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Development of novel extraction techniques for the determination of polycyclic aromatic hydrocarbons in wastewater and sludge



Somandla Ncube

A thesis submitted to the Faculty of Science, University of the Witwatersrand in fulfilment of the requirements for the degree of Doctor of Philosophy

February 2018

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any university

S.Ncube

Somandla Ncube

23rd day of February 2018 at Johannesburg

PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following papers in which the candidate was the principal author:

1. **Recent advances in the adsorbents for isolation of polycyclic aromatic hydrocarbons (PAHs) from environmental sample solutions**
   Somandla Ncube, Ewa Cukrowska, Luke Chimuka
   Published in *Trends in Analytical Chemistry 99 (2018) 101- 116*
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2. **Synthesis and characterization of a molecularly imprinted polymer for the isolation of the 16 US-EPA priority polycyclic aromatic hydrocarbons (PAHs) in solution**
   Somandla Ncube, Phumlile Kunene, Nikita T. Tavengwa, Hlanganani Tutu, Heidi Richards, Ewa Cukrowska, Luke Chimuka.
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3. **Development of a single step membrane assisted solvent extraction-molecularly imprinted polymer technique for extraction of PAHs in wastewater followed by gas chromatography mass spectrometry determination**
   Somandla Ncube, Nikita Tavengwa, Ewa Cukrowska, Luke Chimuka
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4. **Development and optimisation of a novel three-way extraction technique based on combination of Soxhlet extraction, membrane assisted solvent extraction and molecularly imprinted polymer using wastewater PAHs as model compounds**
   Somandla Ncube, Goitsemang Lekoto, Ewa Cukrowska and Luke Chimuka
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CANDIDATE CONTRIBUTION TO ARTICLES

1. Recent advances in the adsorbents for isolation of polycyclic aromatic hydrocarbons (PAHs) from environmental sample solutions

   Somandla Ncube, Ewa Cukrowska, Luke Chimuka

   Candidate contribution: Principal author, conceptualized the idea, planned and wrote the review. Co-authors reviewed the manuscript.

2. Synthesis and characterization of a molecularly imprinted polymer for the isolation of the 16 US-EPA priority polycyclic aromatic hydrocarbons (PAHs) in solution

   Somandla Ncube, Phumlile Kunene, Nikita T. Tavengwa, Hlanganani Tutu, Heidi Richards, Ewa Cukrowska, Luke Chimuka

   Candidate contribution: Principal author, did experiments and wrote the manuscript. Co-authors helped with analysis of results. Phumlile Kunene was an Honours student who assisted with experimentation.

3. Development of a single step membrane assisted solvent extraction-molecularly imprinted polymer technique for extraction of PAHs in wastewater followed by gas chromatography mass spectrometry determination

   Somandla Ncube, Nikita Tavengwa, Akhona Soqaka, Ewa Cukrowska, Luke Chimuka

   Candidate contribution: Principal author, did experiments and wrote the manuscript. Co-authors helped with analysis of results. Akhona Soqaka was an intern who assisted with experimentation

4. Development and optimisation of a novel three-way extraction technique based on combination of Soxhlet extraction, membrane assisted solvent extraction and molecularly imprinted polymer using wastewater PAHs as model compounds

   Somandla Ncube, Goitsemang Lekoto, Ewa Cukrowska and Luke Chimuka

   Candidate contribution: Principal author, involved in conceptualizing the idea, did experiments, analyzed data and wrote the review. Co-authors helped with analysis of results

CONFERENCE OUTPUTS

1. Analysis of total sludge PAHs using a novel single-step extraction technique based on combination of Soxhlet extraction, membrane assisted solvent extraction and molecularly imprinted polymer (SE-MASE-MIP) (ChromSA student seminar, University of Pretoria, Pretoria, 08 September 2017)

2. Statistical method validation for test laboratories (Holiday Inn, East Rand, Johannesburg, March 2017)

3. Optimization of the Soxhlet apparatus for extraction of PAHs in sludge (Water Research Commission of South Africa, Pretoria, South Africa, Feb 2016)

4. Multivariate optimization of the hollow fibre liquid phase microextraction of muscimol from human urine samples (Chromsaams2016, Vanderbijlpark, Gauteng, 11 – 14 September 2016)

5. Column choices for separation of polar hallucinogenic alkaloids (42nd International Convention of the South African Chemical Institute, Durban, South Africa, 29 Nov - 04 Dec 2015)

6. Liquid phase microextraction of hallucinogenic compounds from biological samples using a single hollow fibre followed by chromatographic determination: A preliminary study (ANALITIKA 2014, Parys, South Africa, 07 – 12 Sept 2014)

OTHER RESEARCH OUTPUTS

1. S. Ncube, A. Poliwoda, H. Tutu, P. Wieczorek, L. Chimuka; Multivariate optimization of the hollow fibre liquid phase microextraction of muscimol in human urine samples. J. Chrom. B. 1033-1034 (2016) 372-381
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2. S. Ncube, A. Poliwoda, E. Cukrowska, P. Wieczorek, L. Chimuka; Comparative Study of Different Column Types for the Separation of Polar Basic Hallucinogenic Alkaloids. S. Afr. J. Chem. 69 (2016) 189-195
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ABSTRACT

This thesis presents development, optimization and application of two novel extraction techniques for the determination of total polycyclic aromatic hydrocarbons in wastewater and wastewater sludge. PAHs belong to an important class of persistent organic pollutants that are commonly found in the environment at low concentrations. The sources of these compounds include anthropogenic activities such as chemical industries, combustion and agriculture activities. Owing to their potential carcinogenicity, mutagenicity and teratogenicity, it is important to determine polycyclic aromatic hydrocarbons in several matrices, particularly complex ones such as wastewater and wastewater sludge. To date, very few studies have looked at the detailed presence and distribution of these compounds within wastewater and wastewater sludge in South Africa and internationally.

Although the official methods approved by international bodies like the US-EPA are able to extract polycyclic aromatic hydrocarbons in wastewater sludge, all of them involve two separate steps. The steps generally include extraction of the target compounds along with other unwanted compounds followed by the clean-up step before analysis. A two-step approach increases the errors in measurements and it is time consuming. Therefore, the primary goal of this study was to develop, optimize and validate novel extraction techniques for selective extraction of polycyclic aromatic hydrocarbons from wastewater and wastewater sludge. The novelty of these techniques is that they combine two or three extraction steps into one thereby minimizing sample turnaround time and method errors that might arise due to non-ideal analytical behaviour of the setup.

The first part of the work involved synthesis and characterization of a molecularly imprinted polymer that would enhance selectivity of extraction of polycyclic aromatic hydrocarbons. Several other adsorbents in the analysis of polycyclic aromatic hydrocarbons have been reported by different researchers. These were presented in this thesis as a review article (**Paper 1**). Key in the synthesis of the polymer was the choice of the appropriate template that could create cavities with

an affinity for all the 16 US-EPA priority polycyclic aromatic hydrocarbons. Several cavity-tuning experiments were done to find the best imprinted polymer. A polymer combination consisting of benzo[k]fluoranthene-imprinted and indeno[1 2 3-cd]pyrene-imprinted polymers mixed at 1:1 (w/w) was successfully screened from these experiments. The polymer mixture showed high selectivity and affinity towards all 16 US-EPA priority polycyclic aromatic hydrocarbons. This work is presented as **Paper 2**. The average extraction efficiency from an aqueous solution was 65 ± 13.3% (n = 16, SD). The maximum binding capacity of the polymer was 5.19 ± 0.392 mg g^{-1} (n = 3, SD) with binding capacities of individual polycyclic aromatic hydrocarbon ranging from 200.8 - 493.9 μg g^{-1}. Batch adsorption and kinetic studies confirmed that the binding of polycyclic aromatic hydrocarbons onto the molecularly imprinted polymer particles resulted in formation of a monolayer and that the binding process was the rate limiting step. The molecularly imprinted polymer performance studies confirmed that the synthesized polymer had an imprinting efficiency of 103.9 ± 3.91% (n = 3, SD). A comparison of the theoretical number of cavities and the experimental binding capacity showed that the overall extent of occupation of the imprinted cavities in the presence of excess polycyclic aromatic hydrocarbons was 128 ± 6.45% (n = 3, SD). The loss of selectivity was estimated at 2.9% with every elution cycle indicating that the polymer can be re-used several times with limited loss of selectivity and sensitivity. The polymer combination was proven to be an effective adsorbent that can be used to isolate all 16 US-EPA priority polycyclic aromatic hydrocarbons in solution.

The combined molecularly imprinted polymer particles were then used to selectively extract the 16 US-EPA priority polycyclic aromatic hydrocarbons during development of two novel techniques for the analysis of these pollutants in wastewater and wastewater sludge. The polymer combination was first used during development of a novel extraction technique referred to as the membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) technique. The approach was to place the MIP particles in a microporous membrane bag of 0.22 μm pore size, 160 μm thickness and 15 mL internal

volume. This work is presented as **Paper 3**. The optimised conditions for the two-way MASE-MIP technique were found to be 25% of dimethyl sulfoxideas an organic modifier in the aqueous sample, 80 mg of MIP particles in the membrane bag, 180 min 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 polycyclic aromatic hydrocarbons and a method detection limit ranging between 0.01 and 0.45 ng mL^{-1}. This developed technique showed remarkable selectivity and extraction capability for the polycyclic aromatic hydrocarbons soluble in wastewater.

