B[a]P
B[b]F	5	Indeno > B[k]F > B[k]F/B[a]P
B[k]F	5	B[a]P/B[ghi]P > Indeno/B[ghi]P ≈ B[k]F > B[k]F/B[a]P
B[a]P	5	Py/B[a]P ≈ Indeno > B[a]P/B[ghi]P ≈ B[k]F/B[a]P
Indeno	6	B[k]F/Indeno > B[a]P/B[ghi]P > Flu/B[k]F
Benzo[ghi]perylene	6	Indeno/B[ghi]P > B[a]P≈ B[k]F/B[a]P > B[k]F
Dibenz[a,h]anthracene	6	B[k]F/Indeno > B[k]F/B[a]P ≈ B[a]P > Pyr/B[a]P

[a] PAH used as a template
Fluorene (Flu); Pyrene (Pyr)

This observation implies that the imprinting PAH and other sterically related PAHs have to seek alternative binding sites. For the five-ringed PAHs, a preference towards MIPs imprinted with the HMW PAHs was observed. Likewise, the four-ringed B[a]A and chrysene seemed to be favoured by MIPs imprinted with five-ringed PAHs. Only the pyrene- and fluoranthene-imprinted MIPs favoured the imprint molecules and other LMW PAHs. The LMW PAHs get easily washed out of the cavities designed with HMW PAHs.

MIP combinations were preferred by most PAHs except anthracene, fluoranthene and B[b]F (Table 7). LMW PAHs (with three to four rings) mostly preferred the fluoranthene/pyrene combination. This was because fluoranthene and pyrene are both four-membered rings, which result in imprint cavities that were just big enough for the three-ring PAHs to easily bind/ adsorb, but too small to be accessible to PAHs of higher molecular weight. The flexibility of the cross-linker might also contribute towards allowing the cavities of the fluoranthene/pyrene MIPs to stretch so they can accommodate bulkier compounds such as B[a]A and chrysene.

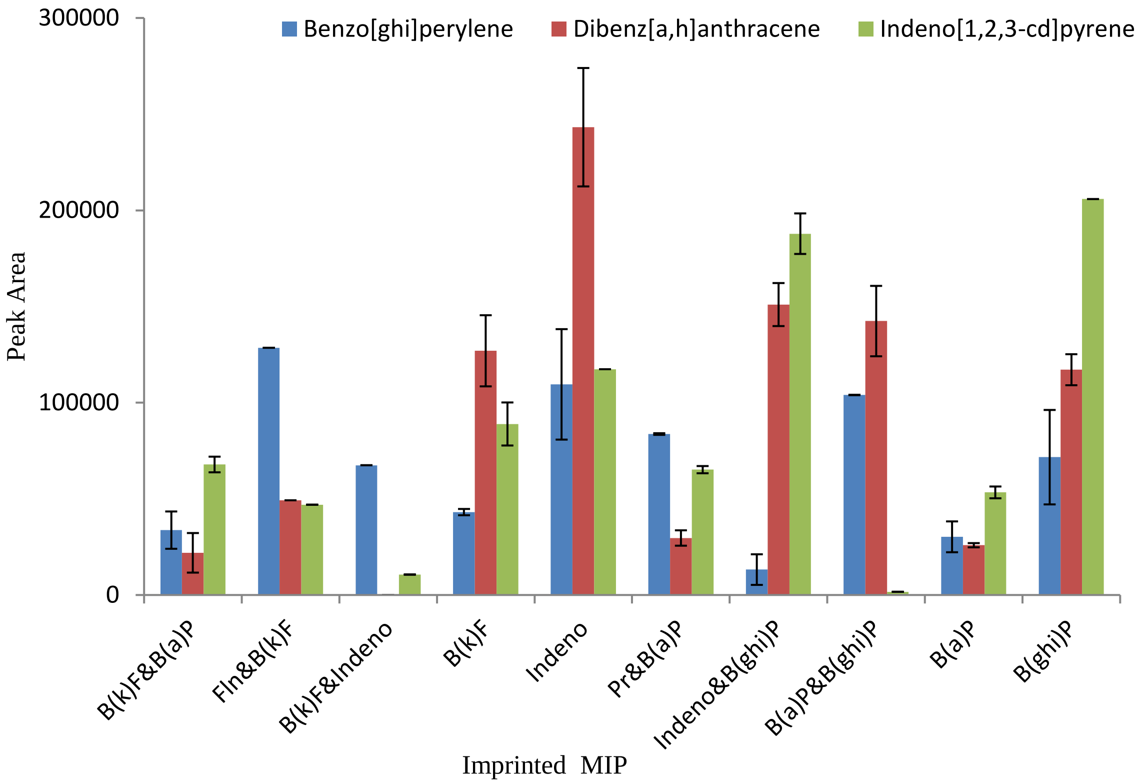


Figure 8: Comparison of the best pyrene- and fluoranthene-based MIPs, and best combination MIPs for extracting the three HMW PAHs. The peak areas correspond to the amount of PAHs remaining in solution after extraction (n = 3, SD)

For the middle and high molecular weight PAHs (with five to six rings), there was a high affinity for the B[k]F/indeno combination and other combinations containing cavities imprinted by these two PAHs. The possible explanation for this observation could be the hydrophobicity (log Kow) of the PAHs, the size and shape of the cavities. When the number of rings of the PAHs increases, the polarity decreases, thus making the PAHs more hydrophobic. Both indeno and B[k]F structures are wide and bulky. In addition to this, the flexibility of the cross-linker would make their cavities stretch to allow for very bulky PAHs like B[ghi]P to bind easily.

With preference biased towards extraction of HMW PAHs, the B[k]F/indeno combination was selected as the best MIP combination for the effective extraction of all 16 PAHs. It was chosen because it was the combination that appeared the most (Table 7). Where this combination is not present, they are preferred as either B[k]F or indeno, or their combinations with other MIPS.

4.2.2 Implications of the selected MIP combination

The B[k]F and indeno-imprinted MIP combination was selected after performing rigorous optimisation experiments aimed at tuning the cavities that would be selective towards the 16 US EPA priority PAHs. The selection experiments were exhaustive with both LMW and HMW, and pyrene- and fluoranthene-based PAHs investigated as potential imprints. Most reported template or mix-template choices to fabricate MIPs for PAHs are unoptimised with researchers using a trial-and-error and/or expert-template selection approach. Other PAH-imprinted MIPs reported were mainly used to target a single PAH or a portion of the 16

US EPA priority PAHs. Multi-template imprinting, especially with all 16 US EPA priority PAHs, is uneconomic and not green considering that the bound templates have to be leached out to create the cavities.

The observation that an imprint PAH is not preferentially sequestrated from a solution containing other PAHs is essential in the analysis of PAHs. This study has shown that size and shape of the cavities are important especially where the target is to analyse multiple PAHs. PAHs generally exist in environmental samples as complex mixtures rather than single compounds. Suitable MIP-cavity tuning experiments are required. The cavity tuning approach has also been used in testing the binding ability of MIPs prepared with six different PAHs (Dickert et al., 1999).

4.2.3 Characterisation

The BET surface area characterisation study was performed with the powder of leached and unleached MIP as well as the washed NIP to know the effect of imprinting on the polymer surface area, pore volume and width. Leached MIP exhibited more surface area (386.6 $m^2 \cdot g^{-1}$) than NIP (360.6 $m^2 \cdot g^{-1}$) and unleached MIP (332.6 $m^2 \cdot g^{-1}$) (Table 8). BET surface area results indicated an enhancement in surface area for MIP due to imprinting. The observed surface area of the non-printed polymer might be attributed to the pores left by the porogen molecules during washing.

Table 8: The BET surface area for the synthesised MIP

Polymer	**Surface area ($m^2 \cdot g^{-1}$)**	**Pore volume ($cm^3 \cdot g^{-1}$)**	**v_m**	**c**
MIP bound	332.5	0.74	75.6	661
MIP eluted	386.6	0.61	86.0	388
NIP eluted	360.6	0.55	81.2	616

The surface morphology and size determination results from SEM are presented in Figure 9. The image of the control polymer (NIP) shows a very smooth surface compared to the MIP image, which has a very rough surface. A rough surface is an indication of the presence of cavities that exist after eluting the template molecule. The surface of the unleached MIP is less rough because the template molecules are still embedded on the cavities (Kawaguchi et al., 2006). These results also confirm that the polymer particles obtained by bulk polymerisation have an irregular shape.

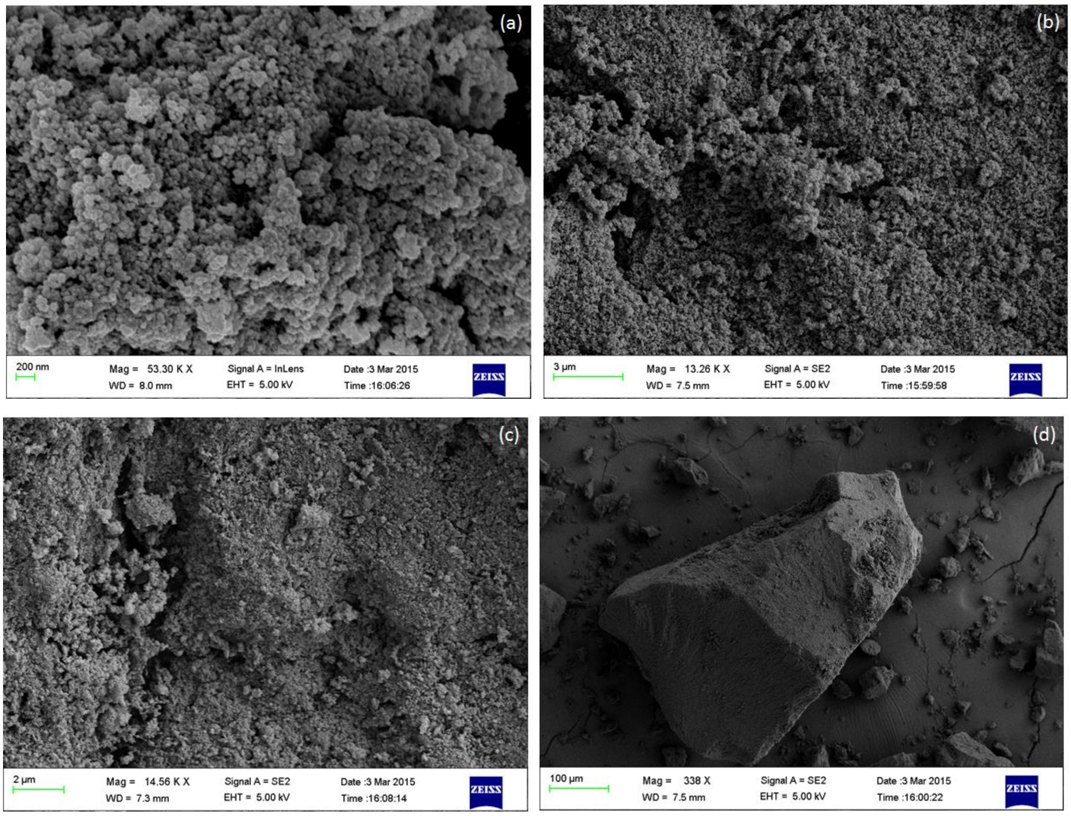
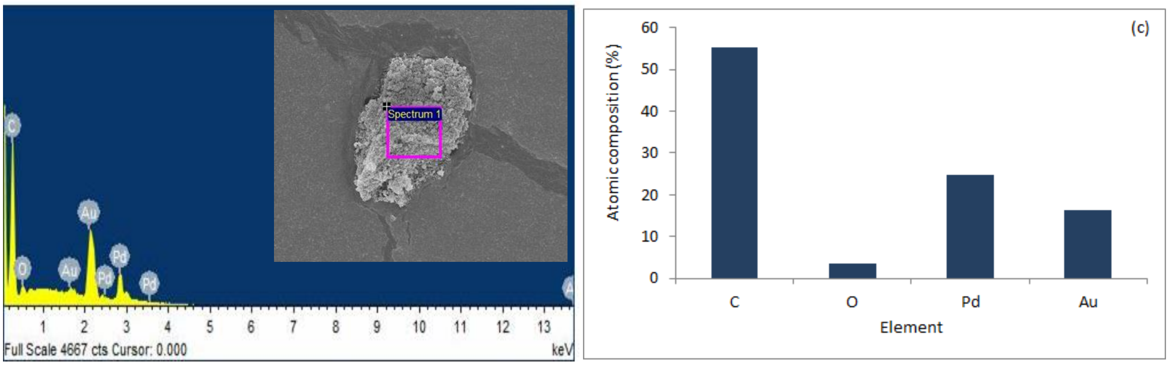


Figure 9: SEM: (a) leached MIP, (b) unleached MIP, (c) washed NIP, (d) unwashed NIP

The EDS measurements were used to determine the elemental composition of the synthesised polymers. Figure 10 shows that the dominant element was carbon, which resulted from the use of organic pre-polymerisation reagents (MAA and EGDMA). Since the initial images showed charging, a phenomenon that compromises the quality of micrographs, the sample specimen was coated with palladium and gold. These two coating agents are also observed along carbon and oxygen in Figure 10(b).



(a) (b)

Figure 10: EDS spectrum of the synthesised MIP and the elemental composition

The TGA results in Figure 11 show that both polymers (MIP and NIP) are stable up to about 370°C. The slight difference between MIP and NIP is due to the removal of template from the MIP. As the temperature increases, the cross-linker compresses the polymers. This compression is shown by the gradual decomposition at 300°C for MIPs and about 330°C for NIPs. The polymers then attain total decomposition from 400°C.