The MASE-MIP technique was further combined with Soxhlet extraction to form a three-way extraction technique referred to as the Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique (**Paper 4)**. The SE-MASE-MIP technique combines the extraction efficiency of the Soxhlet extractor, the selectivity of a size exclusion membrane and the specificity of a molecularly imprinted polymer into a single stem format for the extraction of polycyclic aromatic hydrocarbons from wastewater sludge. This technique was optimised for various parameters such as extraction solvent, reflux time and membrane acceptor phase. The developed technique was optimised using a wastewater sludge certified reference material and then tested on real wastewater sludge samples. The extraction efficiencies of the 16 US-EPA polycyclic aromatic hydrocarbons from wastewater sludge samples using the developed technique ranged from 17 to 48% with %RSD values ranging from 0.78 to 18%. The method detection limits ranged from 0.14 to 12.86 ng g^{-1}. Thus, despite relatively low extraction efficiencies, the extraction process was reproducible and showed remarkable selectivity. The developed technique is a promising prospect that can be applied in the analysis of organic pollutants from complex solid samples. In this case, extraction of PAHs from Soxhlet was found to be the rate limiting factor.

The impact of the MASE-MIP and the SE-MASE-MIP techniques was demonstrated in the clean chromatographic patterns generated for both certified reference materials and real samples, indicating an effective removal of impurities

(interferences) for final chromatographic instrumental analysis. The two developed techniques were therefore considered quite novel and innovative for handling complex matrices such as wastewater sludge, where current approved standard methods are still affected by matrix effects and the existence of target compounds in the nano-scale.

DEDICATION

In memory of my beloved late grandmother

Mazizela Ngenge Mnkandla

1924 – 2001

Kukho konke engikwenzayo; khanyisa sibane sikaMazizela

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LIST OF ABBREVIATIONS

CXL	CO_2-expanded liquid
EU	European Union
GC-TOF/MS	Gas chromatography - time-of-flight mass spectrometer
GPC	Gel permeation chromatography
HF-LPME	Hollow fibre liquid phase micro-extraction
HMW	High molecular weight
MAE	Microwave-assisted extraction
MASE	Membrane assisted solvent extraction
MASE-MIP	Membrane assisted solvent extraction-molecularly imprinted polymer
MIP	Molecularly imprinted polymer
PAHs	Polycyclic aromatic hydrocarbons
PLE	Pressurized liquid extraction
QUECHERS	Quick Easy Cheap Effective Rugged Safe
SFE	Supercritical fluid extraction
SLM	supported liquid membranes
SPE	Solid-phase extraction
SPME	Solid phase micro-extraction
US-EPA	United States Environmental Protection Agency
WRC	Water Research Commission
WWTP	Wastewater treatment plant

CHAPTER ONE

1 INTRODUCTION

South Africa is generally lagging behind in the evaluation of organic pollutants in wastewater and/or wastewater sludge from municipal wastewater treatment plants (WWTPs). The Water Research Commission (WRC) has previously supported research on sludge leading to five report volumes that deal with guidelines for the utilisation and disposal of wastewater sludge (WRC, 2009, 2006). However, none of these reports looks at organic pollutants 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 wastewater sludge (Snyman and van der Waals, 2004). This implies that many of the dangers of agronomic sludge due to organic pollutants are unassessed.

Currently South Africa has no legislation concerning organic pollutants in its sludge disposal and wastewater effluent guidelines. This is mainly due to lack of data on the contamination of these samples by organic pollutants. Studies in the northern hemisphere have also observed a similar level of ignorance of potential impacts of unmonitored sludge disposal among European Commission countries (Suciu et al., 2015; Wenzl et al., 2006). The assumption of this research is that this stems from lack of viable quantitation methods for organic pollutants in sludge and hence limited insights on the detrimental effects of sludge used in agriculture or in land refill programmes. Development of viable quantitation approaches for organic pollutants in wastewater and wastewater 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 the development of improved wastewater treatment methods, disposal options and monitoring protocols that will assist in protecting the environment and minimizing human exposure to organic pollutants.

Among trace organic pollutants are a group of compounds with two or more fused aromatic rings called polycyclic aromatic hydrocarbons (PAHs) which have received considerable attention because of their documented potential

carcinogenic, teratogenic and mutagenic nature to human health and persistence in the environment (Chimuka et al., 2015; Singh and Agrawal, 2008). The European Union (EU), for example, has set 6 mg kg^{-1} as the maximum total concentration of their priority PAHs in sewage sludge. Denmark and Sweden have set 3 mg kg^{-1} while United State of America has set 9.5 mg kg^{-1} (Smith, 2009). Knowing the actual concentration of these pollutants is very important in 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 the country's wastewater treatment plants (WWTPs) to remove these compounds during treatment is unknown. It has been observed that 80% of wastewater treatment facilities in South Africa dispose their sewage sludge on dedicated land disposal sites (Snyman and van der Waals, 2004) while the wastewater effluent is pumped into river systems and eventually end up in dams. Unmonitored introduction of PAHs to the environment without fully understanding the chemical composition of the wastewater and sludge can result in adverse consequences in the near future. The long-term impacts of PAHs in the environment cannot be underestimated hence the need for extensive scientific evidence on the sludge PAH content.

The WRC has continued to support research into developing analytical methods to assess the occurrence, fate and environmental risk of organic pollutants in South Africa's WWTPs but not much has been reported on presence of PAHs in wastewater and sludge. The occurrence of selected polybrominated diphenyl ethers, phenols and phthalates esters has 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 wastewater treatment works in Pietermaritzburg (Manickum and John, 2014). A study on the levels of ibuprofen, naproxen and triclosan in several Johannesburg and Durban WWTPs has also been reported (Amdany et al., 2014b; Madikizela and Chimuka, 2016). This research has focussed on the quantitation of PAHs in wastewater effluents and wastewater sludge.

Although PAHs method development and application has been reported in SA, very little has been reported on wastewater sludge. Most of the studies have reported only a portion of the priority PAHs mainly in water and sediment\soil samples (Chimuka et al., 2015). Sludge acts as a sink for many pollutants like PAHs that are not degraded during treatment processes. Analysis of these pollutants is very important in planning future use of end products of wastewater treatment especially sludge for agronomic purposes. However, sludge is one of the most difficult samples to analyze which might explain the limited studies on quantitation of organic pollutants. Development of selective sample preparation techniques remains a priority for an effective quantitation of organic pollutants in these complex samples.

In this project, two novel sample preparation techniques were developed and applied in the analysis of organic pollutants in wastewater and wastewater sludge samples using PAHs as model compounds. The approach was an attempt to minimize the number of analytical steps involved by integrating the extraction and clean-up steps into a single step format for the extraction of PAHs in wastewater 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 extraction from sludge while the combination of the membrane and the MIP was developed for isolation from wastewater. For analysis in wastewater, the set-up was combined into a single entity with the MIP particles placed inside a membrane and used as an advancement of the membrane assisted solvent extraction technique (**Paper 3**). The MIP-carrying membrane was also placed inside a Soxhlet apparatus for analysis of PAHs in wastewater sludge (**Paper 4**). The combination of a Soxhlet extractor, a membrane and a polymer in our approach is the first of its kind for application in complex solid samples such as wastewater sludge. The two developed techniques are unique, simple and selective. They allow for the use of cheap extraction systems to isolate PAHs followed by chromatographic determination.

The applicability of the developed techniques was tested on samples taken from Northern Works and Goudkoppies WWTPs in Johannesburg, South Africa. The premise was to use the two techniques to provide scientific evidence of the presence of PAHs in wastewater and wastewater sludge in South Africa. Evidence of their presence might provide a rationale for considering PAHs and other organic pollutants in legislature and quality assurance systems for wastewater effluent and sewage sludge disposal protocols. The city of Johannesburg was targeted because it has a bulging population, the highest incidence of vehicles in the streets and the busiest airport in Africa coupled with complex restaurants and industrial activities all of which are potential anthropogenic sources of PAHs yet its PAH-sludge content is unknown.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Background

Sample preparation and clean up steps are viewed as of paramount importance prior to analysis of any chemical in aqua and biota samples (Chimuka et al., 2011a; Martín-Esteban, 2016; Ncube et al., 2016; Turiel and Martín-Esteban, 2010). Extraction of trace organics from complex samples (aqueous and solid) has many challenges like low concentrations, matrix interferences and the general similarity of organics. The need for efficient cost-effective sample preparation procedures requires development of efficient, simple and, if possible, solvent-free or solvent-minimized 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. These approaches were non-selective and require a lot of manual shaking. A further clean-up step was then introduced using selective adsorbents that were packed in columns or solid-phase extraction (SPE) cartridges. This approach has been accepted in sample preparation of chemicals in complex samples and is still in widespread use. With the advent of more selective sorbents like MIPs and immunosorbents, the effectiveness of the clean-up step has been greatly improved (Hennio, 1999; Turiel and Martín-Esteban, 2010). Using selective sorbents has the advantage in that only target compounds are favoured during extraction. The effect of interfering compounds is thus reduced.

The effectiveness of the selective sorbents is however still affected especially in the analysis of organics from 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 is most pronounced if the target compound is also hydrophobic in nature 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 in order to prevent the matrix components especially the HMW ones from coming into contact with the sorbent. The matrix compounds that manage to cross the membrane are either too small and become easily washed out of the MIP cavities or their functional groups and structural orientation does not permit their binding. The use of 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 membrane-polymer technique is a prevention-is-better-than-cure approach as matrix components are prevented from adsorbing on the sorbent altogether.

2.2 Polycyclic Aromatic Hydrocarbons

2.2.1 Physico-chemical properties and fate

PAHs also referred to as polynuclear aromatic hydrocarbons or polyaromatic hydrocarbons 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 carbon and hydrogen atoms only. Depending on the number of aromatic rings, the compounds can be classified either as low or high molecular weight PAHs with the former defined by having less than four aromatic rings and the latter having more than four rings. Over a hundred individual and substituted PAHs are documented, and they generally occur as complex mixtures rather than single compounds. PAHs are found in all environmental compartments and have a tendence 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 presence of bay regions in their structural appearance as represented by the structure of benzo[a]pyrene in Figure 1 (Cai et al., 2007; Nekhavhambe et al., 2014; Oleszczuk, 2007; Sun et al., 2009; Wenzl et al., 2006). Environmental concerns of PAHs have necessitated their inclusion in lists of priority pollutants by several regulatory agencies such as the United States-Environmental Protection Agency (US-EPA), European Union 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 potential risks to human health has been the focus of much 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 high boiling point and low 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 are the least soluble in water.

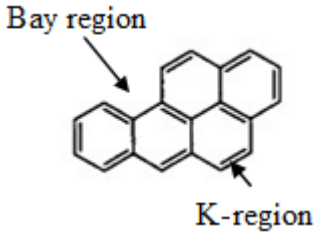


Figure 1 Structural features of PAHs that contribute to toxicity

2.2.2 Sources of PAHs

The introduction of PAHs into the environment mainly via natural and anthropogenic combustion of bituminous products (Amdany et al., 2014b; Nielsen et al., 1983). Examples of natural sources include volcanic eruptions, degradation of biological materials and forest fires.

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

Compound	Rings	MW	Solubility mg L^{-1}	B.P (°C)	Log Kow	Log Koc	Vapour pressure (Pa)
Acenaphthylene	3	152	1.93	265 -275	4.07	1.40	0.029
Acenaphthene	3	154	3.93	279	3.98	3.66	4.47×10^{-3}
Fluorene	3	166	1.68 - 1.98	295	4.18	3.86	3.2×10^{-4}
Phenanthrene	3	178	1.20	340	4.45	4.15	6.8×10^{-4}
Fluoranthene	3	202	0.20 - 0.26	375	4.90	4.5	5.0×10^{-6}
Anthracene	3	178	0.076	342	4.45	4.15	1.7×10^{-5}
Pyrene	4	202	0.077	393	4.88	4.58	2.5×10^{-6}
Benz[a]aanthracene	4	228	0.010	400	5.61	5.30	2.2×10^{-8}
Chrysene	4	228	2.8×10^{-3}	448	5.16	5.30	6.3×10^{-7}
Benzo[a]pyrene	5	252	2.3×10^{-3}	495	6.06	6.74	5.6×10^{-9}
Benzo[a]fluoranthene	5	252	0.0012	No data	6.04	5.74	5.0×10^{-7}
Benzo[b]fluoranthene	5	252	0.0012	No data	6.04	5.74	5.0×10^{-7}
Benzo[k]fluoranthene	5	252	7.6×10^{-4}	480	6.06	5.74	9.59×10^{-11}
Benzo[ghi]perylene	6	276	2.6×10^{-4}	550	6.50	6.20	1.03×10^{-10}

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 for energy supply (e.g. coal, oil, gas, and wood), 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 wastes (Maliszewska-Kordybach, 1999). Other sources of PAHs include unregulated agricultural burning, smoke from crematoria, cigarette smoking, recreational fires, volatilisation from soils etc. 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 like roads, parking areas and construction sites, industrial effluents from the manufacture of chemicals. They then enter the WWTPs through the sewerage. PAHs tend to accumulate in sludge during wastewater treatment processes owing to their high affinity for carbonaceous particles. Sewage sludge is the semisolid material separated during wastewater 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 (Chimuka et al., 2015) showed that these compounds are present in various South African environmental compartments. This is not surprising because South Africa being one of the largest economies in Africa has a lot of 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 with also the largest number of informal settlements without proper solid waste management. It has the largest

number of vehicles on the road contributing to the PAH environmental burden. Most of the PAHs end up in sludge and wastewater because of the open sewer system that takes storm water to WWTPs. The reported concentrations of PAHs in South African environments are more focused in air, water, soil and sediment samples. Very little has been reported in wastewater and sludge despite being major routes for the fate of PAHs in the environment.

2.2.3 Exposure and health effects

Exposure

PAHs are strongly lipid-soluble organic compounds, as indicated by their high log Kow values. Their entry routes into animal and human bodies primarily occur 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 mostly absorbed into the blood stream unaltered (Boström et al., 2016). Uptake through the gastrointestinal tract is through consumption of contaminated dietary lipids. Their absorption from the intestinal tract is aided by the presence of bile salts. PAHs tend to concentrate most in the liver and the fat-rich tissues of the body. Bio-concentration of PAHs in humans is not much pronounced because most of the PAHs are quickly metabolized by the liver and excreted through faecal matter and urine.

Health effects

The general adverse health effects of PAHs to humans and animals as well as a variety of flora and fauna species is 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 are of major concern. The major health and ecological concerns of PAHs are their carcinogenic potential (Baird et al., 2005; Boström et al., 2016) resulting. Animal studies have revealed PAHs' potential to cause gene mutations, developmental defects and tumors. Many individual PAHs have been tested for tumorigenicity and it was observed that PAHs with bay regions were likely to be potent carcinogens (Boström et al., 2016).

Benzo[a]pyrene is the most potent carcinogens of amongst the 16 US-EPA priority PAHs and 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 effect on humans. These are benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[ah]anthracene, and indeno[1,2,3-cd]pyrene.

2.3 Various techniques for extraction of PAHs

During analysis of organics in biological and environmental samples, a series of critical steps are involved. These procedures start with sample collection, storage and treatment of the sample. This is followed by extraction, isolation and identification of the target compound(s). The final step is quantitation and data handling. Amongst these steps, the choice of extraction method is viewed as a crucial step towards 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 and 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, the conventional solid–liquid extraction techniques like Soxhlet extraction and ultra-sonication extraction are still used extensively in present analytical procedures (Lau et al., 2010; 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 go to completion. New modern sample preparation techniques such as supercritical fluid extraction, pressurized liquid extraction and microwave-assisted extraction have been developed as an alternative to these classical techniques for analysis of 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 sonication technique is one of the most used for extraction of organic pollutants from solids like PAHs (Banjoo and Nelson, 2005; Liu et al., 2007). Its application in sludge samples for the extraction of all the US-EPA PAHs has been reported (Abad et al., 2005; Busetti et al., 2006). Other techniques that are a modification of the sonication technique include 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 in the extraction of organic pollutants from soils and sediments mainly because of its high extraction efficiency compared to modern techniques (Banjoo and Nelson, 2005; Martinez et al., 2004). The Soxhlet extractor has therefore been vastly used as a benchmark technique in the extraction of organics from soils and sediments. It is still the reference technique for almost all new extraction. 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). In addition, the US-EPA 3540C Soxhlet extraction (US-EPA, 1996a) 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

Pressurized extraction techniques form part of the green analytical extraction techniques because they either use less organic solvents or green solvents like water and CO_2. Examples of these modern sample extraction techniques for solid samples include the supercritical fluid extraction (SFE), pressurised liquid

extraction (PLE), microwave accelerated extraction (MAE) and pressurised hot water extraction.

The PLE technique has been used in the extraction of the 16 US-EPA PAHs in sludge samples (Aemig et al., 2016; Zielińska and Oleszczuk, 2016). The technique has also been applied in 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 in the extraction of 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, 2005). The resultant supercritical CO_2-solvent mixture is referred to as a CO_2-expanded liquid (CXL) (Al-Hamimi et al., 2016; Yusty, 2005). These CXLs have performed better than when CO_2 alone is used and better efficiencies have been recorded compared to the classical SE in the extraction of especially HMW PAHs.

2.3.3 Membrane based extraction techniques

Membrane based extraction techniques include membrane assisted solvent extraction (MASE), a technique first used by Hauser and Popp (2001) (Hauser and Popp, 2001; Rodil et al., 2007; Salgueiro-González et al., 2015; Schellin and Popp, 2003) and the supported liquid membranes (SLMs) such as the miniaturized hollow fibre liquid phase micro-extraction (HF-LPME) first reported by Jönsson and Mathiasson (1999) (Jönsson and Mathiasson, 1999; Kocherginsky et al., 2007; Ncube et al., 2016; Pedersen-Bjergaard and Rasmussen, 1999), emulsion liquid membranes (Davoodi-nasab et al., 2017) and ionic liquid membranes (Pabby et al., 2017). Selectivity in MASE depends on polarity and size of the compounds which is similar to SLM in a two phase system unless a carrier or a three phase system is used. The hydrophobic and size-exclusive properties of the micromembranes used during these techniques are responsible for eliminating

both hydrophilic compounds and higher molecular weight hydrophobic matrix compounds. Other advantages include high enrichment power and the potential for automation through online interfacing with chromatographic instruments (Barri and Jönsson, 2008; Hyötyläinen and Riekkola, 2008; Kocherginsky et al., 2007; Megersa et al., 2001). The main drawback of membrane-assisted extraction techniques is that extraction is not exhaustive (Hyötyläinen and Riekkola, 2008). Membrane memory effects are also possible due to clogging of the surface and the pores of the membrane by high molecular weight compounds and other hydrophobic compounds (Jakubowska et al., 2005).