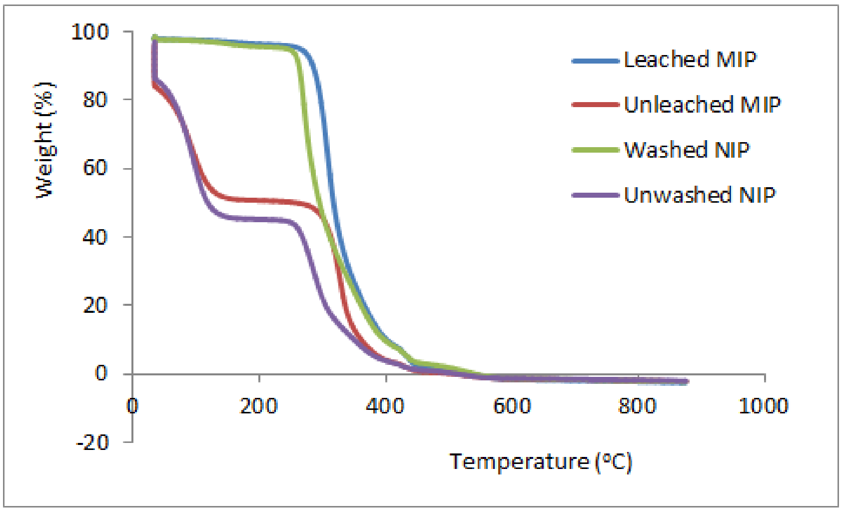


Figure 11: Thermal stability of the synthesised polymer

4.2.4 Swelling of MIPs

The swelling behaviour of the MIP and the NIP in various organic solvents is shown in Figure 12. The general higher swelling of MIPs in the presence of solvents than NIPs can be attributed to the presence of cavities. The swelling of the MIP was at least 200% for all solvents investigated. The flexibility of the EGDMA cross-linker may be responsible for this observed behaviour. Knowledge of the swelling behaviour of the MIP helps to explain the maximum amount of MIP that can be placed inside a membrane bag. The high swelling behaviour for DCM is because DCM is highly volatile compared to other solvents.

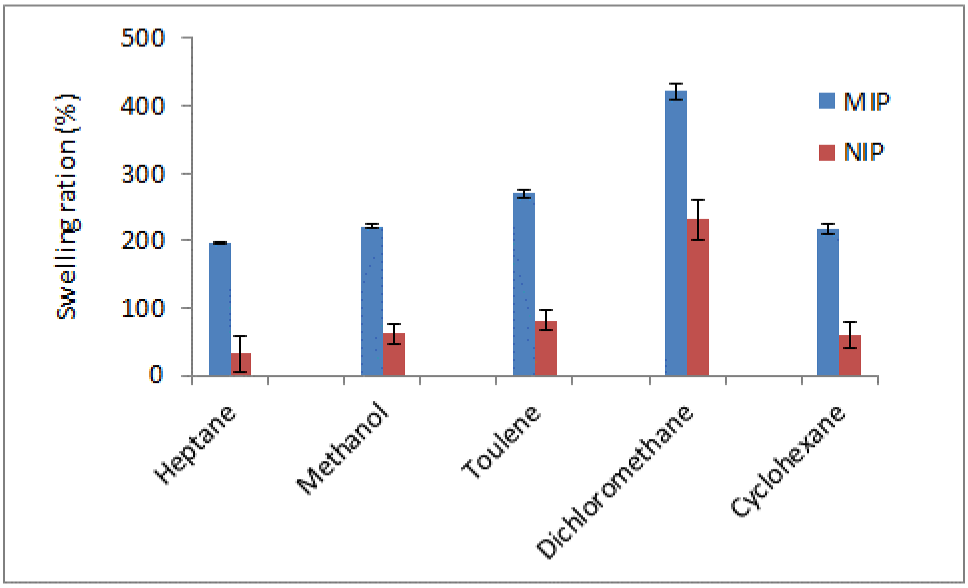


Figure 12: Swelling behaviour of the MIP in different solvents (n = 3, SD)

4.2.5 Batch adsorption

4.2.5.1 Adsorption isotherms

A comparison of the r^2 values in Figures 13 and 14 shows that the Langmuir isotherm is the dominant mechanism of adsorption when compared to the Freundlich. This implies that the adsorption results in formation of a monolayer on the MIPs. This was expected since the MIPs have cavities engineered to bind PAHs. The Langmuir isotherm was then accepted and its parameters were used to further explain the extent of adsorption of PAHs onto the MIP surface.

A MIP particle that forms a monolayer as per the Langmuir isotherm model is bound to attain a maximum adsorption capacity. The adsorption capacity can be viewed as the maximum amount of PAHs that can be adsorbed onto the cavities of a unit mass of the MIP. Its value is related to the slope of the Langmuir graph as shown in Equation 6. The calculated maximum adsorption capacity of the MIP obtained after a 60-minute contact time was 3.33 mg·g^{-1}.

$$slope = \frac{1}{Q} \tag{6}$$

Where Q is the maximum adsorption capacity (mg·g^{-1}) at time, t (min).

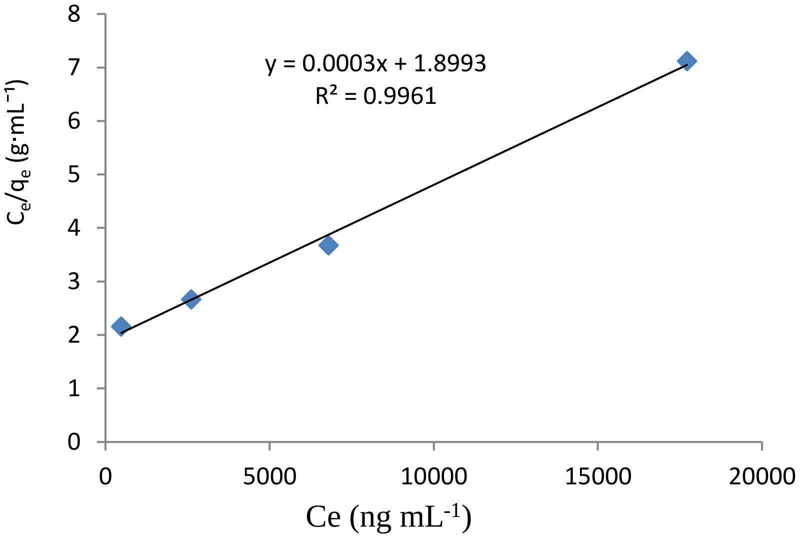


Figure 13: Langmuir adsorption isotherm plotted using Equation 4

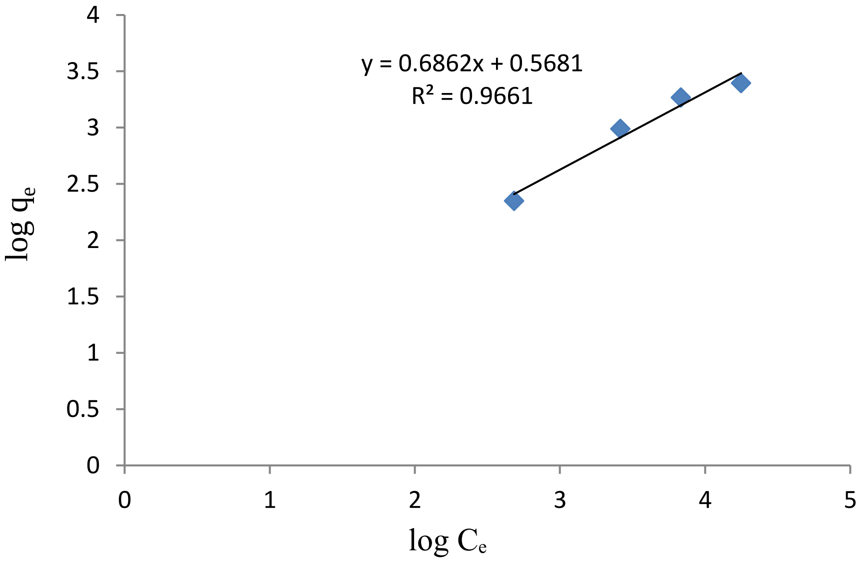


Figure 14: Freundlich isotherm model plotted using Equation 5

4.2.5.2 Effect of contact time

The effect of contact on the binding capacity of the MIP is summarised in Figure 15. Generally, the graph shows a strong dependence of adsorption on contact time. The MIP attained its maximum binding capacity within 80 minutes. When a polynomial of 5th order with a 0.9348 coefficient of determination was inserted through the data points (Figure 15), the estimated maximum binding capacity was 5.19 ± 0.392 $mg \cdot g^{-1}$. The binding preferences summarised in Table 9 show a higher affinity towards HMW PAHs. This is an indication that the PAH-MIP interactions are dependent on the molecular weight and number of rings. This has also been observed by other researchers (Baggiani et al., 2007; Ho et al., 2011; Lai et al., 2004).

The stabilised MIP-analyte effect due to larger PAHs might be related to the increased hydrophobicity and steric orientations on the cavities. More rings implied availability of a reservoir of delocalised electrons that participate in the π–π interactions with the functional monomers. However, the synthesised MIP still displayed a broad selectivity towards the 16 PAHs with no imprinting effect observed (Table 9). The individual binding capacities ranged from 200.8 $\mu g \cdot g^{-1}$ for fluoranthene to 493.9 $\mu g \cdot g^{-1}$ for B[ghi]P. This was related to the extraction efficiencies for individual PAHs that ranged from 40.2-98.8%. The average binding capacity was 317 ± 45.1 $\mu g \cdot g^{-1}$ (n = 12, SD). The average extraction efficiency of the MIP to extract all 16 PAHs from an aqueous solution was 65 ± 13.3% (n = 12, SD). The efficiency was calculated using Equation 14.

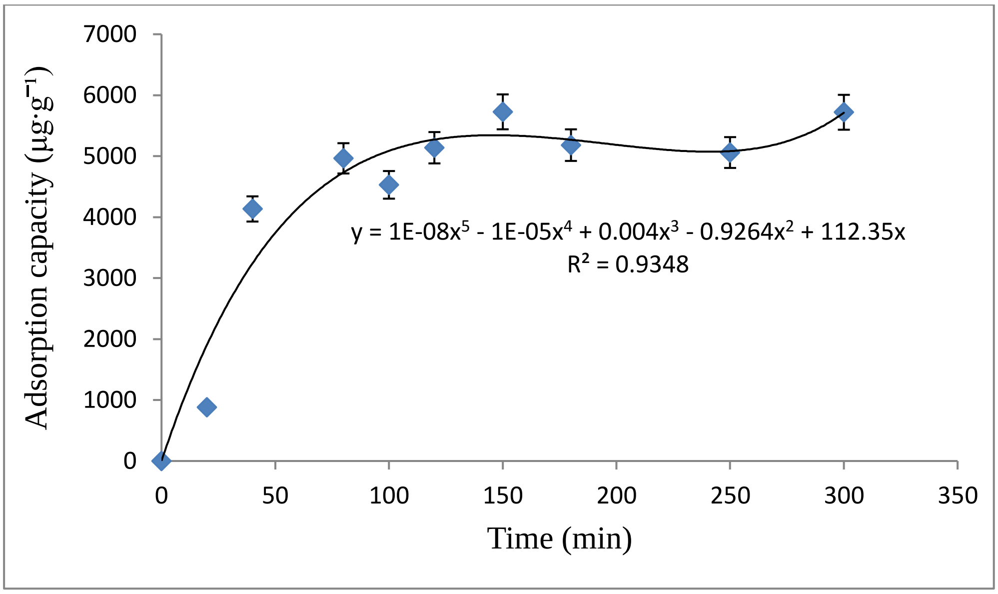


Figure 15: Effect of contact time on adsorption capacity (n = 3, SD)

Table 9: MIP adsorption preferences at maximum binding capacity (n = 3)

No. of rings	No. of PAHs	Adsorption capacity ∑PAHs (ng·mg^{-1})	Average (ng·mg^{-1})	Percentage preference (%)
2 & 3	6	1883.7	313.9	33.0
4	4	1057.5	264.4	27.7
5 & 6	6	2247.6	374.6	39.3
All PAHs	**16**	**5188.8**	**317.6**	**100.0**

4.2.5.3 Adsorption kinetic modelling

The kinetic modelling results showed that the adsorption process follows pseudo-second order kinetics. A Lagergren pseudo-first order model had an r^2 value of 0.7171 compared to 0.9830 for the pseudo-second order model. A plot of the linearised Lagergren pseudo-second order equation is presented in Figure 16. The implication of the pseudo-second order model is that the chemisorption of the PAHs with the MIP cavities through hydrophobic and π–π interactions is the rate-limiting step. The adsorption capacity was estimated from the gradient of the Lagergren pseudo-second order linear equation in Figure 16 and was found to be 5.00 mg·g^{-1}. This value is within the standard deviation of the experimental adsorption capacity implying there was an agreement of the experimental and the calculated values. The pseudo-second order kinetic results and the Langmuir adsorption isotherm modelling results can be accepted when describing the extent of adsorption of PAHs in MIP cavities. The rate constant (k_2) predicted from the intercept of the model in Figure 16 was 0.784 min^{-1}. This value implies that there is fast mass transfer of PAHs into the imprinted cavities. The Lagergren pseudo-first order rate constant was 0.049 min^{-1}.

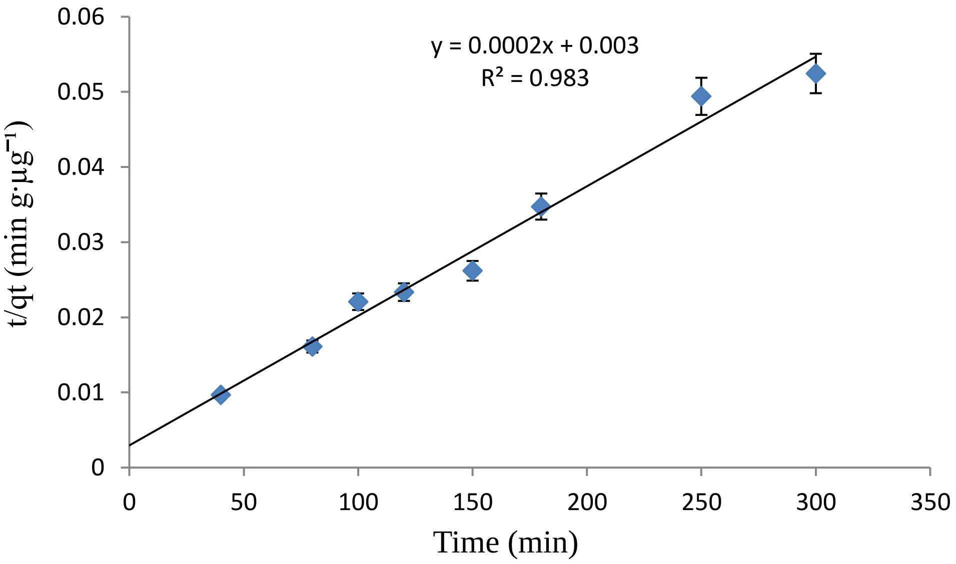


Figure 16: Pseudo-second order kinetic adsorption of PAHs on the surface of the MIP (n = 3, SD)

Several articles have reported the performance of PAH-imprinted MIPs. Song et al. (2012) synthesised a multi-template MIP for PAHs with a binding capacity of 2.7 $mg \cdot g^{-1}$. In this rare approach, all 16 US EPA priority PAHs were used for imprinting the MIPs. The imprinting factor was 2.25. A similar multi-template MIP synthesised using all 16 PAHs by Krumpadam et al. (2009) had a binding capacity of 6.93 $mg \cdot g^{-1}$, which is comparable with the 5.19 $mg \cdot g^{-1}$ obtained in this study. When the same authors used a five-template MIP, the binding capacity was 1.93 $mg \cdot g^{-1}$. When a sixth PAH was added to produce a six-template MIP, the binding capacity dropped to 0.69 $mg \cdot g^{-1}$. The imprinting factors were 4.5, 2.4 and 5.8 respectively (Krupadam et al., 2010a). Ho et al. (2010) reported 0.02 $mg \cdot g^{-1}$ for a B[a]P-imprinted MIP synthesised for the extraction of B[a]P.