Recent advances in the application of membranes has been to incorporate nanosorbents and immunoassays on the SLM in order to enhance further selectivity (Basheer et al., 2006; Davoodi-nasab et al., 2017; Thordarson et al., 2000). Electro-membranes have also been reported as a way of facilitating transfer across SLMs (Carasek and Merib, 2015; Eibak et al., 2014; Marothu et al., 2013; Pedersen-Bjergaard et al., 2017; Rezazadeh et al., 2013; Yamini et al., 2014). While the extract is free of matrix effects, the electro-membrane approach has a low sample throughput. Other studies have reported a non-porous polymeric membrane in combination with pressurized liquid extraction (Rodil et al., 2009; Streck et al., 2008). Another strategy has been to follow up the membrane technique with isolating the target analytes on a solid adsorbent. This is common with the MASE technique where the sorbent material can be placed in the lumen of the membrane or used as an SPE packing material (Barri and Jönsson, 2008; Bhadra and Mitra, 2013; Chimuka et al., 2011b; Demeestere et al., 2007; Iparraguirre et al., 2014).

The MASE technique for PAHs from aqueous samples has been reported before (Alcudia-León et al., 2009; March et al., 2011; Prieto et al., 2008; Rodil et al., 2007; Zuin et al., 2006). This technique alone is not a favourable approach in the analysis of PAHs with March et al. (2011) recording recoveries of 12.5 - 23% for 7 PAHs from sea water (March et al., 2011), Zuin et al. (2006) recording 13.6% for benzo[a]pyrene in sugarcane solution samples (Zuin et al., 2006) while Alcudia-Leon et al. (2009) did not report recoveries for their analysis of 5 PAHs

in water samples (Alcudia-León et al., 2009). However, Rodil et al. (2007) recorded recoveries above 65% for all the 16 US-EPA priority PAHs using the MASE method coupled with large volume injection programmed temperature vaporisation (Rodil et al., 2007). The same technique was reported by Prieto et al. (2008) and obtained recoveries of 81 – 116% in the analysis of the same PAHs in estuarine water samples (Prieto et al., 2008). Amdany et al. (2014) have used a semipermeable membrane device as a passive sampler for PAHs in dam water with recoveries of 55 - 123% (Amdany et al., 2014a). The HF-LPME technique has been reported for the extraction of PAHs from wastewater effluent samples (Charalabaki et al., 2005) and in seawater samples (López-López et al., 2017) An adsorbent-assisted MASE technique in the analysis of six PAHs from water samples has been reported by Ge et al. (2012) with recoveries recorded at 88.1 – 110.9% (Ge and Lee, 2012).

The MIP-assisted membrane approaches have been reported in the analysis of other organic compounds. 17β-estradiol has been extracted from aqueous solutions using both membrane bags (Chimuka et al., 2011b) and flat membranes (Mhaka et al., 2009; Nemulenzi et al., 2009) in combination with MIPs. Other studies that have reported membranes and MIPs include Barahona et al. (2016) who used an HF-LPME coated with MIPs for extraction of triazines from aqueous samples (Barahona et al., 2016). Takeda et al. (2005) used a molecularly imprinted nylon membrane for enhanced selective extraction of L-phenylalanine (Takeda et al., 2005).

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 investigate the potential of combining membrane assisted solvent extraction and a molecularly imprinted adsorbent into a single step format for analysis of PAHs in wastewater.

2.3.4 Other extraction methods

Other methods that have been reported for the extraction of PAHs include QUECHERS (Ben 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 from 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 bio-charred green coconut and goose eggshells have been applied in isolating PAHs in aqueous samples (Crisafully et al., 2008; Nuhu et al., 2012). Commercialized techniques like the fexIKA 200-control series extractor have been reported (Gfrerer et al., 2002). Solid phase micro-extraction (SPME) with mainly polydimethylsiloxane fibres for the extraction of 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 in the extraction of PAHs from aqueous and solid samples. A review on the techniques that have been used in the extraction of PAHs in soil samples has recently been done (Lau et al., 2010). Most of the studies have reported comparable performances of both the classical and modernized techniques in the extraction of 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 pressurized techniques have better recoveries than the likes of Soxhlet extraction, ultra-sonication 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 traditional techniques in the extraction of PAHs (Shu et al., 2003; Tomaniová et al., 1998).

Conversely, classical techniques especially the Soxhlet and ultra-sonication techniques have in some instances gave better recoveries than the 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 the performance of HF-LPME and SPME (Charalabaki et al.,

2005), and that of the matrix solid-phase dispersion method and the MAE technique (Pena et al., 2008).

2.5 Isolation and pre-concentration of PAHs

Almost all techniques used in the extraction of PAHs are followed by a clean-up step in which the PAHs are isolated from the matrix effects and finally pre-concentrated for detectability in chromatographic determination (Rawa-Adkonis et al., 2006). This step is almost always through solid 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 and functionalized nanoparticles have been introduced (Cacho et al., 2003; Chimuka et al., 2011a; Ferrer and Barceló, 1999; Hussen et al., 2007). The adsorbents that have been reported are summarized as a review article (**Paper 1)** while the membrane-based clean-up is discussed in **Paper 3 and 4**. These two articles report the combination of membranes and MIPs into a single step format.

The review article (**Paper 1**) offers a critical review on the materials that have been applied as adsorbents in the isolation of PAHs from matrix solutions. The review looks at adsorbents that have been used during the clean-up stage to isolate PAHs from aqueous samples and organic solvent extracts from treatment of solid samples. Articles that report the synthesis and application of adsorbents in the analysis of PAHs in the past decade stretching from 2007 were considered. The review focussed mainly on recent advances that have been done to enhance the applicability of both traditional adsorbents like silica, and modern ones like molecularly imprinted polymers and nanoparticles. The application of each modified adsorbent is critically evaluated with areas for possible improvement suggested. Finally, the review offers areas of exploration that can allow the analysis of PAHs to reach new heights.

2.6 Chromatographic instruments

The quantitation of PAHs is usually done using chromatographic instruments like gas chromatography (GC) and high-performance liquid chromatography (HPLC). In GC determination of PAHs, the time of flight mass spectrometry (TOF/MS) is

the preferred method of detection with most of the recent advances reported in the analysis of PAHs using the GC-TOF/MS (**Paper 1 – Review article**). The latest advancements that include two-dimensional separation make the GC-TOF/MS an ideal instrument in the analysis of multiple analytes that also exist in complex samples and in trace levels (Dallüge et al., 2002). The detection of the *m/z* ions is unlimited and any co-eluting peaks are detected and identified as separate responses. The HPLC-based systems reported in the analysis of PAHs involve coupling with ultra-violet, fluorescence and diode array detectors. These approaches are currently losing ground in the analysis of organics because they are easily affected by matrix effects and co-eluting compounds cannot be distinguished. HPLC coupled to quadrupole mass spectrometers has been introduced in the analysis of organics as a solution to the traditional detectors. This approach offers better advantages that include reproducibility, better signal-to-noise ratio and better sensitivity. However, the GC-TOF/MS has a better scan rate, better deconvolution ability and can identify analytes instantly from installed libraries (Krone et al., 2010). In view of the above, the GC-TOF/MS was used in this study for quantitation purposes.

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CHAPTER 3

3 AIM AND OBJECTIVES

3.1 Aim of the study

To develop two extraction techniques for the determination of polycyclic aromatic hydrocarbons in wastewater and wastewater sludge

3.2 Objectives

- To synthesize a molecularly imprinted polymer for the selective binding of the 16 US-EPA priority PAHs
- To develop and optimize a membrane assisted solvent extraction-molecularly imprinted polymer technique for analysis of PAHs in wastewater effluents
- To develop and optimize a Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer technique for analysis of PAHs in wastewater sludge
- To apply the two techniques in the determination of PAHs in wastewater effluents and sludge samples from wastewater treatment plants

3.3 General approach

- The first step was to do a thorough literature search on the techniques and adsorbents that have been applied in the analysis of PAHs in wastewater and wastewater sludge. It was observed that there is lack of review articles that critically evaluate the performance of adsorbents for PAHs. A review article that reports advances in the application of adsorbents for isolation of PAHs was then done and is presented as **Paper 1** already published in *Trends in Analytical Chemistry*.
- Then a molecularly imprinted polymer was synthesized to be used as a selective adsorbent for PAHs. Rigorous and exhaustive MIP cavity tuning experiments were done to identify a polymer with an affinity for all the 16 US-EPA priority PAHs. This research targeted the 16 US-EPA priority PAHs and a polymer that could effectively extract all of them was needed. The selected polymer was characterized for stability and pore related

parameters. Batch adsorption studies were also done to understand its molecular recognition behaviour. This part of the research led to **Paper 2** already published in the *Journal of Environmental Management*.

- The optimized polymer was then applied in the development of a membrane assisted solvent extraction-molecularly imprinted polymer herewith referred to as the MASE-MIP technique. This technique was optimized for several parameters and its performance reported as extraction efficiency, limit of detection and relative standard deviation values.
- The MASE-MIP technique was finally applied in the extraction of PAHs from wastewater effluent followed by quantitation using a GC-TOF/MS system. Optimization and application in the analysis of real wastewater samples was summarized as **Paper 3** and submitted to *Journal of Chromatography A* for peer review
- The MASE-MIP technique was then combined with the Soxhlet extractor and optimized for extraction of PAHs from wastewater sludge. The approach was referred to as the Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique.
- The SE-MASE-MIP technique was then applied in the extraction of PAHs from wastewater sludge samples followed by GC-TOF/MS determination. The optimization and application results were used to produce **Paper 4** already published in *Journal of Separation Science*.

CHAPTER 4

4 PUBLICATIONS AND MANUSCRIPTS

This section presents the published articles and manuscripts that must be considered for examination of the thesis. The referencing in each manuscript is formatted according to requirements of the journal in which they were published or submitted for peer review. The design including topics, subtopics and referencing styles are therefore different for all the four documents. However, all the published articles have been formatted to suit the requirements of this thesis submission. For manuscripts submitted for peer review, line numbers have been removed and the page numbers formatted to cater for the thesis submission requirements. The Figures and Tables have been inserted in appropriate positions close to where they are mentioned in the body of the text. Cover letter and Highlights submitted to each journal are not included in this thesis. Page numbers for published manuscripts have been removed to cater for the page format requirements for the thesis. **Paper 1, 2** and **4** have already been published while **Paper 3** is still under peer review.

4.1 PAPER 1

This paper entitled "Recent advances in the adsorbents for isolation of polycyclic aromatic hydrocarbons (PAHs) from environmental sample solutions" has been published in *Trends in Analytical Chemistry*. This is a review article that critically evaluates the materials that have been applied as adsorbents in the isolation of polycyclic aromatic hydrocarbons from matrix solutions

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Recent advances in the adsorbents for isolation of polycyclic aromatic hydrocarbons (PAHs) from environmental sample solutions

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Abstract

This article offers a critical review on the materials that have been applied as adsorbents in the isolation of polycyclic aromatic hydrocarbons from matrix solutions. The review looks at adsorbents that have been used during the clean-up stage to isolate polycyclic aromatic hydrocarbons from aqueous samples and organic solvent extracts from treatment of solid samples. Articles that report the application of adsorbents for solid phase extraction and solid phase microextraction of PAHs in the past decade stretching from 2007 were considered. The review focussed mainly on recent advances that have been done to enhance the applicability of both traditional adsorbents like silica, and modern ones like molecularly imprinted polymers and nanoparticles. The application of adsorbents modified with functional organic groups is critically evaluated with areas for possible improvement suggested. Finally, the authors offer areas of exploration that can allow the analysis of polycyclic aromatic hydrocarbons to reach new heights.

Keywords

polycyclic aromatic hydrocarbons, solid phase extraction, solid phase microextraction, periodic mesoporous organic silica, metal organic frameworks, molecularly imprinted polymers, carbon nanoparticles

Abbreviations

C_{18}, octadecyl; CNP, carbon nanoparticle; EGDMA, ethylene glycol dimethacrylate; Fe_3O_4, magnetite; GPC, gel permeation chromatograph; LOD, limits of detection; MIL-101(Cr), Cr-doped Material of Institute Lavoisier no. 101; MIL-100(Fe), Fe-doped Material of Institute Lavoisier no. 10; MIP,

molecularly imprinted polymer; MOF, metal or metal oxide organic framework; MWCNT, multiwalled carbon nanotube; NP, nanoparticle; PAH, polycyclic aromatic hydrocarbon; PMO, periodic mesoporous organic silica; SPE, solid phase extraction; SPME, solid phase microextraction; TBCD, 1,4,7,10-tetrabenzyl-1,4,7,10-tetraazacyclododecane

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that consist of fused benzene rings. These compounds have low solubility and tend to adsorb on solid and carbonaceous materials in the environment. PAHs are ubiquitous in the environment with several studies reporting their presence in both aqueous (mainly surface water, plant oils and petroleum products), air and solid samples that include sediments, soils and wastewater sludge [1,2]. Several studies have also reported the presence of PAHs in food samples [3–7]. For analysis of PAHs in solid samples, the PAHs firstly need to be transferred into a solvent. Most of the extraction techniques, both conventional and modern, are non-selective and result in an extract with a large amount of matrix effects. A further clean-up step is therefore needed in which the PAHs are isolated from the matrix effects and finally preconcentrated for detectability in quantitation instruments. Analysis of water samples also requires that the PAHs be isolated first before introduction into the chromatographic instrument for quantitation. Solid phase extraction (SPE) and gel permeation chromatography (GPC) are commonly used during the isolation stage. Isolation is achieved by binding on various adsorbent phases that are packed on the SPE and GPC systems. For analysis of water samples, the adsorbent may also be dropped directly into the solution. Suitable organic solvents are then used to elute the PAHs from the sorbent followed by chromatographic determination. Another important isolation technique in the analysis of PAHs that uses adsorbents is solid phase microextraction (SPME).

Challenges in the application of adsorbents for PAHs are related to lack of functional groups on PAHs that can be targeted for binding. They are hydrophobic and tend to co-extract with large amounts of matrix effects. In addition, they have a very low charge, exist in the environment as mixtures and in trace amounts.

Mass spectrometry is arguably the best detection technique yet it exhibits low sensitivity towards PAHs due to lack of soft spots that can easily be fragmented. These undesirable characteristics have made the search for adsorbents that can effectively isolate PAHs from environmental samples a priority. In this regard, several potential adsorbents have been investigated. These include traditional adsorbents such as the synthetic silica and alumina while molecular imprinting and nanoparticles (NPs) have been tried as modernized adsorbents. This review discusses the impact of each adsorbent type in the analysis of PAHs.

2 Silica gel and alumina adsorbents

The traditional silica gel and alumina are still reported as effective clean-up adsorbents in the isolation of PAHs from matrix effects [8–12]. Silica and alumina are micro-powdered synthetic adsorbents with a nano-porous structure made of silicon dioxide and aluminium oxide, respectively. These classical adsorbents are still common during the clean-up step after PAHs had been extracted from solid samples using organic solvents. They have been used for sample clean-up and pre-concentration of PAHs after Soxhlet extraction [13], ultrasound assisted solvent extraction (ultra-sonication) [4,14], saponification [15,16] and microwave assisted extraction [11,17,18]. The SW-846 US-EPA 3630C silica gel clean-up approved by the US-EPA has been used by Cai et al. (2007) while Bojes and Pope (2007) have used a modified SW-846 US-EPA Method 3611B that uses alumina as an adsorbent [19,20]. Other researchers have combined alumina and silica as SPE sorbents [14,21,22] or GPC column gels [11,23]. Fromberg and Højgård (2007) have combined GPC using commercial Bio-Beads S-X3 and the silica based SPE for cleaning PAHs while Hossain and Salehuddin (2012) have used silica as both the fractionating GPC column and the SPE adsorbent for isolation of PAHs from oil samples [24,25]. Comparative studies have shown that better selectivity and reproducibility are achieved with activated silica gel. Generally, the applicability of silica in the adsorption of PAHs is restricted by the presence of -OH groups on the surface. These groups interact with water molecules that result in formation of a hydration film around silica particles. The interaction of the silica surface and a PAH molecule therefore has to suppress this water phase. As such, focus has shifted to modifications of the silica surface using organic

functional monomers mainly the ringed organic groups that can complement the structure of PAHs. Attempts have also focussed on down-scaling the particle size to the nano scale for the purposes of improving surface area. Herein we discuss the applications of functionalized silica and silica NPs in the analysis of PAHs in environmental samples.

2.1 Mesoporous organic silica

One of the major advances in the application of functionalized silica is the use of periodic mesoporous organic silica (PMO). This is silica that has been functionalized with organic derivatives to provide mesoporosity on its surface. PMOs are characterized by periodically ordered pores of 2 – 50 nm sizes and large surface areas of up to 1884 m^2 g^{-1} recorded by Xia et al. (2005). This PMO had a pore size distribution of 2.2 – 2.6 nm [26]. However, the highest surface area recorded for any silica-based PMO is 2370 m^2 g^{-1} which was reported by Wei et al. (2016) for PMO nanocubes with a 200 nm pore size [27]. Functionalization occurs by covalently linking the organic functional groups with siloxane domains.

Several studies have reported the application of PMOs. Cyclodextrin-functionalized PMOs are very common as sorbents for extracting PAHs. β-cyclodextrin, a doughnut-shaped oligosaccharide consisting of 7 D-glucopyranoside units, is documented as the functional coating agent of choice. Its cyclic structure in addition to the cyclic repeating units makes it a better choice for targeting cyclic hydrophobic molecules like PAHs. A β-cyclodextrin-functionalized PMO has been used by Mauri-Aucejo et al. (2016) for analysis of trace levels of 8 PAHs in different water samples with limits of detection (LOD) values of 3 – 800 ng L^{-1} and efficiency levels of 54 – 145% [28]. The observed spread in the efficiency values was attributed to matrix influences. The authors further suggested that a standard addition calibration approach can be used to counteract these matrix effects using the proposed method. This is an indication that there is still need for improvement in the synthesis of cyclodextrin-based PMOs to produce a sorbent with a selective affinity for priority PAHs. Topuz et al. (2017) used a similar PMO based on silica NPs to isolate 5 PAHs in aqueous samples. The adsorption capacities ranged from 0.3 to 1.65 mg g^{-1} [29]. Soler-

Segui et al. (2016) gave LODs of 1.2 – 38 ng L^{-1} for 7 PAHs in water samples using a dextrin-silica hybrid composite [30]. Pentynyl β-cyclodextrin as the organic moiety for a PMO adsorbent for phenanthrene has recently been reported with extraction efficiencies above 95% [31].