Adsorption capacities higher than that of the MIP synthesised during this study have been reported. In most cases, such binding capacities were tested on the imprinting molecule only. Hassan et al. (2015) got a binding capacity towards anthracene as 374.3 $mg \cdot g^{-1}$ for an anthracene-imprinted MIP. In another study, the same authors reported a relatively lower adsorption capacity of 0.0078 $mg \cdot g^{-1}$ for the same B[a]P-imprinted MIP towards B[a]P in the presence of other PAHs. In this research, B[a]P had a contributing adsorption capacity of 0.319 $mg \cdot g^{-1}$. Li et al. (2013) reported 15.57 $mg \cdot g^{-1}$ for phenanthrene using a phenanthrene-imprinted MIP while Tiu et al. (2016) recorded a binding capacity of 11.37 $mg \cdot g^{-1}$ for pyrene. In all these studies, the binding capacity was obtained by extracting individual PAHs from solutions spiked with the target PAH only.

Calculating the binding capacity value was essential in determining the amount of MIPs to be used for a routine PAH analysis either from waste water or waste water sludge. The procedure used in calculating the MIP-binding capacity was also used to determine the NIP-binding capacity. The imprinting factor was then calculated as a ratio of the binding capacity of the MIP to that of the NIP (Equation 3). The imprinting factor value helps in evaluating the adsorption effectiveness of the MIP.

4.2.6 MIP-PAH elusion solvent

A good choice of eluting solvent is that which maximises elution of all the PAHs from the MIP cavities with minimal volume used. For LMW PAHs, hexane was the best elution solvent as shown in Figure 17. However, the five- and six-ringed PAHs were eluted better by the least polar solvent, namely, heptane.

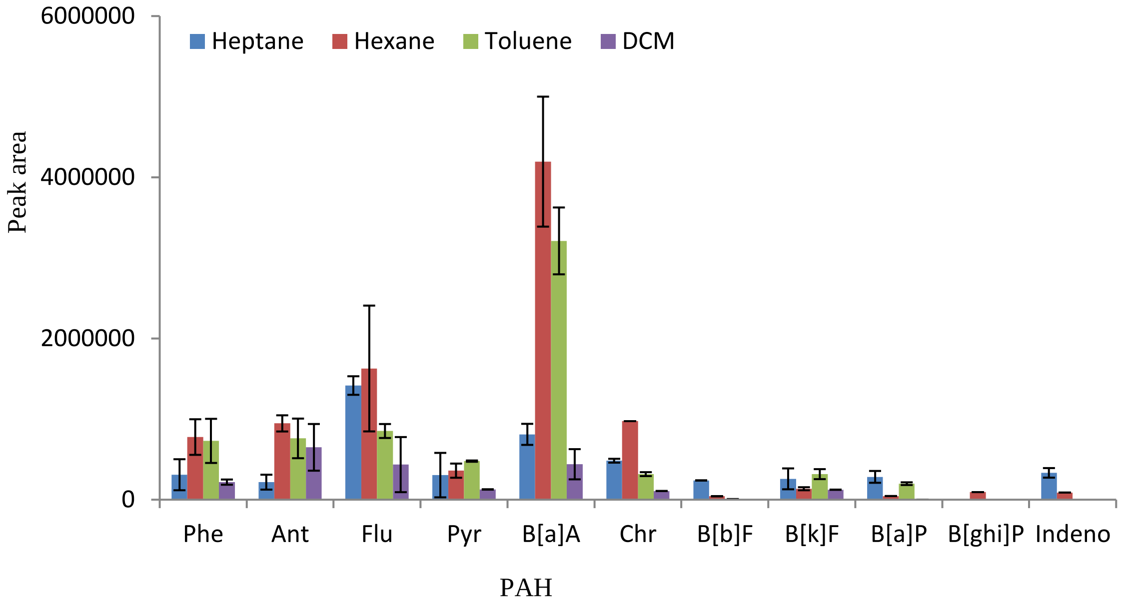


Figure 17: Performance of four solvents in eluting PAHs from MIP cavities (n = 3, SD). Phe (Phenanthrene); Ant (Anthracene); Flu (Fluorene); Pyr (Pyrene); Chr (Chrysene)

Hot hexane still performed well for naphthalene, acenaphthylene, acenaphthene and fluorene. Hot heptane was adopted for further studies. The use of DCM in combination with other solvents like acetic acid (Song et al., 2012) and hexane (Ho et al., 2011) followed by gas chromatography determination has been reported in elution of PAHs from MIP cavities. However, DCM has been used without comparing its extracting power with other theoretically relevant non-polar solvents like hexane. When the hot heptane and hexane maintained at 40°C were further investigated on synthesised MIPs, the effect of both hexane and heptane was greatly enhanced (Figure 18). The results show that heptane was more effective in eluting most PAHs from MIP cavities.

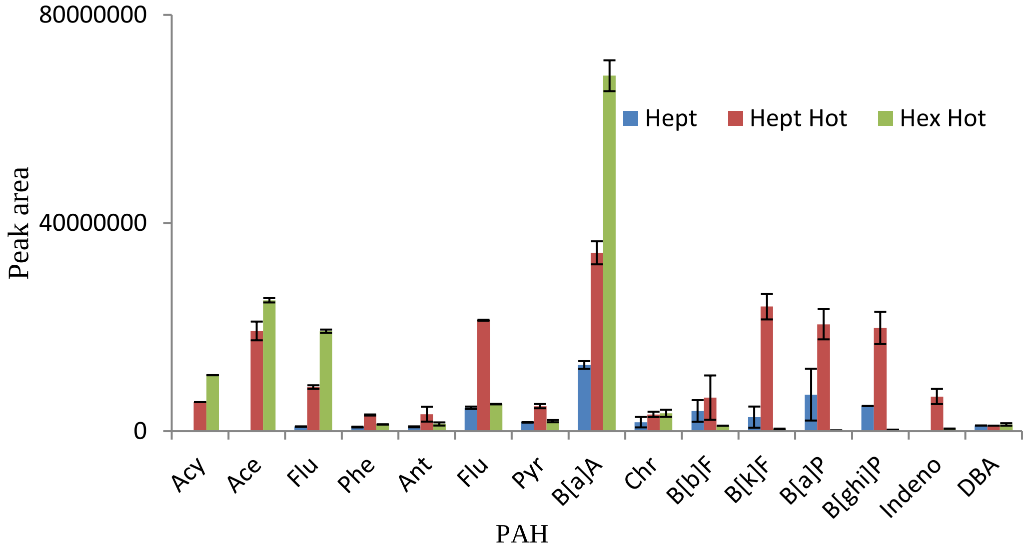


Figure 18: Performance of hot solvents in eluting PAHs from MIP cavities(n = 3, SD). Hept (Heptane); Hex (Hexane); Acy (Acenaphthylene); Ace (Acenaphthene); Flu (Fluorene); Phe (Phenanthrene); Ant (Anthracene); Pyr (Pyrene); Chr (Chrysene)

4.2.7 MIP performance and cost results

4.2.7.1 Imprinted cavity efficiency

A 10 mg mass of imprinting PAH resulted in synthesis of an average of 2.002 ± 0.0753 g of the polymer. This mass was obtained after the MIP had been washed to remove the template. The summary of results in Table 10 shows that 5 mg of an imprinting PAH is needed to synthesise a gram of the MIP. The experimental adsorption capacity value implies that a gram of the MIP will conversely adsorb a total of 5.188 g of PAHs from an aqueous solution. This gives an imprinting efficiency of 103.9%. This level of efficiency sounds impractical and was not expected. The experimental adsorption capacity does not eliminate the effect due to those PAHs that adsorb on the hydrophobic backbone of the MIP. Assuming that one imprint molecule results in formation of a single cavity, 5 mg of the imprinting B[k]F would give a total of 1.19×10^{19} cavities per gram of MIP. The experimental number of cavities calculated from the maximum adsorption capacity was 1.53×10^{19} per gram of the synthesised MIP. This value is greater than the theoretical value, an indication that adsorption is not entirely due to analyte penetration of the cavities. This too can be explained in terms of the hydrophobicity and lack of functional groups in PAHs. The overall extent of occupation of the imprinted cavities in the presence of excess PAHs was found to be 128%.

Table 10: Imprinting efficiency results (n = 3)

Parameter	Average	%RSD
Mass of MIP (g)	2.002	3.76
Mass of imprint (mg)	10.00	–
Imprinting ability ($mg \cdot g^{-1}$)	5.002	3.79
Experimental adsorption capacity ($mg \cdot g^{-1}$)	5.188	7.56
Imprinting efficiency (%)	103.9	3.76
Predicted no. of cavities (g^{-1})	1.19×10^{19}	3.79
Experimental no. of cavities (g^{-1})	1.53×10^{19}	7.56
Cavity efficiency (%)	128	*5.04

** Calculated as a propagation of errors*

4.2.7.2 Regeneration and reuse of the MIP

When the polymer combination was used repeatedly to extract PAHs at maximum conditions, there was a general decrease in the maximum adsorption capacity (Figure 19). A decrease might be related to the warm heptane solvent used to elute the PAHs from the cavities. The eluting and washing conditions might result in destruction or collapse of some binding sites. However, this effect was less pronounced with an average 2.9% in the binding capacity with every washing cycle. After the MIP had been regenerated seven times, the maximum binding capacity had dropped from 5188 $ng \cdot mg^{-1}$ to 4139 $ng \cdot mg^{-1}$. This was considered insignificant because the target analytes exit in trace amounts and the 80 mg amount of MIP used would still have a binding capacity of 331 125 ng of PAHs. The results of the regeneration studies have shown that the MIP can be reused with minimal loss in its specificity and selective.

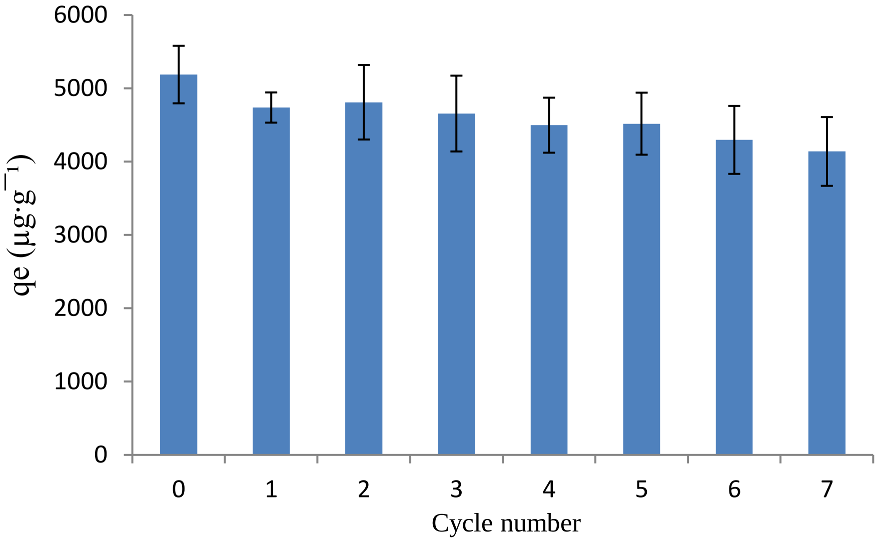


Figure 19: Effect of MIP reuse on its binding capacity (n = 3, SD)

4.3 Development of the MASE-MIP for PAHs in Waste Water

4.3.1 Influence of addition of the organic modifier in the sample

The addition of miscible organic solvents to aqueous samples has been reported to minimise adsorption of organic analytes on the walls of glass flasks (Benanou et al., 2003). Figure 20 shows the results obtained when various organic modifiers were used. It is evident that DMF gave the maximum PAHs extraction. March et al. (2011) found that the presence of ethanol increased the extraction efficiency and found 5% to give the optimum uptake. The amount of ethanol for maximum extraction seems to be related to the analyte polarity. Addition of organic modifier in this case is very important especially for heavier PAHs that have poor solubility in water. With an organic modifier, they are forced to be in solution and thus get extracted into the membrane bag.

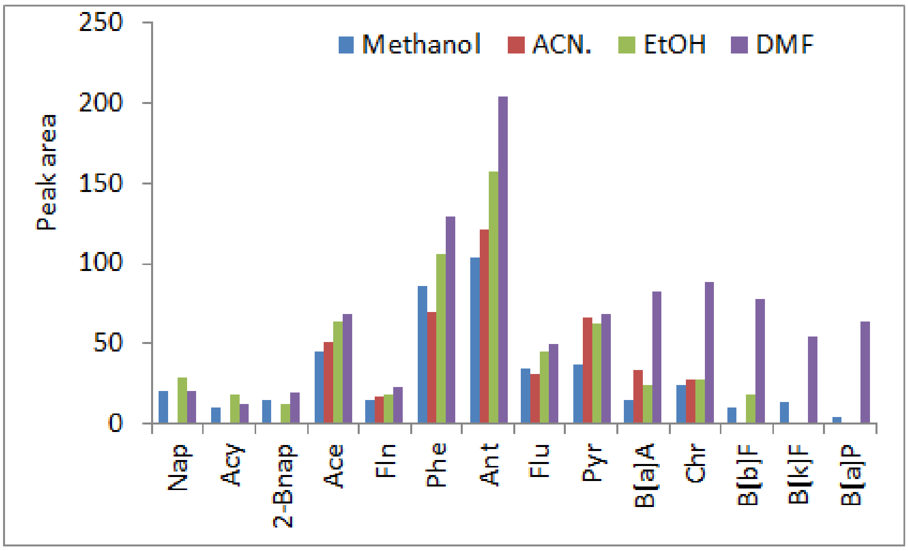


Figure 20: Influence of type of organic modifier in the aqueous donor phase solution. Nap (Naphthalene); Acy (Acenaphthylene); Ace (Acenaphthene); Fln (Fluoranthene); Phe (Phenanthrene); Ant (Anthracene); Flu (Fluorene); Pyr (Pyrene); Chr (Chrysene)

4.3.2 Influence of the amount of organic modifier content in the sample

In this study, the influence on the amount of organic modifier was studied in the range of 1-25% (v/v) of DMF. The extraction behaviour of the PAHs followed two trends. The effect of the amount of the organic modifier had a maximum uptake at 15% DMF composition for LMW and medium molecular weight (MMW) PAHs. However, the heavier PAHs were extracted more with a 25% DMF organic modifier content (Figure 21). This was because of their increased dissolution at high organic modifier content. At low DMF content, there was no imprinting phenomenon as the target template, B[k]F, was unavailable in solution due to its low dissolution. Thus, a 25% DMF was considered optimum.