Other organic coating agents for silica include an aminopropyl imidazole-modified silica sorbent reported by Wang et al. (2014) in water samples with LODs of 6.5 – 500 ng L^{-1} and efficiencies of 63.2 – 112.3% [32]. Zhao et al. (2016) have used a macrocyclic tetraazacalix[2]arene[2]triazine functional agent with efficiencies of 94.3−102.4% and LODs of 0.4 - 3.1 ng L^{-1} in the analysis of 5 PAHs in water samples [33]. Vidal et al. (2011) have functionalized silica with phenyl groups for extraction of 5 PAHs in aqueous solutions with removal efficiencies of 40 – 70 % and adsorption capacity of 0.72 – 1.69 mg g^{-1} [34]. Haemoglobin-coated mesoporous silica by Laveille et al. (2010) yielded removal efficiency of above 82% for 11 PAHs in water samples [35].

A novel approach has been reported by Liu et al. (2014) in which a macrocyclic polyamine functionalized with an ionic liquid, 1,3-dibutylimidazolium bis[(trifluoromethyl)sulfonyl]imide with LOD values of 2 - 100 ng L^{-1} and efficiencies of 80.9 - 120.1% was used in the determination of 5 PAHs in water samples [36]. Other improvements in the application of silica include doping the PMO with magnetite (Fe_3O_4). Li et al. (2017) have magnetized silica coated with polydopamine with LOD values recorded at 0.185 – 0.367 mg g^{-1} for PAHs in water samples [37]. Tetraethyl orthosilicate magnetized with maghemite (Fe_2O_3) NPs with a binding capacity of 0.39 mg g^{-1} has been applied by Huang et al. (2016) for the isolation of acenaphthene with removal efficiencies reported at 85% [38].

2.2 Mesoporous silica nanoparticles

Mesoporous silica NPs have also been reported as adsorbents for isolation of PAHs from aqueous solutions and environmental water samples. An Si–MCM–41 (Silica-functionalized Mobil Composition of Matter or Mobil Crystalline Material No. 41) has been reported in the analysis of PAHs in aqueous samples [39,40]. This is a silica-based mesoporous molecular sieve prepared from a silica source in

the presence of a surfactant. Once the surfactant is washed out, a hexagonal framework with a high surface area and specific pore size is formed. Dodecylamine and tetramethylammonium have been reported as the surfactants with adsorption capacity values of 18.35 – 18.78 μg g^{-1} and 0.69 – 1.09 mmol g^{-1}, respectively [39,40]. An NH_2-SBA-15 (amine-functionalized Santa Barbara Amorphous No.15) hybrid with an adsorption capacity range of 0.76 – 1.92 mg g^{-1} towards PAHs has been reported by Balati et al. (2015). SBA-15 differs from MCM-41 in that its pores are round and pluronic acid is used as a surfactant [41]. Studies by Li et al. (2017) have shown that the geometric framework of SBA-15 renders it a better adsorbent for PAHs than MCM-41 [42]. Zhang et al. (2017) have recently reported a magnetized form of a mesoporous silica NP called Fe-SBA-15 for adsorption of pyrene [43]. Magnetized silica NPs using cholesterol as a functionalizing agent for the extraction of 7 PAHs in traditional Chinese medicine has been reported by Yan et al. (2013). LODs were between 0.50 and 1.0 ng g^{-1} [44]. Galan-Cano et al. (2013) have used N-methylimidazole-coated magnetized silica NPs to effectively isolate 13 PAHs with LODs and extraction efficiencies recorded at 0.06 – 1.11 ng mL^{-1} and 75 – 102%, respectively [45].

3 Nanosorbents for polycyclic aromatic hydrocarbons

NPs are known to have extraordinary properties owing to their distinctively increased surface area and sorption capacity and likewise their application as nanosorbents has shown great promise in the isolation of PAHs from matrix effect sample solutions [46,47]. Nanosorbents that have been reported in the analysis of PAHs include the NPs of carbon, metal and metal oxide. Novel advancements include functionalizing the surface of NPs with organic groups that have a high affinity towards PAHs as a way of improving selectivity. Another advancement is the application of magnetic NPs and magnetized NPs to improve extraction of the adsorbent from the matrix solution. The widely used magnetic nanoparticle is Fe_3O_4 because it exhibits a tremendous paramagnetic behaviour which becomes essential during separation of the nanosorbent from an aqueous solution. Magnetized NPs involve synthesizing a composite that consist of Fe_3O_4 and another NP. The direct application of Fe_3O_4 requires that its surface be functionalized to improve selectivity towards target analytes while the magnetized

NP can still be used in its unfunctionalized form. The following sections discuss the applicability of the common NPs that have been used in the analysis of PAHs since 2007. NPs that have been used in the synthesis of sensors for detection and identification purposes were not considered in this discussion. The novel advances in the applications of carbon nanoparticles (CNPs), quantum dots and metal (and metal oxide) nanoparticles in the analysis of PAHs are discussed.

3.1 Carbon nanoparticles

Several analogues of carbon-based NPs that include graphene, graphene oxide and carbon nanotubes have all been reported as sorbents in the analysis of PAHs from environmental samples. The abundance of π-electrons and high hydrophobicity of the surface of CNPs allow PAHs to bind through π – π and hydrophobic stacking interactions. Generally, a study by Zhao et al. (2014) has shown that graphene has better sorption capacities towards PAHs than nanotubes and nanosheets [48]. The closed interstitial spaces through folding and rearrangement of graphene sheets to form tubes or fibres and the stacking of sheets tend to reduce the surface area and micro-pore volume. Graphene oxide has a weakened π electron system and its surface is hydrophilic in nature affecting its applicability for interaction with PAHs. Despite its high adsorption capacity towards hydrophobic compounds, graphene is hardly used as a naked adsorbent due to its tendency to aggregate. Several novel solutions have been developed in which graphene and other CNPs are either embedded on a support or coated with organic functional groups for an efficient application in the analysis of PAHs in environmental aqueous samples.

3.1.1 Recent applications of carbon nanoparticles in the analysis of PAHs

Wang et al. (2013) have used a graphene-based SPE disk for 9 PAHs in water samples and recorded LODs and extraction efficiencies of 0.84 – 13 ng L^{-1} and 72.8 - 106.2%, respectively [49]. The multiwalled carbon nanotube (MWCNT) is another form of a CNP that has been applied as adsorbents in the analysis of PAHs in environmental water samples with LOD values ranging from 2 to 58 ng L^{-1} and recoveries of 70 – 127% [47,50]. Recent improvements include embedding CNPs on a silica support. Huang et al. (2014) reported graphene immobilized on silica for determination of 6 PAHs in water and milk samples

with LODs and recoveries of 2.9 - 52 ng L^{-1} and 89.0 - 115.4%, respectively [51]. Graphene oxide embedded on silica has also been reported for 14 PAHs in cigarette with LOD values of 0.05 - 0.36 ng per cigarette [52]. Other attempts to minimize aggregation have seen Zhao et al. (2011) introducing hydrophilic sulfonic acid groups to the surface of graphene. Sulfonation was explained to have prevented interlaminations due to aggregations. The effect was adsorption capacities as high as 2.326 mmol g^{-1} for naphthalene and 2.407 mmol g^{-1} for 1-naphthol [53].

Novel approaches aimed at improving the ease of using CNPs include incorporating Fe_3O_4 as a magnetizing agent. Several studies have reported merging Fe_3O_4 into graphene. This provides a double effect in that the surface area of graphene is increased and an external magnetic field can be used to remove the NPs from the sample [54–57]. Zhao et al. (2011) used a magnetic MWCNT adsorbent for isolating 8 PAHs in oil samples with LOQs and extraction efficiencies of 0.34 - 2.9 ng g^{-1} and 87.8 - 122.3%, respectively [58]. Approaches aimed at improving the selectivity of CNPs towards PAHs have led to functionalization of their surface with organic groups that have an affinity for PAHs. Vinyl alcohol [59] and tetraethoxysilane [60] have been used as functional agents on the surface of graphene with LOD values of 5 – 8 and 100 – 300 ng L^{-1}, respectively. Graphene oxide functionalized with brilliant blue for the analysis of 2 PAHs gave an LOD and extraction efficiency of 2.212 mmol g^{-1} and 93.2%, respectively for fluoranthene [61]. The most noted novelty in the application of CNPs is the combination of magnetization and functionalization. Ji et al. (2017) have used magnetized graphene oxide functionalized with phytic acid (LOD range of 0.06–0.15 ng g^{-1} and recoveries of 85.6–102.3% for PAHs in vegetable oils) while Mehdinia et al. (2015) have used thiophene as an organic coating agent for graphene with LOD values and recoveries of 9 – 20 ng L^{-1} and 83–107%, respectively in the extraction of PAHs from water samples [62,63]. Recently, Mahpishanian et al. (2017) have used a magnetized silica-graphene composite functionalized with 2-phenylthylamine to isolate 10 PAHs from aqueous matrices with LOD values ranging from 5 to 100 ng L^{-1} and extraction efficiencies of 71.7

– 106.7% [64]. The introduction of silica was reported to provide stability of the organic coating.