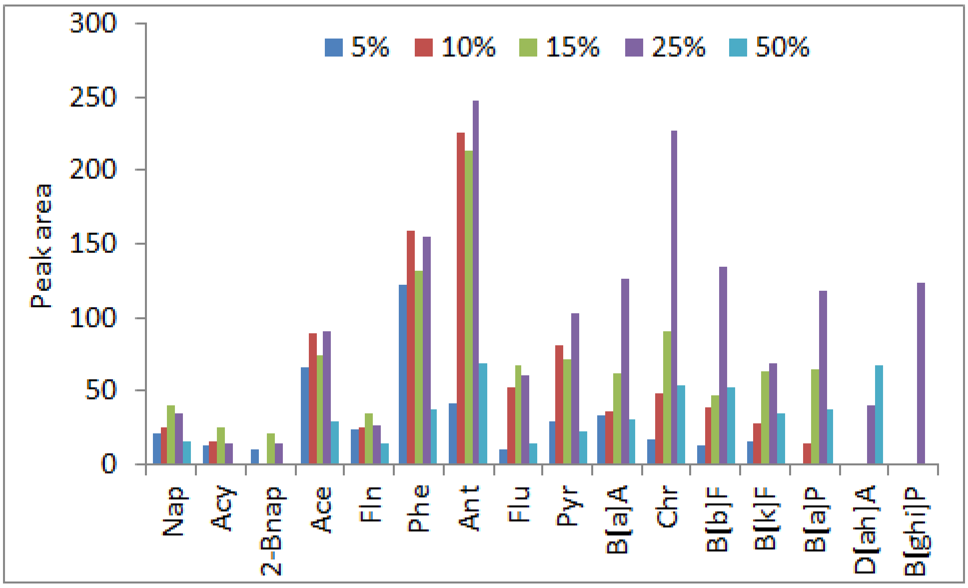


Figure 21: Effect of variation of the DMF donor phase content in the extraction of PAHs. Nap (Naphthalene); Acy (Acenaphthylene); Ace (Acenaphthene); Fln (Fluoranthene); Phe (Phenanthrene); Ant (Anthracene); Flu (Fluorene); Pyr (Pyrene); Chr (Chrysene)

4.3.3 Influence on the amount of MIPs in the membrane bag

In Figure 22, it was observed that an increase in the amount of the MIP sorbent increased the extracted amount of the PAHs. This was the case for all 17 analytes investigated. The sorbent mass was only investigated up to 80 mg. The capacity of the MASE membrane bag would have been exceeded especially when factoring the swell capacity, which was observed to be at least 200% for toluene used as the receiver phase. A MIP mass of 80 mg was therefore selected for use in further optimisation experiments.

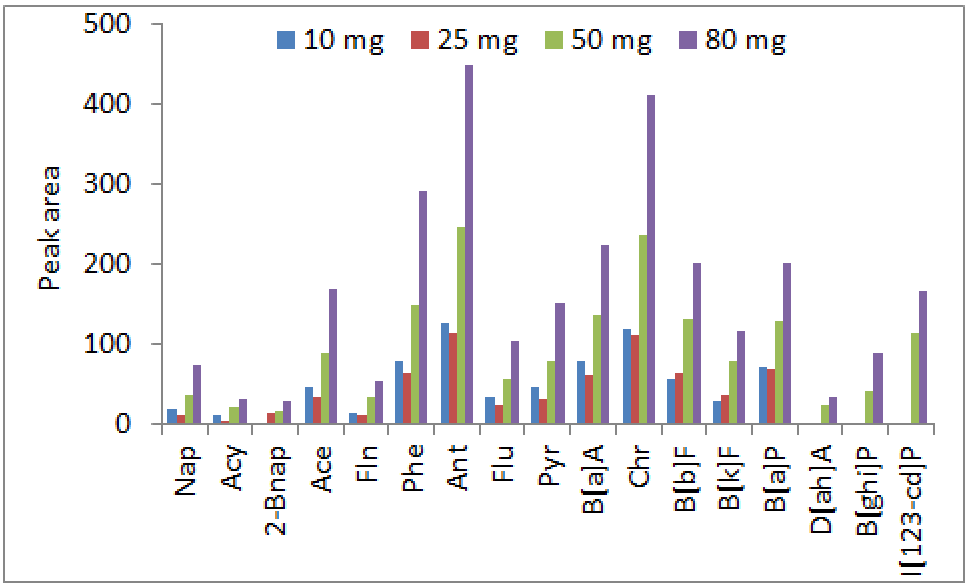


Figure 22: Effect of amount of MIPs in the membrane bag. Nap (Naphthalene); Acy (Acenaphthylene); Ace (Acenaphthene); Fln (Fluoranthene); Phe (Phenanthrene); Ant (Anthracene); Flu (Fluorene); Pyr (Pyrene); Chr (Chrysene)

4.3.4 Effect of contact time

The effect of time on the extraction of PAHs from an aqueous solution across the membrane is illustrated in Figure 23. Extraction was done from 5 ng·mL^{-1} spiked deionised water with a 1000 rpm stirring rate. The results show that most LMW PAHs reached equilibrium within two hours. This might be related to the ease of transfer across the membrane. The bulky PAHs attained optimum extraction in the three- to four-hour range. HMW PAHs generally have low mobility and this might have contributed to their slow transfer across the membrane. The optimum extraction time was taken as four hours, which was used in further experiments.

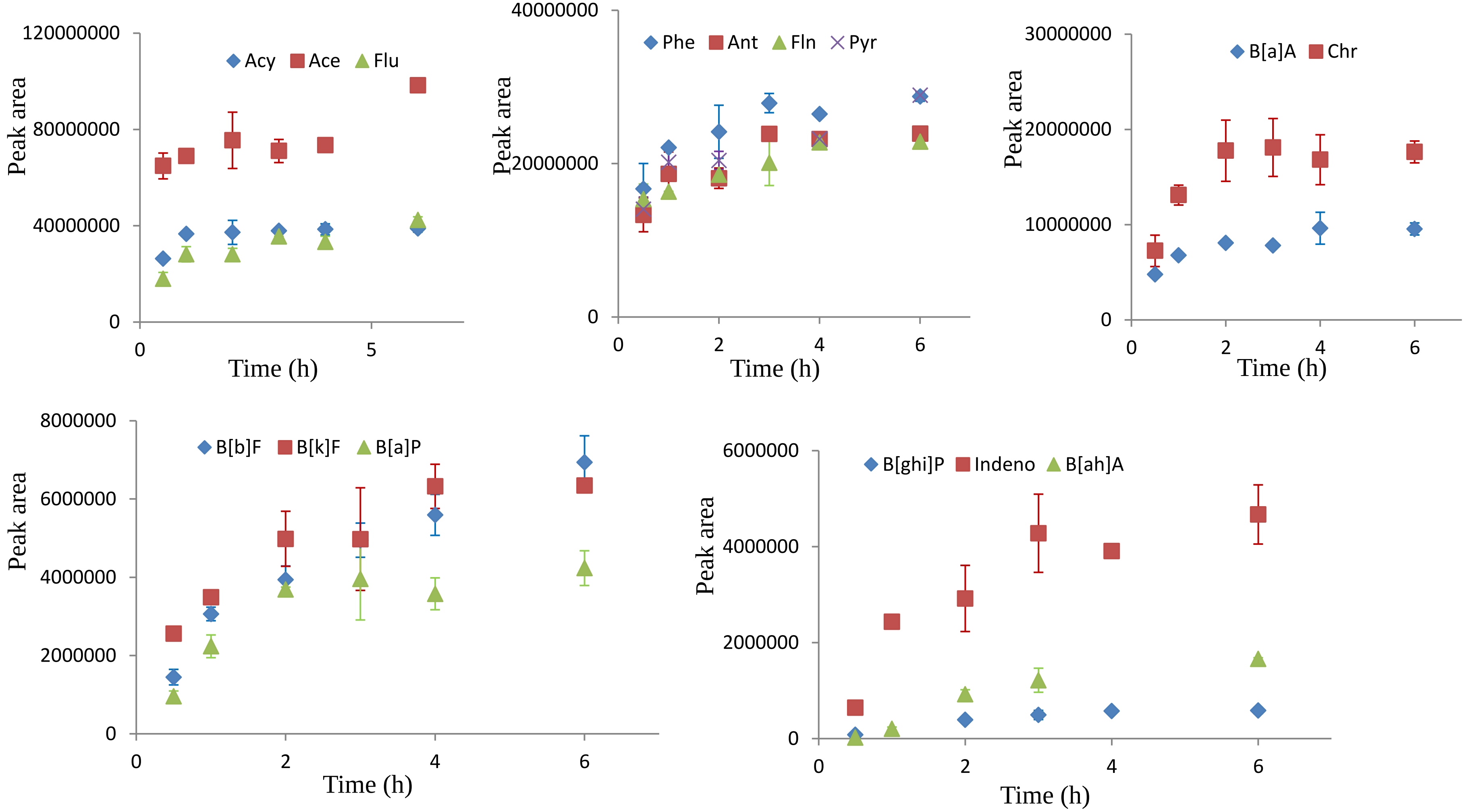


Figure 23: Effect of contact time on the extraction of PAHs. GC-qTOF/MS was used for quantitation (n = 3, SD)

4.3.5 Effect of the stirring rate

Figure 24 shows the results obtained when the stirring rate was varied while keeping other parameters constant. There was a general increase in the amount of extracted PAHs as the stirring speed was increased from 420 rpm to 1000 rpm. Stirring increases mass transfer of the PAH analytes from the donor phase across the membrane into the MIPs suspended in the receiver phase. The stirrer instrument used had a 1200 rpm limit, hence 1000 rpm was chosen for further experiments.

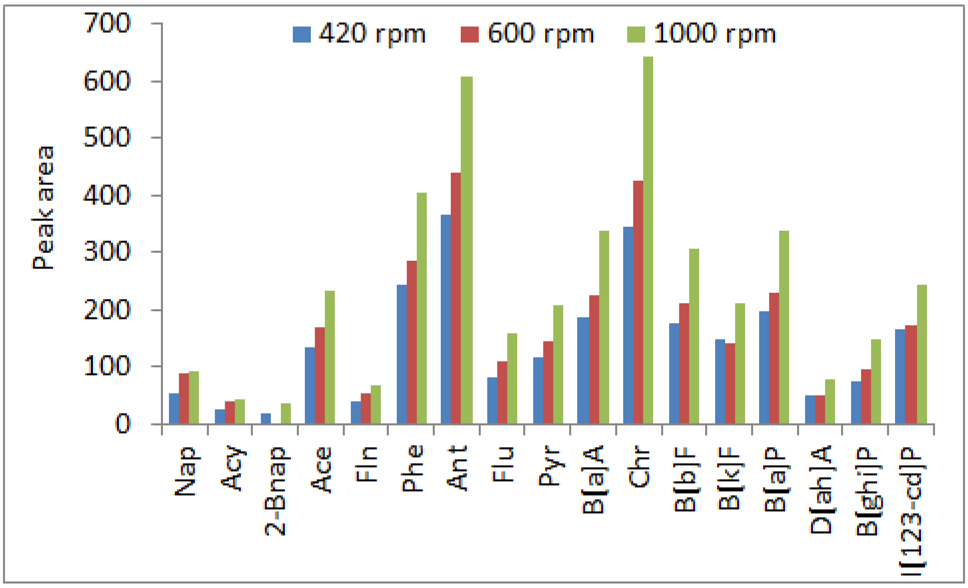


Figure 24: Influence of stirring rate on the uptake of PAHs. HPLC-UV was used for quantitation. Nap (Naphthalene); Acy (Acenaphthylene); Ace (Acenaphthene); Fln (Fluoranthene); Phe (Phenanthrene); Ant (Anthracene); Flu (Fluorene); Pyr (Pyrene); Chr (Chrysene)

4.3.6 Method validation

4.3.6.1 MASE-MIP extraction efficiency

The extraction efficiency for individual PAHs from a spiked water sample are presented in Table 11. The results show that the extraction efficiency ranged from 62.8-96.8% with an average of 77.4%. The average extraction efficiency for the HMW PAHs with five and six rings was 84.7%. This might be explained by their hydrophobicity that enhances their transfer from the aqueous solution into the heptane acceptor phase. In addition, they are slightly favoured for binding in the MIP cavities. The LMW and MMW PAHs were also transferred efficiently with average efficiency values of 75.1% and 69.7% respectively. The relative standard deviation (RSD) value for the average extraction efficiency is a confirmation of the absence of the imprinting factor. The reduced selectivity while specificity of the cavities towards a class of analytes remains high is essential where numerous analytes is targeted.

The average efficiency of the MASE-MIP method is slightly higher than the efficiency calculated when the MIP reached its maximum adsorption capacity (77.4% and 65% respectively). This might be an indication that extraction efficiency tends to increase as the ratio of the concentration of the analytes to the mass of MIPs used increases. It might also be an indication that extraction from aqueous solutions is higher than when analytes are dissolved in non-polar solvents.

Table 11: Extraction efficiency of the MASE-MIP method (n = 3)

PAH	Efficiency	EF	RSD
Naphthalene	66.2	13.2	0.152
Acenaphthylene	74.6	14.9	0.081
Acenaphthene	78.3	15.7	0.076
Fluoranthene	77.0	15.4	0.063
Phenanthrene	76.4	15.3	0.154
Anthracene	78.2	15.6	0.123
Fluorene	62.8	12.6	0.353
Pyrene	75.9	15.2	0.182
B[a]A	69.2	13.8	0.224
Chrysene	71.1	14.2	0.365
B[b]F	86.4	17.3	0.104
B[k]F	69.2	13.8	0.353
B[a]P	83.1	16.6	0.122
B[ghi]P	84.2	16.8	0.059
Indeno	88.4	17.7	0.027
DBA	96.8	19.4	0.007
Average	**77.4**	**15.5**	**0.153**

Despite the fairly high extraction efficiencies, the EF values are quite low with an average of 15.5. This is mainly because the donor phase volume is only viginti-fold the acceptor volume. The EF values are important when calculating method detection and quantitation limits.

4.3.6.2 MASE-MIP method detection and quantitation limits

The limits of detection and quantitation for the 16 US EPA priority PAHs are given in Table 12. Both the MDL and MQL values were in the nanogram scale with the MDL ranging from 0.01-0.45 ng·mL^{-1}. The MQL values ranged from 0.02-1.49 ng·mL^{-1}. Generally, the LMW PAHs had better limit values than HMW PAHs. This might be related to the low response of the mass spectrometry towards these PAHs.

Table 12: Method detection and quantitation levels for the developed MASE-MIP technique (n = 3, RSD < 15%)

PAH	Limit of detection (ng·mL^{-1})	Limit of quantitation (ng·mL^{-1})	EF	MDL (ng·mL^{-1})	MQL (ng·mL^{-1})
Naphthalene	0.07	0.21	13.2	0.01	0.02
Acenaphthylene	0.26	0.85	14.9	0.02	0.06
Acenaphthene	0.39	1.30	15.7	0.02	0.08
Fluoranthene	0.49	1.62	15.4	0.03	0.11
Phenanthrene	0.61	2.02	15.3	0.04	0.13
Anthracene	0.79	2.61	15.6	0.05	0.17
Fluorene	3.33	11.0	12.6	0.26	0.87
Pyrene	0.88	2.91	15.2	0.06	0.19
B[a]A	2.05	6.78	13.8	0.15	0.49
Chrysene	2.03	6.69	14.2	0.14	0.47
B[b]F	2.46	8.11	17.3	0.14	0.47
B[k]F	2.50	8.25	13.8	0.18	0.60
B[a]P	7.50	24.8	16.6	0.45	1.49
B[ghi]P	3.75	12.4	16.8	0.22	0.74
Indeno	5.36	17.7	17.7	0.30	1.00
DBA	4.55	15.0	19.4	0.23	0.77

4.3.7 Application of the MASE-MIP technique in waste water

The developed MASE-MIP technique was applied to quantitate PAHs in effluent waste water obtained from Goudkoppies WWTP. The deuterated surrogate standards used were naphthalene-D8, acenaphthene-D10 and phenanthrene-D10. The extraction efficiencies of the developed MASE-MIP towards the surrogate standards are given in Table 13. The results show that the efficiencies of the deuterated standards were comparable with those of the analytes as given in Table 11. The efficiencies in Table 11 were therefore accepted and used in quantitation calculations.

Table 13: Extraction efficiencies of the surrogate standards (n = 3, SD)

Surrogate standard	Calibration linearity	Efficiency	%RSD
Naphthalene-D8	0.9977	68.1	8.6
Acenaphthene-D10	0.9934	79.8	10.0
Phenanthrene-D10	0.9944	82.2	14.1

Generally, LMW PAHs were detected in waste water samples in a concentration range of 3.4-1829.8 ng·mL^{-1} (Table 14). The highest amount found was acenaphthene. Acenaphthene has one of the highest solubility values in water (3.93 ng L^{-1}), which might explain its levels in waste water. Of the quantified PAHs, pyrene and anthracene have the lowest solubility at 0.077 ng·mL^{-1} and 0.076 ng·mL^{-1} respectively. Naphthalene and acenaphthene contributed 67% of the 3230.3 ng·mL^{-1} total PAH concentration. This is an indication that the WWTP under investigation is releasing insignificant amounts of the toxic PAHs in its effluent.

Table 14: Quantities of PAHs in Goudkoppies effluent waste water samples (n = 3)

PAH	Concentration (ng·mL^{-1})	%RSD
Naphthalene	343.1	12.08
Acenaphthene	1829.8	4.36
Fluorene	679.6	10.06
Phenanthrene	80.0	6.76
Anthracene	9.8	5.29
Fluoranthene	284.6	13.21
Pyrene	3.4	20.14
∑PAHs	**3230.3**	**10.27**

The HMW PAHs have low solubility in water and were below limits of quantitation. This observation is essential considering that HMW PAHs have known health effects rather than LMW PAHs. Their absence (or low concentration) in waste water is an indication that they are less bioavailable because they do not dissolve into aqueous environments. The extraction was repeated the second day in which the regenerated MIPs were used. The inter-day reproducibility of the developed method is presented in Table 15. The results had RSD values ranging from 0.6-24.9%.

Table 15: Quantities of PAHs in waste water samples (n = 3)

PAH	Concentration (ng·mL^{-1})		Inter-day
	Day 1	Day 2 ± %RSD	%RSD
Naphthalene	343.1	459.9 ± 12.6	11.9
Acenaphthene	1829.8	1 804.7 ± 5.0	0.6
Fluorene	679.6	641.8 ± 10.9	2.3
Phenanthrene	80.0	62.7 ± 12.2	9.9
Anthracene	9.8	5.2 ± 0.0	24.9
Fluoranthene	284.6	418.3 ± 6.9	15.5
Pyrene	3.4	3.3 ± 13.4	1.6
∑PAHs	**3230.3**	**3 396.1 ± 8.7**	**9.5**

4.4 Development of the SE-MASE-MIP for PAHs in Waste Water Sludge

4.4.1 Effect of temperature on extraction of PAHs

The results show that increasing the temperature improves the binding ability/capability of PAHs on MIPs (Figure 25). The possible explanation might be that increasing temperature increases the MIP-PAH collision frequency thus increasing the chances of successful collisions. Similar results have been observed with other PAH extraction techniques (Librando et al., 2004; Miege et al., 2003; Shu and Lai, 2001; Xu and Lee, 2008). However, above 60°C there was a decrease in the extraction efficiency for LMW PAHs. Interesting to note was that the sequestration of HMW PAHs was minimal at low temperatures. B[a]P, DBA, B[ghi]P and indeno could not be detected from elution of MIPs at 20°C and 40°C. The HMW PAHs have limited mobility and polarity compared to LMW PAHs (Chimuka et al., 2015; Sun et al., 2009; Zhang et al., 2007). This might have affected the diffusion and sequestration kinetics and could explain the preference of MIPs for LMW PAHs at lower temperatures.

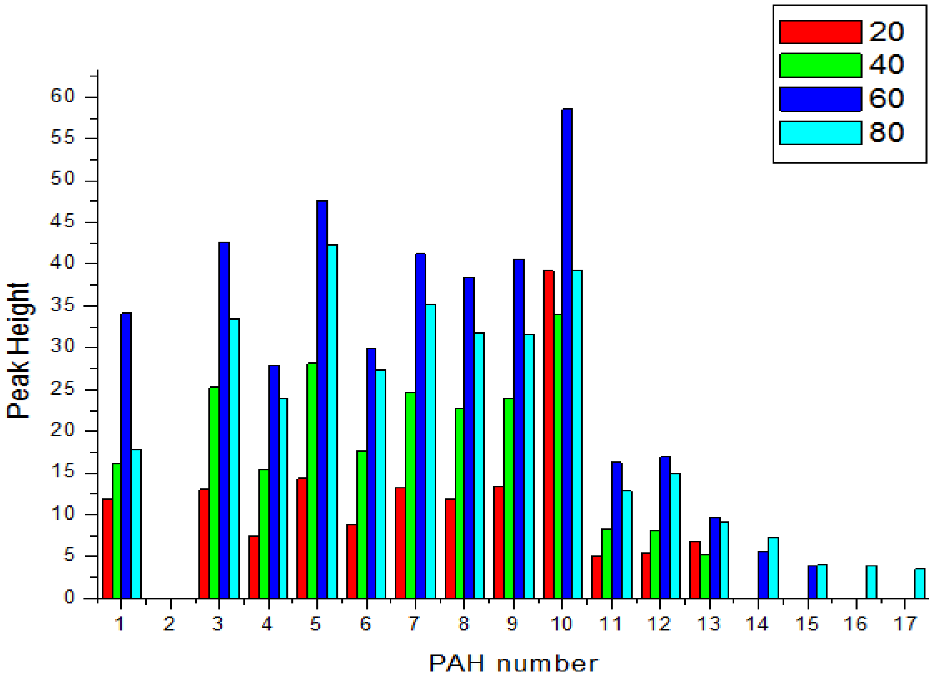


Figure 25: Effect of temperature on the transfer and sequestration of PAHs. 1. Naphthalene, 2. Acenaphthylene, 3. 2-Bnap, 4. Acenaphthene, 5. Fluoranthene, 6. Phenanthrene, 7. Anthracene, 8. Fluorene, 9. Pyrene, 10. B[a]A, 11. Chrysene, 12. B[b]F, 13. B[k]F, 14. B[a]P, 15. DBA, 16. B[ghi]P, 17. Indeno

When the binding temperature was investigated in the absence of a membrane in which the MIPs were directly added to the donor phase, all 16 PAHs appeared to bind better at lower temperatures (Figure 26). This observation was in total contrast to the results observed when the MIPs were placed in the membrane. The slightly reduced binding capacity at higher temperature could be attributed to the mobility associated with the kinetic energy of the PAHs.

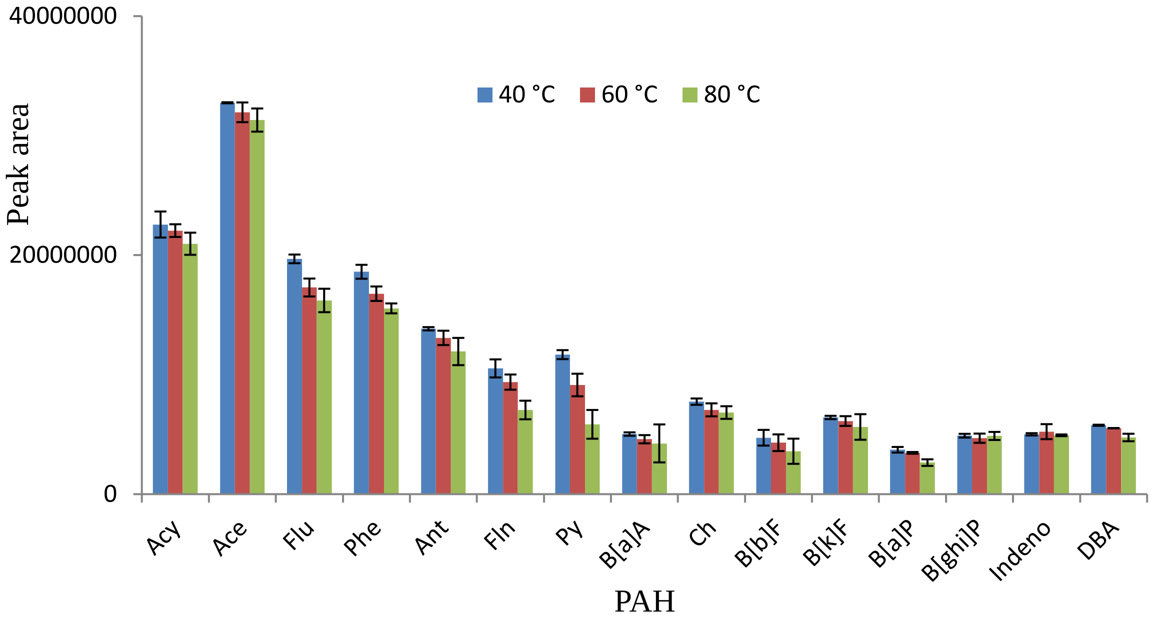


Figure 26: Influence of stirring rate on the uptake of PAHs (n = 3, SD). Acy (Acenaphthylene); Ace (Acenaphthene); Flu (Fluorene); Phe (Phenanthrene); Ant (Anthracene); Fln (Fluoranthene); Py (Pyrene); Ch (Chrysene)

The higher temperature weakens the PAH-MIP interactions and the PAHs are somehow washed out of the MIP cavities. However, an analysis of variance (ANOVA) showed that temperature was not significant in the sequestrating ability of the MIP into its cavities. In the presence of a membrane, an increase in PAH sequestration might be attributed to improved transfer of PAHs across the membrane. This effect is better observed with HMW PAHs whose mobility and polarity are very low. An idea of the temperature dependence of the transfer of PAHs across the membrane and the subsequent binding on to the MIP cavities gave us a snap view of how to approach solvent choice optimisation.

4.4.2 Choice of extracting solvent

4.4.2.1 Preliminary results

Sludge is a semi-wet solid that agglomerates into many compact clusters when drying and contains considerable amounts of bound water (Jin et al., 2004; Lee and Hsu, 1995; Siuris, 2011; Zorpas et al., 2011). From preliminary results in which extraction was done from spiked soil samples, hexane and the mixture of hexane/THF and hexane/acetone performed fairly well. The performance of hexane in combination with a relatively polar solvent can be related to the de-wetting ability of the water-soluble solvent. THF and acetone enhance extraction by entering the pores of the sample and breaking up the soil aggregates, thus exposing the PAHs to the less polar hexane (Heemken et al., 1997). The polar solvent also helps reduce the water content of the sample as the reflux progresses, thereby allowing the non-polar solvent to come into contact with the PAHs.

The hexane/THF mixture gave a better extracting capacity. This might also be related to the enhancing effect of high temperature on the sequestration of PAHs onto the MIPs observed during temperature studies. The boiling temperature difference between the two solvents is only 3°C and the mixture refluxed at about 60°C. The hexane/acetone solvent mixture refluxed at 45°C while the hexane/THF refluxed at 60°C. Hexane and acetone have a 13°C boiling point difference. Even though acetone is more polar than THF and was thus expected to penetrate the sample clumps better than THF, there is a possibility that only acetone was refluxing. Thus, it had reduced extraction efficiency compared to the hexane/THF mixture.