3.1.2 Future of carbon nanoparticles in the analysis of PAHs

The applicability of unfunctionalized CNPs in real samples is affected by several environmental conditions. Studies by Zhao et al. (2014) have shown that presence of surfactants reduces selectivity towards PAHs through competition [48]. On the other hand, studies by Zhang et al. (2012) have revealed that the sorption of PAHs on CNPs is reduced in the presence of humic acids [65]. This therefore limits the applicability of unfunctionalized CNPs in real environmental samples where humic acids exist in abundance. Another major challenge with CNPs is the tendency to aggregate in aqueous solutions due to the hydrophobic nature of their surface. Preventive measures that include coating on other nanoparticle materials like silica and magnetite are promising. Novel improvements that incorporate small hydrophilic groups such as sulfonic acid to solvate the CNPs have shown great potential. Inducing exfoliation using surfactants to alleviate aggregated CNPs during analysis and exfoliation of the layers in case of multiwalled CNPs to create more surfaces that can be utilized for adsorption is another area of interest.

Magnetizing CNPs has helped in their separation from the sample solution using an external magnetic field. Functionalization and silica supports for CNPs have greatly increased the surface area for an effective extraction of PAHs using minimal amounts of the nanosorbent. It can therefore be postulated that functionalization acts by preventing aggregation of the CNPs while at the same time providing an alternative functional surface. It is observed that minimal number of potential organic coating moieties have been explored as nanocarbon organic framework functional agents for adsorption of PAHs. The challenge remains with the fact that both PAHs and graphene lack functional groups that can be targeted in the search for perfect coating agents.

3.2 Quantum dots

The application of quantum dots as adsorbents in the analysis of PAHs has not been well explored. The few cases in which they have been applied as nanosorbents has involved functionalization with cyclodextrin. Qu and Li (2009)

reported β-cyclodextrin-functionalized cadmium telluride quantum dots for 2 PAHs with LODs of 13.1 and 94.3 μg L^{-1} while Han and Li (2008) recorded an LOD of 1.6 x 10^{-8} mol L^{-1} for a single PAH using a cadmium selenide/zinc sulfide quantum dot composite functionalized with β-cyclodextrin [66,67]. Studies involving quantum dots and PAHs have generally been scarce owing to the comparatively high detection values observed in the mentioned studies. The applicability of quantum dots is in sensor devices for PAHs owing to their large band gap that relate to excellent optical properties.

3.3 Metal and metal oxide nanoparticles

Unfunctionalized metal and metal oxide NPs have been sparingly applied in the isolation of PAHs from environmental samples. Gold and titanium oxide NPs have been reported with LOD values and recoveries of 0.9 – 59 ng L^{-1} and 83.3 – 100%, respectively [68,69]. The commonly used approach has therefore been through metal or metal oxide organic frameworks (MOFs). This involves incorporating metal ions or metal oxides into organic ligands that have an affinity for the target analytes. The result is a three-dimensional porous structure with a large surface area ranging between 3 000 and 7 000 m^2 g^{-1} [27]. The functional organic moiety acts by providing interaction sites for analytes while the metal and metal oxide NPs increase the surface area of interaction. The result is enhanced interactions between the functionalized nanosorbent and the PAHs.

3.3.1 Metal and metal oxide organic frameworks

Several organic moieties have been reported in combination with NPs of gold, silver, titanium and titanium oxide in the isolation of PAHs from environmental samples. Cetyltrimethylammonium has been reported as a functionalizing agent for silver by Romanovskaya et al. (2011) and titanium oxide by Huang et al. (2011) [70,71]. Huang et al. (2011) recorded 26 – 820 ng L^{-1} LOD values and 75.0 – 114% recoveries for the 16 US-EPA priority PAHs in environmental water samples. These LOD values were however lower than the 17 – 59 ng L^{-1} values recorded for unfunctionalized titanium oxide applied by Kefi et al. (2011). A titanium nanotube functionalized using n-octadecanethiol supported on silver NPs has been reported with LOD values of 0.0015 – 0.4 ng g^{-1} and recoveries of 70.32

- 115.51% [72]. The organic groups that have been reported for functionalizing gold NPs include the silane-based 3-mercaptopropyltrimethoxysilane [73] and, the thiolated agents such as 1-pentanethiol [74] and 3-(trimethoxysilyl-1-propanthiol) [75]. The thiol-based coated NPs recorded LODs of 0.8 – 60 ng L^{-1} for 16 PAHs and 100 – 890 ng L^{-1} for 4 PAHs, respectively. The respective recoveries were 44.6 – 90.5 and 84 -106%. A further novelty is by Mehdinia et al. (2014) who used magnetized gold NP functionalized with 3-aminopropyltriethoxysilane. LOD and recovery values ranged from 2 to 4 ng L^{-1} and 91.4 to 104.2%, respectively for the extraction of 5 PAHs from seawater samples [76]. A magnetized silver nanosorbent by Khajeh et al. (2016) achieved LODs of 10 – 90 ng L^{-1} and recoveries of 92.8 – 96.5% for 3 PAHs in water samples [77].

3.3.2 Functionalized magnetic nanoparticles

Magnetic and magnetized MOFs are the most used nanosorbents in the isolation of PAHs. Magnetic MOFs involve embedding the organic agents on Fe_3O_4. A magnetized MOF involves doping another functionalized material with Fe_3O_4. This can be a metal/metal oxide, silica or graphene organic functionalized framework. Magnetizing the adsorbents has several advantages over traditional SPE as it avoids time-consuming and tedious SPE cartridge procedures [78,79]. It provides a rapid and simple separation of the nanosorbent from the sample solution using an external magnet. This approach avoids the need for centrifugation or filtration steps. The magnetic NPs loose magnetism when the magnetic field is removed from their proximity implying that they will not agglomerate after separation from the matrix solution.

3.3.3 Common organic coating agents

Most of the organic groups that have been used as MOF functionalizing agents contain a characteristic ringed component, an indication that adsorption may be layered through π-π interactions and hydrophobic effects between rings of the functional agent and the PAHs. Figure 1 shows a list of the ringed organic agents that have been reported in the synthesis of MOFs for adsorption of PAHs. These include alkaloids, sugars, benzyl carboxylic acids and vinyl benzenes among others. Figure 2 lists the acyclic functional ligands that include alkoxysilanes and

substituted aliphatics. The aliphatic groups that have been utilized most are the long-chain hexadecyl and octadecyl groups connected to a thiol, ammonium, imidazolium or phosphate group as shown in Figure 2. The methoxy- and ethoxy-groups of silanes have an O molecule for anchoring while the alkyl groups take part in hydrophobic interactions with PAHs. Figure 3 shows the macromolecular organic groups that have been applied in MOF fabrication. All the macromolecular functional moieties are characterized by six/five-ringed components involved in non-covalent layered interactions with PAHs. Some novel advancements in MOFs have seen researchers modifying the NP surface with a combination of organic functional groups that all have an affinity for PAHs. Examples of organic ligand combinations in the synthesis of MOFs for PAHs are shown in Figure 4.

The performance values due to ligand selection in Table 1 show that recovery values due to different functional organic moieties are similar while the detection limits are affected. Considerations for method detection limits are important especially for trace analysis and where complex samples are involved. In Table 1, it is observed that the lowest LOD values have been achieved for functional moieties that contain an unsubstituted phenyl ring. A phenyl group can be viewed as a benzene ring substituted at one position. Diphenyl, triphenylamine and 1,4,7,10-tetrabenzyl-1,4,7,10-tetraazacyclododecane (TBCD) all had LOD values of 0.04 – 0.39 (16 PAHs), 0.04 - 3.75 (6 PAHs) and 0.03 - 1.2 (7 PAHs), respectively. Good recoveries of 56 – 112, 80 – 108 and 81 – 116% were obtained respectively. In turn, triphenylamine is the better functional group among other alkaloids that have been reported (Figure 1), diphenyl among other benzyl-based moieties (Figure 1) and TBCD among other macromolecular functional ligands (Figure 3). An unsubstituted benzyl group has a stable system of π-electrons and its surface is not hindered sterically to allow PAHs to bind in a layered format. Another notably functional group is the hexadecylsilane moiety with LOD values of 0.14 – 0.31 ng L^{-1} and recoveries of 88 – 96% for 5 PAHs in water samples. This group is the most performing amongst all silanes and substituted aliphatics. The organic silanes act by combining with hydroxyl groups on the surface of silica. This effect eliminates hydrophilicity on the surface due to hydroxyl groups

while at the same time inducing hydrophobicity for effective interaction with PAHs. The long-chain hexadecyl group offers greater hydrophobicity. Amongst the ringed moieties, the other ligands that have performed well include dopamine, pyrrole, divinylbenzene and glucose (Table 1).