4.4.2.2 CRM-based optimisation

Hexane, and the mixtures of hexane and the polar THF and acetone were investigated using sludge CRM. The MIPs were not included in the Soxhlet set-up. The results in Figure 27 show the extracting power of hexane for all PAHs except for naphthalene (not shown in Figure 27), phenanthrene, chrysene and B[b]F, which were extracted better by the hexane/THF mixture. This behaviour of the hexane/THF mixture could not be explained as the four compounds are not closely related structurally or in terms of number of rings and polarity. An ANOVA was also carried out on the peak areas of PAHs extracted using hexane and the hexane/THF mixture. Naphthalene, acenaphthene, pyrene, chrysene and B[b]F had $F_{obs} > F_{crit}$ and $P < 0.05$. This implied that only the peak areas of these PAHs due to hexane were significantly greater than when extracting using the hexane/THF mixture.

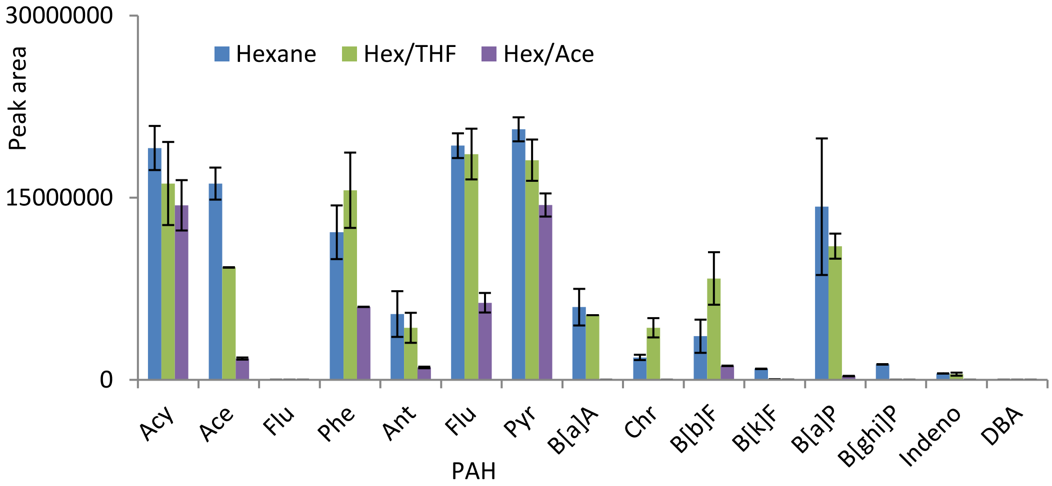


Figure 27: Peak areas representing the amounts of the 16 PAHs extracted by different solvents (n = 3, SD). Acy (Acenaphthylene); Ace (Acenaphthene); Flu (Fluorene); Phe (Phenanthrene); Ant (Anthracene); Pyr (Pyrene); Chr (Chrysene)

It appeared that the extracting power of hexane and the hexane/THF mixture was similar at 95% significance level for most PAHs. The generally enhanced effect of hexane on the sludge CRM (while the effect of using the hexane/THF reduces) might be related to the water content and particle size of the sludge CRM compared to the spiked soil sample that had been made slurry with water. Thus, the impact of the polar THF on extraction becomes less important for dried samples. Such is the observation with the impact of acetone in the hexane/acetone mixture. The results of this optimisation step show that both hexane and the hexane/THF mixture can be used to extract PAHs from a sludge sample. Further extractions were therefore done using a hexane/THF (4:1 v/v) solution.

4.4.3 Selection of the membrane acceptor solvent

The ANOVA results showed that in Figure 28, hexane performed better than heptane and toluene in that order as an acceptor phase for LMW PAHs specifically naphthalene, acenaphthylene and acenaphthene. The same behaviour was observed for pyrene. For most MMW and HMW PAHs, heptane became dominant as an acceptor phase especially with the transfer of chrysene and the four PAHs containing a five-membered ring. Hexane was then accepted as the accepting solvent and used for further studies.

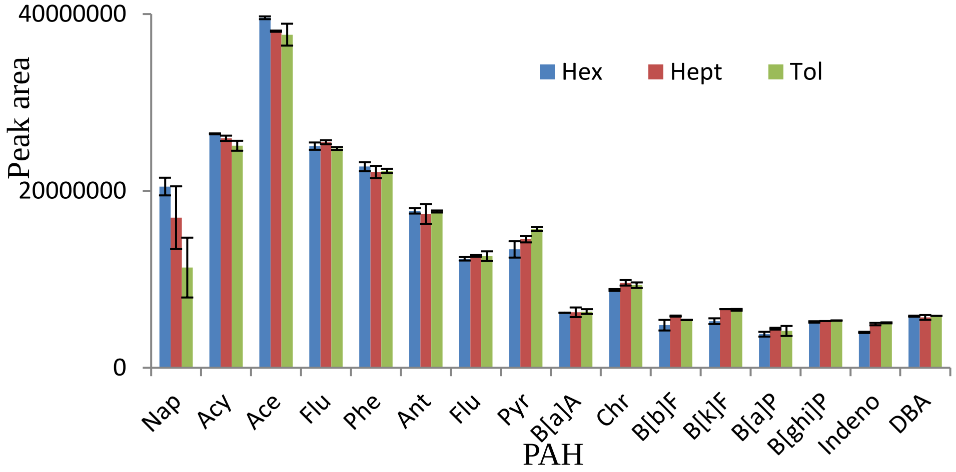


Figure 28: PAH peak areas for different membrane acceptor solvents (n = 3, SD). Nap (Naphthalene); Acy (Acenaphthylene); Ace (Acenaphthene); Flu (Fluorene); Phe (Phenanthrene); Ant (Anthracene); Pyr (Pyrene); Chr (Chrysene)

4.4.4 Extraction efficiency across the membrane

The extent of the transfer of PAHs across the membrane, assuming that the PAHs are now in the Soxhlet flask, is summarised in Table 16. The results were obtained by extracting PAHs from a 1000 ng·mL^{-1} spiked hexane/THF solution mixture placed in the Soxhlet flask while refluxing at 60°C. The EFs ranged from 8-15. The average value was 12. This value was less than the value obtained during optimisation of the MASE-MIP technique (EF = 15.5) in which the PAHs were spiked in water. The PAHs are more soluble in hexane and are expected to stay in solution rather than transfer across the membrane.

4.4.5 Efficiency of the SE-MASE-MIP method

An initial visual assessment of the chromatograms obtained through Soxhlet extraction only and through the developed method show that the developed method is very effective in eliminating the matrix effects (Figures 29). Severe baseline drift is observed for the Soxhlet extract chromatogram as shown in Figure 29(b). The effect of baseline fluctuations is more important especially for quantitation of analytes that exist in trace levels where it becomes difficult to identify the small peaks. In Figure 30, the chromatogram due to the 128-quantitation ion appears to refer to several fragments that might belong to other compounds. Its impact is more pronounced in the chromatogram from the Soxhlet extract that was injected directly into the GC-TOF/MS system. In the chromatogram obtained by injecting the extract from the developed method, only the effect of the 128-quantitation ion is observed at the retention times of the LMW PAHs. The insert in Figure 31 shows where the peaks for the 276 and 278 quantitation ions for B[ghi]P, indeno and DBA appear. Generally, the developed SE-MASE-MIP technique is effective in eliminating matrix effects from a sludge sample. A chromatogram showing retention times and quantitation ions for the 16 PAH standards is shown in Figure 32.

Table 16 shows the efficiency results where extraction was done from spiked hexane (the extraction solvent), which indicate a general reduction in transfer of PAHs compared to when the PAHs are dissolved in aqueous solution. This is expected because hydrophobicity enhanced transfer across the membrane into the non-polar acceptor phase. The average extraction efficiency was 60% with EFs ranging from 3-23.

Table 16: EFs across a membrane placed in a spiked extraction solvent in the Soxhlet extractor. Conditions: 80 mg MIP, 1000 ng·mL^{-1} spiking, 60°C, three-hour extraction (n = 3)

PAH	EF	%RSD
Naphthalene	10	14.8
Acenaphthylene	12	9.3
Acenaphthene	12	7.4
Fluoranthene	12	9.1
Phenanthrene	11	10.8
Anthracene	14	7.6
Fluorene	13	8.0
Pyrene	10	10.1
B[a]A	13	8.4
Chrysene	12	23.0
B[b]F	9	21.3
B[k]F	11	7.5
B[a]P	14	20.8
B[ghi]P	16	16.4
Indeno	8	3.0
DBA	15	5.8

The extraction efficiency of the entire SE-MASE-MIP system dropped significantly to 17-48% (Table 17). This was expected as extractions were optimised using sludge CRM, which also caters for matrix effects. These efficiency vales equated to EF values that were all below 1. Heptane has a density of 0.685 g·mL^{-1} at 20°C. Efficiency was obtained by extracting and transferring PAHs from a 1 g sludge CRM using SE-MASE-MIP and eluting and concentrating them into 1 mL of heptane. The reported EF values were therefore considered a fair result. They were applied in sludge PAH quantitation calculations.

Table 17: Extraction efficiency of PAHs from a sludge CRM using the developed SE-MASE-MIP technique (n = 3, SD)

PAH	**In 1 mL eluent (µg·mL^{-1})**		**Extraction efficiency**	**Enrichment factor**
	Theoretical (CRM values)	**Experimental**		
Naphthalene	0.33 ± 0.15	0.11 ± 0.004	34.6 ± 1.27	0.5 ± 0.02
Acenaphthylene	0.2	0.06 ± 0.010	29.7 ± 4.86	0.4 ± 0.07
Acenaphthene	0.1 ± 0.04	0.02 ± 0.003	22.1 ± 2.89	0.3 ± 0.04
Fluorene	0.19 ± 0.06	0.07 ± 0.007	37.2 ± 3.90	0.5 ± 0.06
Phenanthrene	1.04 ± 0.27	0.38 ± 0.027	36.4 ± 2.64	0.5 ± 0.04
Anthracene	0.17 ± 0.06	0.03 ± 0.003	16.9 ± 2.00	0.2 ± 0.03
Fluoranthene	1.81 ± 0.45	0.52 ± 0.086	28.9 ± 4.76	0.4 ± 0.07
Py	1.53 ± 0.47	0.28 ± 0.038	18.3 ± 2.47	0.3 ± 0.04
B[a]A	0.66 ± 0.24	0.23 ± 0.019	34.5 ± 2.95	0.5 ± 0.04
Ch	0.84 ± 0.18	0.12 ± 0.007	14.5 ± 0.78	0.2 ± 0.01
B[b]F	0.95 ± 0.27	0.33 ± 0.056	34.4 ± 5.92	0.5 ± 0.09
B[k]F	0.45 ± 0.10	0.18 ± 0.012	39.9 ± 2.74	0.6 ± 0.04
B[a]P	0.59 ± 0.14	0.24 ± 0.036	39.9 ± 6.04	0.6 ± 0.09
B[ghi]P	0.62 ± 0.31	0.24 ± 0.046	38.6 ± 7.42	0.6 ± 0.11
Indeno	0.58 ± 0.14	0.19 ± 0.092	32.5 ± 15.87	0.5 ± 0.23
DBA	0.07	0.03 ± 0.010	47.9 ± 14.11	0.7 ± 0.21

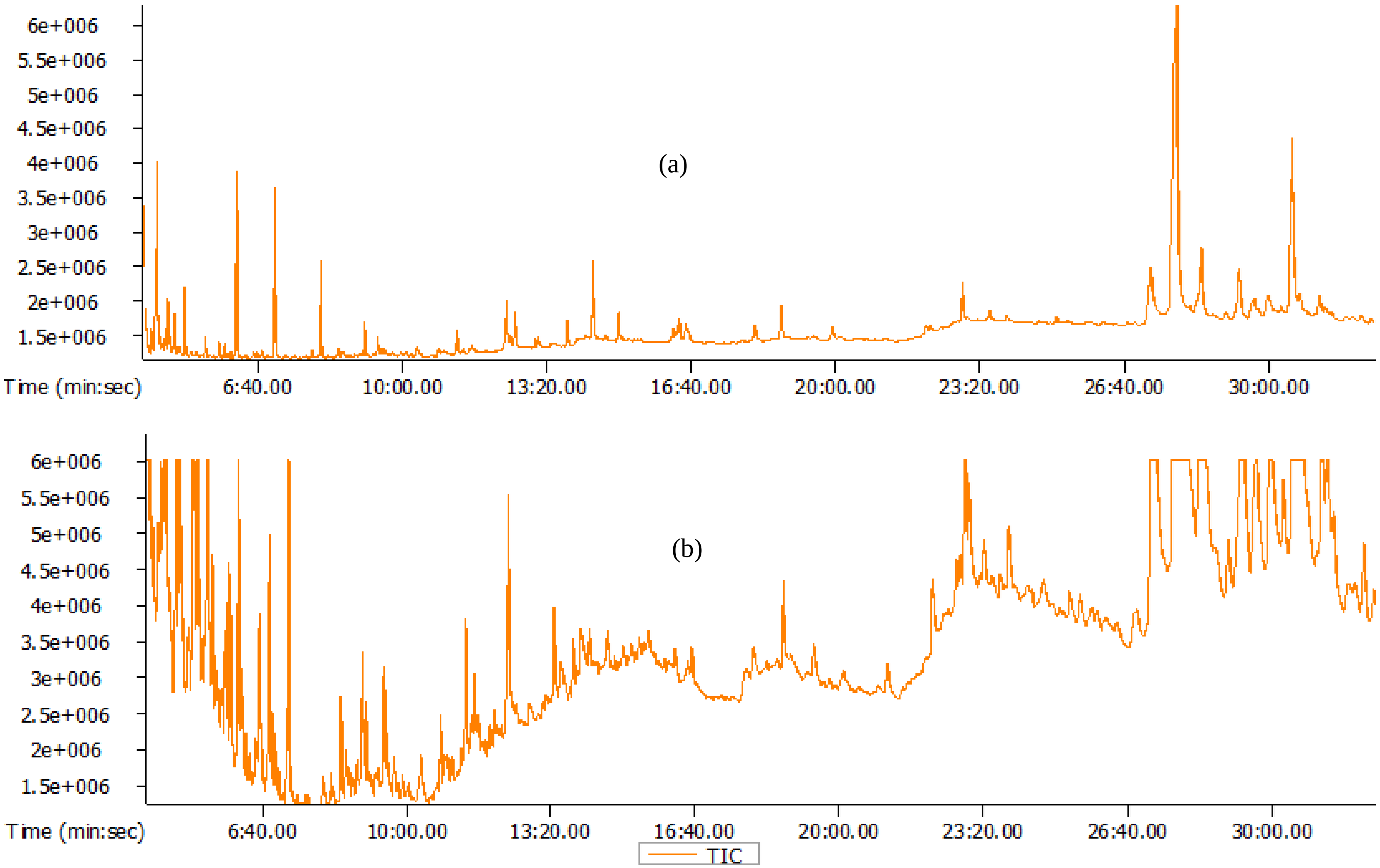


Figure 29: Total ion chromatograms to show efficiency of the developed method (a) against the Soxhlet extraction technique without a further clean-up step (b)

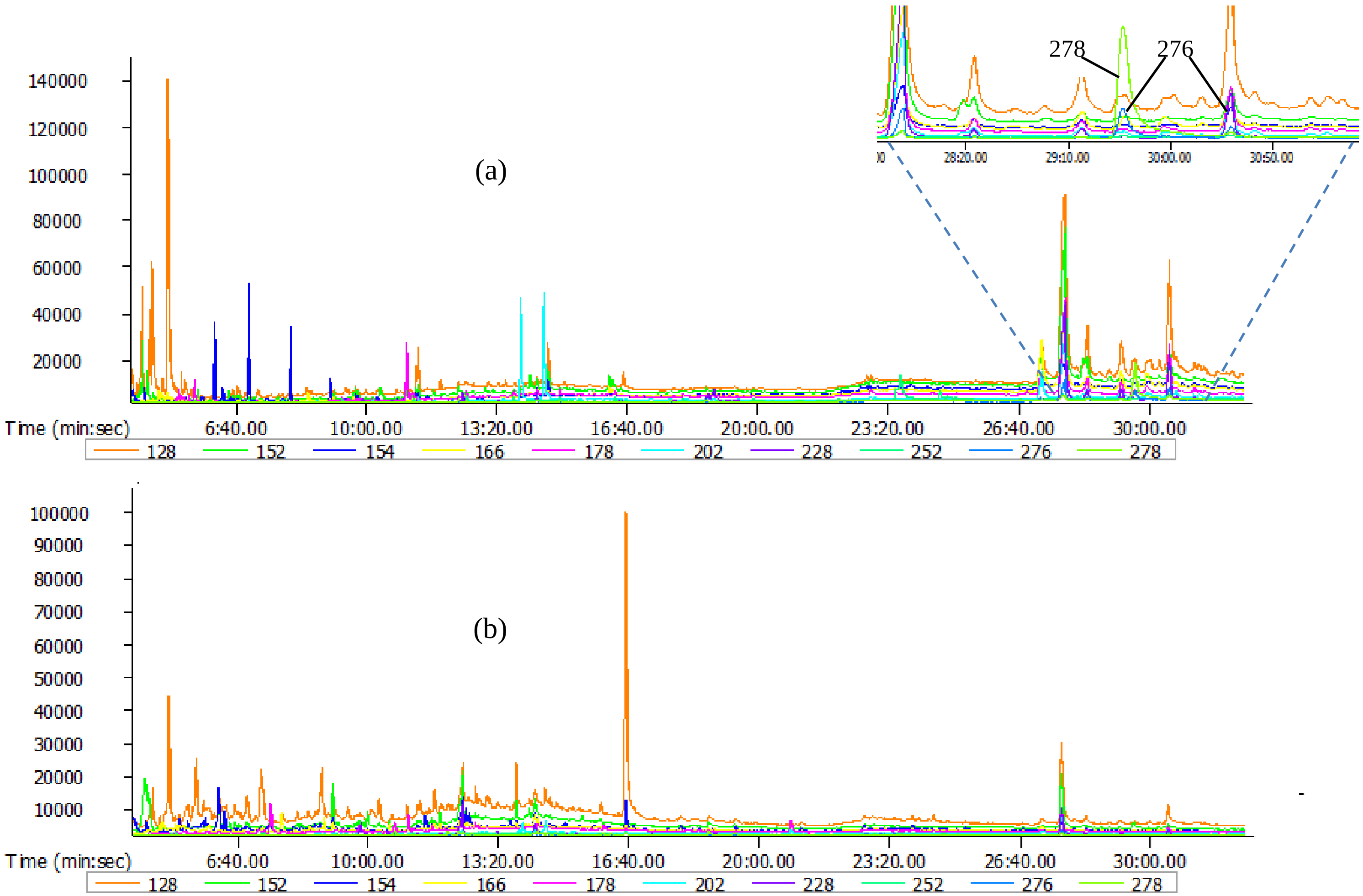


Figure 30: Specific quantitation ion chromatograms for the developed method (a) and the Soxhlet extraction only (b)

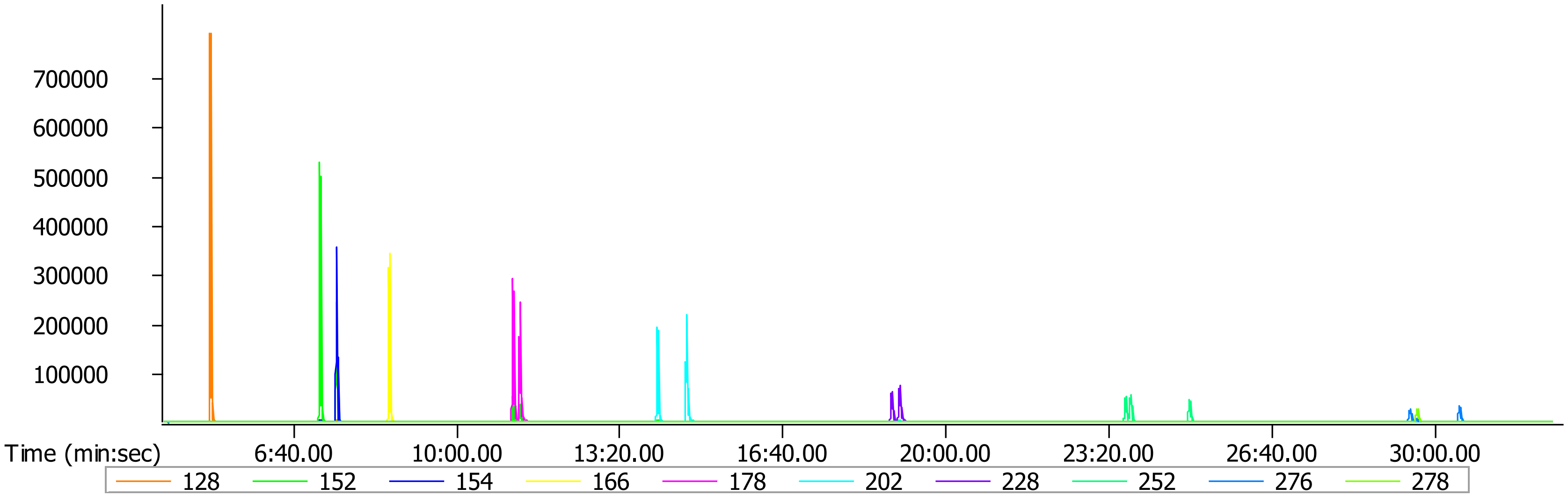


Figure 31: Chromatogram showing the peaks of the 16 PAHs. The values 128-278 represent the quantitation ions

4.4.6 SE-MASE-MIP limits of detection and quantitation

Table 18 shows the detection and quantitation limits for the developed SE-MASE-MIP technique. The MDL and MQL values were affected by the EF values resulting in method values higher than the instrument values. However, the limits were still in the nanogram level.

Table 18: Method detection and quantitation levels for the developed SE-MASE-MIP technique (n = 3, RSD < 15%)

PAH	Limit of detection ($ng \cdot mL^{-1}$)	Limit of quantitation ($ng \cdot mL^{-1}$)	EF	MDL ($ng \cdot mL^{-1}$)	MQL ($ng \cdot mL^{-1}$)
Naphthalene	0.07	0.21	0.5	0.14	0.42
Acenaphthylene	0.26	0.85	0.4	0.60	1.96
Acenaphthene	0.39	1.30	0.3	1.21	4.02
Fluoranthene	0.49	1.62	0.5	0.90	2.98
Phenanthrene	0.61	2.02	0.5	1.15	3.80
Anthracene	0.79	2.61	0.2	3.21	10.59
Fluorene	3.33	11.0	0.4	7.88	26.04
Pyrene	0.88	2.91	0.3	3.29	10.88
B[a]A	2.05	6.78	0.5	4.06	13.43
Chrysene	2.03	6.69	0.2	9.56	31.51
B[b]F	2.46	8.11	0.5	4.89	16.11
B[k]F	2.50	8.25	0.6	4.28	14.13
B[a]P	7.50	24.8	0.6	12.86	42.53
B[ghi]P	3.75	12.4	0.6	6.64	21.96
Indeno	5.36	17.7	0.5	11.27	37.22
DBA	4.55	15.0	0.7	6.49	21.41

4.4.7 Application of the SE-MASE-MIP technique in waste water sludge

The results of using the developed SE-MASE-MIP technique in the quantitation of total PAHs in sludge samples obtained from the Goudkoppies and the Northern Works WWTPs, Johannesburg, South Africa are presented in Table 19. Generally, HMW PAHs appeared in larger quantities than LMW PAHs in both WWTPs.

Northern Works WWTP recorded the highest amount of indeno (2.82 $mg \cdot kg^{-1}$ dw), while the highest molecular weight DBA with a value of 5.40 $mg \cdot kg^{-1}$ was the prominent PAH in Goudkoppies WWTP sludge samples. Overall, the five- and six-membered ring PAHs contributed 53% and 59% of the total PAH content in sludge samples from Northern Works and Goudkoppies WWTPs respectively. Similar trends have been reported by other publications (Hafidi et al., 2008) These PAHs have very low solubility (0.01-0.0026 ng L^{-1}) and are expected to concentrate in sludge during sewage treatment. These results agree with the waste water quantitation results (Section 4.3.7) in which all the HMW PAHs were below the detection limits of the MASE-MIP technique.

The total content of the 16 US EPA priority PAHs for sludge samples from the two WWTP were above the maximum total concentrations permitted by the EU, Denmark and Sweden (6 $mg \cdot kg^{-1}$ and 3 $mg \cdot kg^{-1}$ respectively). The total PAH content is at the edge of the 9.5 $mg \cdot kg^{-1}$ limit set by the USA. The implication of these results is that the PAH content of the two WWTPs has exceeded international standards and will continue to rise as long as there is no legislature or sludge treatment processes that aim to reduce the release of PAHs (and other POPs) back into the environment. Furthermore, the total concentrations of the carcinogenicity of the seven PAHs, namely, B[a]A, chrysene, B[b]F, B[k]F, B[a]P, indeno and DBA, were found to be 4.48 $mg \cdot kg^{-1}$ and 6.73 $mg \cdot kg^{-1}$ for Northern Works and Goudkoppies WWTPs respectively.

B[a]P, which is regarded as the most carcinogenic PAH, contributed only 13.6% and 1.6% of the total carcinogenic PAH content in the two WWTPs respectively. Some studies have reported up to 27% contribution of B[a]P (Cai et al., 2007). Dai et al. (2007) reported as high as 6.1 $mg \cdot kg^{-1}$ for B[a]P. It should, however, be noted that the recorded amounts of B[a]P at Northern Works and Goudkoppies WWTPs sludge samples were below the maximum permissible limits for land sludge application. China, for example, has set 3 $mg \cdot kg^{-1}$ as its limit. The potential risks associated with the use of sludge from these WWTPs as a soil amendment is therefore a cause of concern and the concentration of PAHs in waste water sludge reported in this study cannot be ignored. More research is underway in which the seasonality of the sludge PAH content is being investigated.

Table 19: Method detection and quantitation levels for the developed SE-MASE-MIP technique (n = 3, RSD < 18%)

PAH	**Amount of PAHs in sludge ($\mu g \cdot g^{-1}$ d.w)**	
	Northern Works	**Goudkoppies**
Naphthalene	3.04	0.81
Acenaphthylene	0.003	0.03
Acenaphthene	0.59	1.75
Fluoranthene	0.03	0.52
Phenanthrene	ND	ND
Anthracene	0.01	0.04
Fluorene	0.003	0.43
Pyrene	0.02	0.44
B[a]A	0.36	0.36
Chrysene	0.19	0.01
B[b]F	ND	0.03

PAH	Amount of PAHs in sludge (µg·g^{-1} d.w)	
	Northern Works	**Goudkoppies**
B[k]F	0.02	0.02
B[a]P	0.61	0.11
B[ghi]P	0.26	0.07
Indeno	2.82	0.73
DBA	1.09	5.40
∑PAH	**9.03**	**10.75**

4.4.8 Comparative studies

The performance of SE-MASE-MIP compared to other techniques for quantitating PAHs in waste water sludge is shown in Table 20. Of the 12 WWTPs sludge samples investigated by Abadi et al. (2005) only 3% of the samples had a total PAH concentration above 6 mg·kg^{-1}. However, the South African sludge content is difficult to compare with others reported by different authors since most of them are not homogeneous in respect to the number of PAH compounds they represent.

4.4.9 Source of PAHs in sludge samples

The results of the ratio calculations of the PAHs with 178, 202, 228 and 276 molecular weights are summarised in Table 21. Phenanthrene was not detected in the two WWTPs sludge samples giving an anthracene to anthracene plus phenanthrene (Ant/178) of 1. This value was higher than the 0.10 threshold ratio, which indicates that the sources of PAHs in sludge samples are pyrogenic. The ratio of B[a]A to B[a]A plus chrysene (B[a]P/228), the ratio of the fluoranthene to fluoranthene plus pyrene and that of the indeno to indeno plus B[ghi]P were all higher than their respective transition points. This was confirmation that the quantified PAHs in sludge originate from combustion of solids such wood, rubber and coal. These observations might imply that the chemical and maybe food industries rather than the transport industry are the major contributors of PAHs in Johannesburg. The ratio values were comparable for the two WWTPs. Further studies can be done to pinpoint the sources considering that Northern Works WWTP receives sewage mainly from domestic and food industries while Goudkoppies receives sewage mainly from the chemical industry.

Table 20: PAH ratios for source identification

WWTP	Ant/178 ratio	Fln/202 ratio	B[a]A/228	Indeno/276
Northern Works	1	0.86	0.65	0.91
Goudkoppies	1	0.51	0.98	0.91

Table 21: Comparison of extraction techniques for sludge PAHs

Technique	**No. of PAHs investigated**	**MDL ($ng \cdot g^{-1}$ d.m)**	**Instrument**	**∑(PAH) ($mg \cdot kg^{-1}$ d.m)**	**Country**	**Reference**
SE-MASE-MIP	16	0.14-12.86	GC-TOF/MS	9.03-10.75	South Africa	Current
Electrokinetic capillary, chromatography	12	1.83-100.76	HPLC-FLD	< 2.5	Spain	(Alzola et al., 2008)
USE	9	NR	GC-qTOF/MS	0.03-7.35	Spain	(Abad et al., 2005)
Soxhlet extraction followed by rotovapor concentration	16	20	HPLC-FLD	14-31	France	(Blanchard et al., 2004)
USE	16	4.1-12.1	HPLC-DAD/FLD	1.44-1.26	Italy	(Busetti et al., 2006)
US EPA 3540C	16	0.21-1.3	GC-qTOF/MS	1.4-33	China	(Cai et al., 2007)
Soxhlet extraction followed by SPE	16	–	GC-qTOF/MS	2.5-25.9	China	(Dai et al., 2007)
Soxhlet extraction followed by rotovapor concentration	16	–	GC-TOF/MS	1.4-16.7	China	(Ning et al., 2014)

4.4.10 Quantitation of bioavailable PAHs in sludge

The bioavailable fraction of the PAHs in sludge was dependent on the amount of dissolved sludge as shown for indeno in Figures 32 and 33. The results show that the amount of PAHs that dissolve into an aqueous solution increases with the amount of sludge that is in contact with water. Table 22 shows PAHs that displayed bioavailability when in contact with an aqueous solution. There seems to be a strong linear relationship between bioavailability and PAH total content in sludge as only those PAHs that existed in larger quantities were identified and quantified in the aqueous solution.

The observations in Figure 33 and Table 22 imply that as more sludge contaminated with high quantities of PAHs come into contact with water, more PAHs are likely to dissolve in the aquatic system. This is worrying especially for sludge used for soil amelioration. However, the results in Figure 34 and Table 21 show that even though PAH release into aqueous solutions increases with an increase in amount of sludge, the overall bioavailability tends to decrease. In reality, overall bioavailability should remain the same as long as the volume of aqueous media increases proportionality with the amount of sludge. Overall bioavailability is very low. This could imply that these compounds in sludge may pose minimal risk to the environment. More studies on bioavailability are continuing to understand the influence of exposure aqueous volume.

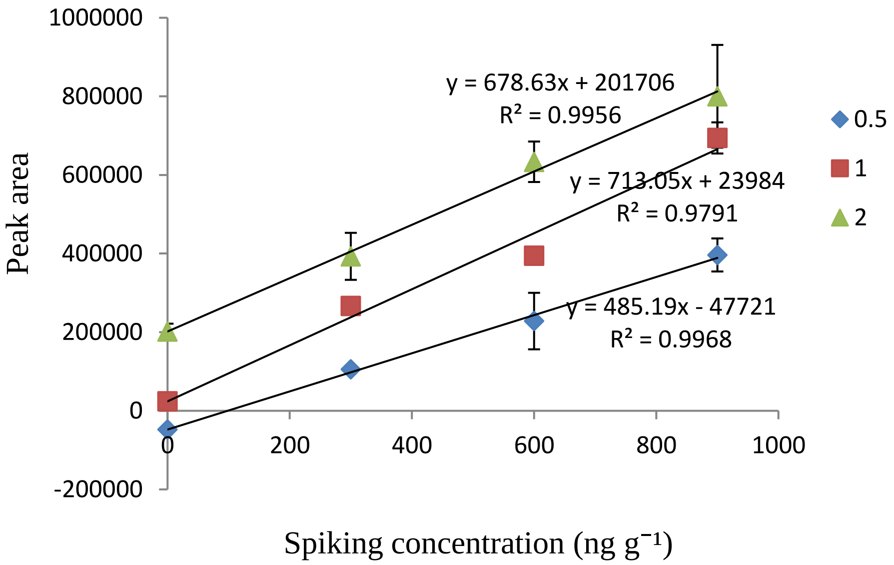


Figure 32: Peak areas showing the effect of increasing amount of dissolved sludge from 1-4% w/v (0.5-2 g in 50 mL) on the bioavailability of indeno. Conditions: Shaking sludge in 50 mL of deionised water for 16 hours and extracting using the developed MASE-MIP technique. Result for Northern Works WWTP sludge sample

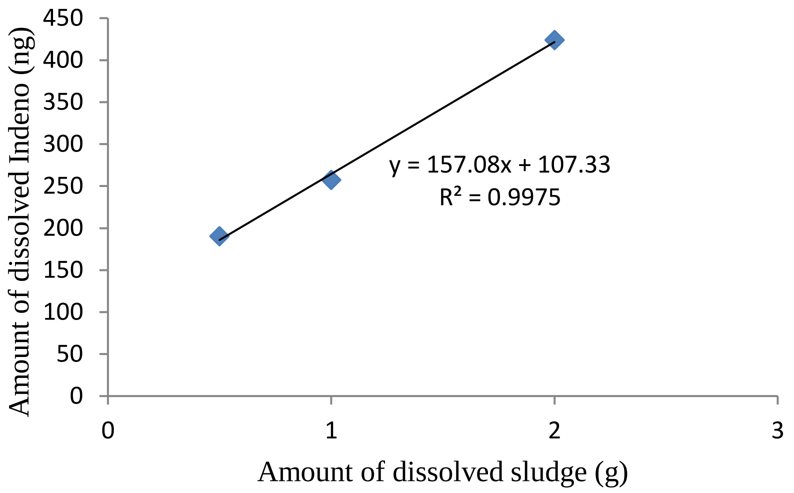


Figure 33: Effect of amount of sludge on release of indeno into an aqueous solution. Conditions: Dissolve 0.5-2 g of sludge in 50 mL of water and extract using the developed MASE-MIP technique. Result for Northern Works WWTP sludge sample

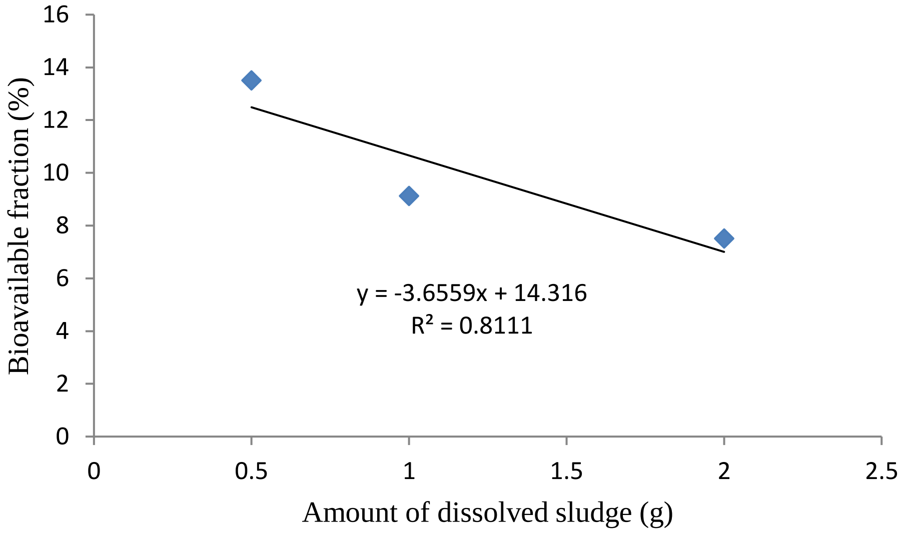


Figure 34: Effect of amount of sludge on bioavailability of indeno expressed as a fraction of total sludge content of indeno. Conditions: Dissolve 0.5-2 g of sludge in 50 mL of water and extract using the developed MASE-MIP technique. Result for Northern Works WWTP sludge sample

Another important observation from Table 22 is the difference in the bioavailability of pyrene and B[k]F in sludge samples from Northern Works WWTP. The two PAHs had the same total amount in sludge, yet it was only pyrene that was quantified after shaking sludge in water. This can be explained in terms of water solubility and organic content of the sludge. This behaviour was also observed for naphthalene and acenaphthene, which had the highest bioavailability values. However, for some PAHs, the bioavailability was not affected by the location where sludge samples were collected. In Table 22, the B[a]A that was found in equal total amounts in the two WWTPs sludge samples (0.36 mg·kg^{-1} dw) also displayed similar bioavailability values of 0.4% and 0.5%.

Table 22: Bioavailability of PAHs in sludge samples from Northern Works WWTP (n = 3, RSD < 21%)

PAH	Amount of sludge (ng)	Total amount of PAH (ng)		Dissolved amount (ng)		Bioavailability (%)	
		Northern Works	Goudkoppies	Northern Works	Goudkoppies	Northern Works	Goudkoppies
Naphthalene	0.5	1 521	407	72.76	33.96	4.8	8.4
	1.0	3 043	813	74.70	43.66	2.5	2.7
	2.0	6 085	1 626	101.90	48.51	1.7	1.5
Acenaphthene	0.5	292.6	877	NQ	105	–	12.0
	1.0	585.2	1 754	3.163	135	0.54	7.7
	2.0	1 170.0	3 507	6.020	150	0.51	4.3
Fluorene	0.5	13.70	258.5	ND	NQ	–	–
	1.0	27.39	517.0	NQ	0.24	–	0.05
	2.0	54.78	1 034.0	NQ	0.21	–	0.02
Anthracene	0.5	6.127	19.3	ND	0.25	–	1.29
	1.0	12.250	38.6	NQ	0.40	–	1.03
	2.0	24.510	77.2	0.9484	0.03	7.7	0.03
Pyrene	0.5	9.749	221.3	NQ	0.26	–	0.12
	1.0	19.500	442.7	0.756	0.39	3.9	0.09
	2.0	39.000	885.4	0.831	1.58	2.1	0.18
B[a]A	0.5	178.4	178.4	0.60	0.89	0.4	0.50
	1.0	356.8	356.8	–	0.59	–	0.17
	2.0	713.7	713.7	1.99	0.74	0.3	0.10

PAH	**Amount of sludge (ng)**	**Total amount of PAH (ng)**		**Dissolved amount (ng)**		**Bioavailability (%)**	
		Northern Works	**Goudkoppies**	**Northern Works**	**Goudkoppies**	**Northern Works**	**Goudkoppies**
Chrysene	0.5	94.06	3.67	NQ	ND	–	–
	1	188.10	7.34	1.482		0.8	
	2	376.20	14.68	1.620		0.4	
B[ghi]P	0.5	132.4	34	NQ	ND	–	–
	1	265.0	69	NQ	NQ	–	–
	2	529.7	138	0.7649	1.20	0.14	0.87
Indeno	0.5	1 410	364	190	ND	13.5	–
	1	2 820	731	258	ND	9.1	–
	2	5 640	1 463	424	NQ	7.5	–
DBA	0.5	542.6	2 699	NQ	124.8	–	4.62
	1	1 085.0	5 399	27.1	27.1	2.5	0.50
	2	2 171.0	10 798	41.2	50.2	1.9	0.47

ND – not detected (below detection limit) *NQ – detected but not quantified (below quantitation limit)*

5 CONCLUSIONS

A MIP combination that consists of B[k]F and indeno mixed at 1:1 (w/w) was successfully synthesised for the effective extraction of 16 US EPA priority PAHs from aqueous solutions. The polymer combination had an adsorption capacity of $5.19 \pm 0.392\ \mathrm{mg \cdot g^{-1}}$. The MIP combination displayed a broad selectivity towards the 16 PAHs with no imprinting effect or template bleeding observed. The average binding capacity towards 16 PAHs was $317\ \mathrm{\mu g \cdot g^{-1}}$. The adsorption and kinetic studies showed that the adsorption process results in the formation of a monolayer that attains a maximum capacity with an increase in the concentration of the analytes in the donor phase. These results were an indication that extraction of PAHs occurs by binding on the engineered MIP cavities. Each gram of a polymer was estimated to contain 1.53×10^{19} cavities. Cavity efficiency in adsorbing the PAH molecules was calculated at 128%, thus implying that a small amount of PAHs was adsorbing on the hydrophobic backbone of the polymer.

A MASE technique was combined with the synthesised MIP to produce a novel technique for extracting PAHs from waste water samples. Under optimised conditions of the developed technique, the 16 US EPA priority PAHs were extracted with an average efficiency of 77.4%. The detection limit of the technique towards the 16 PAHs ranged from $0.1\ \mathrm{ng \cdot mL^{-1}}$ to $0.45\ \mathrm{ng \cdot mL^{-1}}$. The developed method was successfully applied in the quantitation of PAHs in effluent waste water samples from Goudkoppies WWTP. LMW PAHs were detected in the concentration range between $3.4\ \mathrm{ng \cdot mL^{-1}}$ and $1829.8\ \mathrm{ng \cdot mL^{-1}}$.

The synthesised MIP was also used in developing another novel technique, namely, Soxhlet extraction membrane-assisted solvent extraction MIP (SE-MASE-MIP), to extract total PAHs in sludge samples. The developed technique is a promising innovative prospect that we have dubbed “Coming back of Soxhlet” in the study of PAHs in complex samples such as sludge samples. Using the developed technique, the 16 US EPA priority PAHs were quantified in sludge samples with precision values for triplicate quantitation recorded at RSD < 18%. The total PAH content for the two WWTPs under study were found to be higher than the maximum total concentration of $6\ \mathrm{mg \cdot kg^{-1}}$ permitted by the EU. The calculated ratios of the PAH pairs with molecular weights of 128, 202, 228 and 276 were indicative that these sludge PAHs are from pyrogenic sources such as the burning of coal and wood as well as incineration of solid waste material.

The MASE-MIP technique was also applied to determine the bioavailable fraction of sludge PAHs. The maximum bioavailability value of sludge PAHs, when sludge was dissolved in water in the 0.5-2% (w/v), was 4.8%. Generally, HMW PAHs had the lowest bioavailability values. It was also observed that the amount of PAHs that dissolve into the aqueous phase increases with an increase in the amount of sludge applied as a soil ameliorant.

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