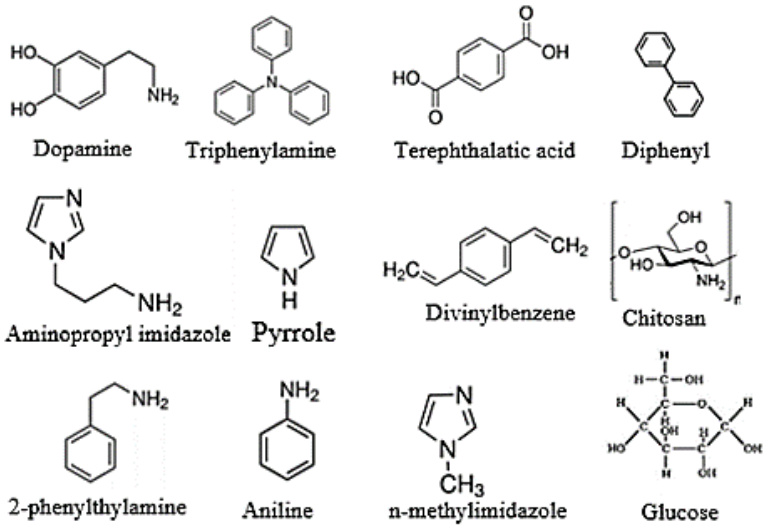


Figure 1: List of cyclic functional agents used in supported organic frameworks for adsorption of PAHs

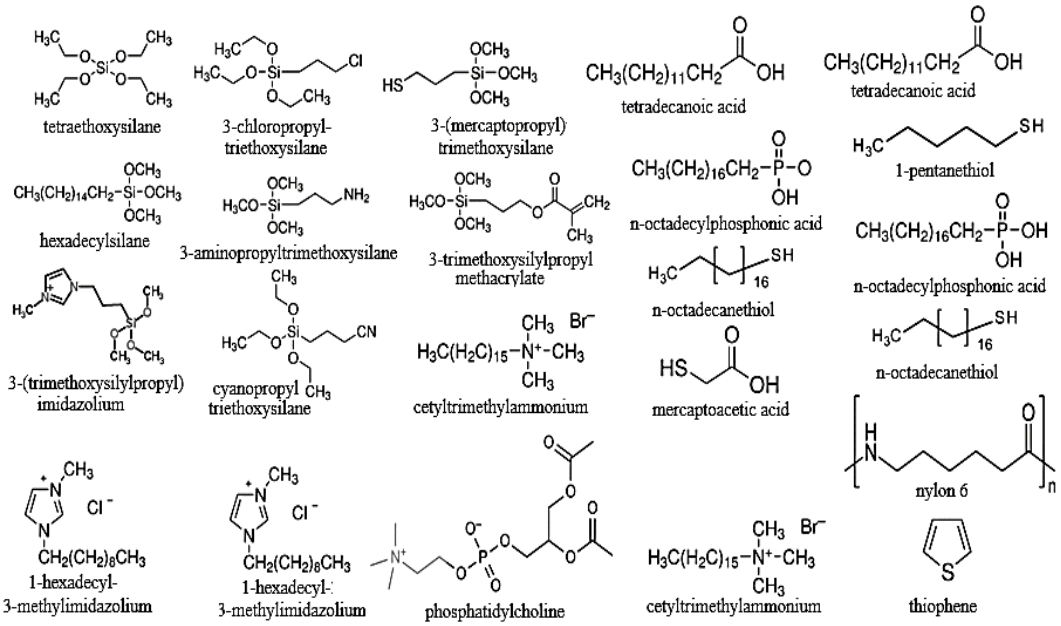


Figure 2: List of silicon and sulphur-based functional groups and other acyclic agents used in supported organic frameworks for adsorption of PAHs

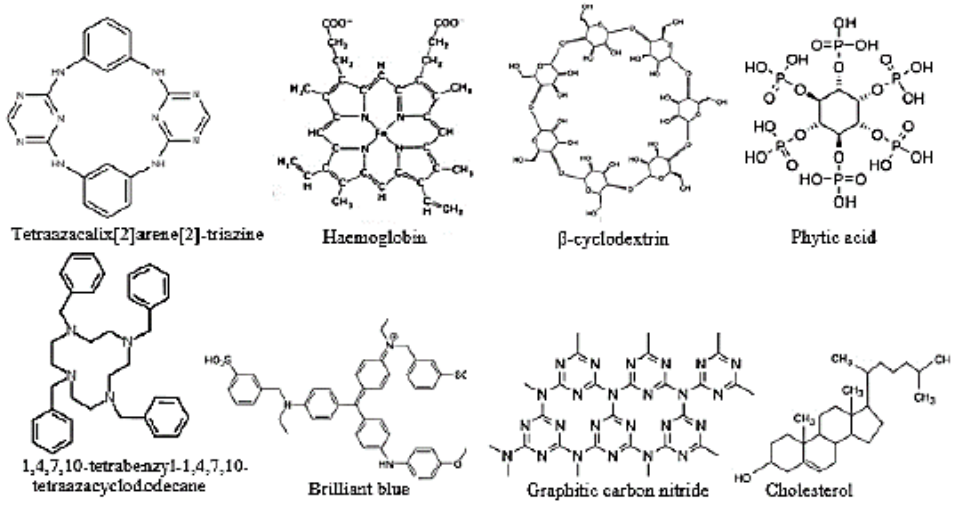


Figure 3: Macromolecular functional agents used in supported organic frameworks for adsorption of PAHs

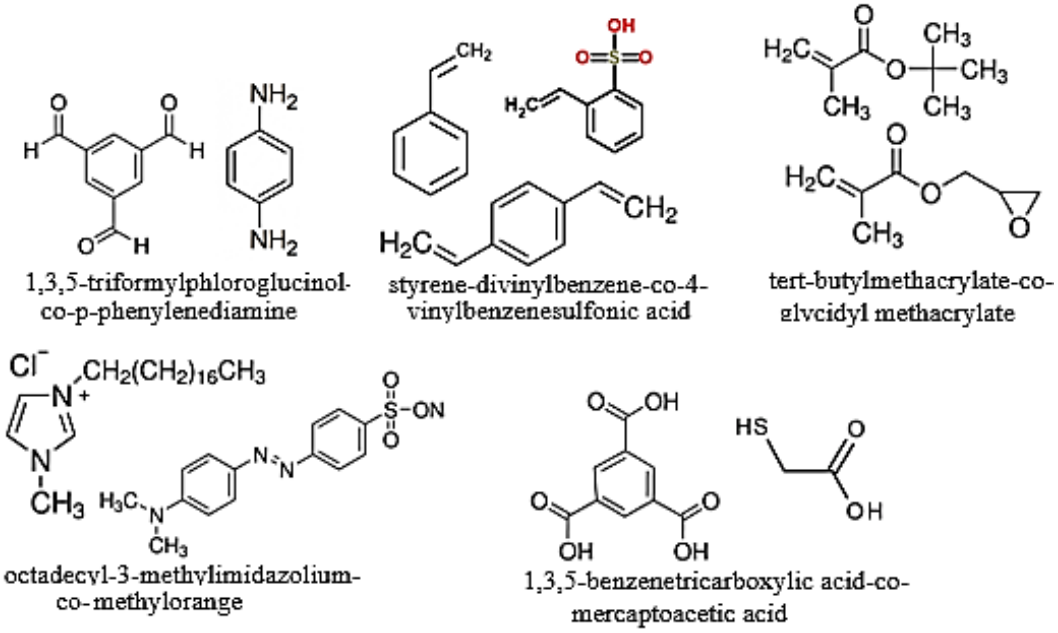


Figure 4 Combination functional agents used in supported organic frameworks for adsorption of PAHs

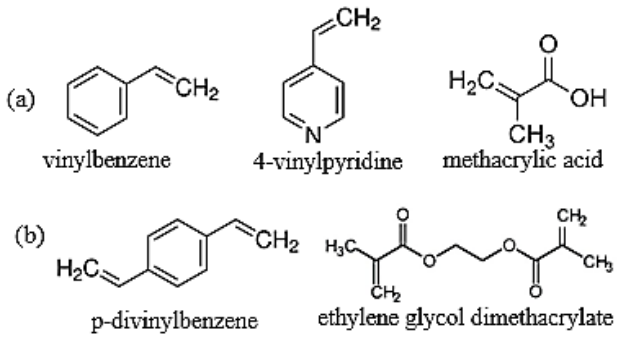


Figure 5: (a) Common monomers and (b) cross-linkers

Ionic liquid organic moieties supported on NPs have also shown good recoveries and low detection limits. These include trimethoxysilylpropyl methacrylate, 1-hexadecyl-3-methylimidazolium and an octadecyl -3- methylimidazolium -co-methylorange ionic liquid surface supported on magnetite as shown in Table 1. A ferrofluid formed through hemimicelles of tetradecanoic acid on the surface of magnetite NPs had limits of quantitation as low as 0.2 ng L^{-1} and recoveries of 85 – 94% (Table 1). The methacrylate group in trimethoxysilylpropyl methacrylate is rich in π-electrons while the hexadecyl, octadecyl and tetradecyl chains have enhanced hydrophobicity. Other functional groups that have been reported to perform well include n-methylimidazole supported on silica with minimum LOD values of 0.06 ng L^{-1} and recoveries of 75 – 102% (Section 2.2). The methyl group in n-methylimidazole (Figure 1) has a stabilizing effect on the imidazole π-electron system.

The performance of some combination ligands towards PAHs is satisfactory and comparable with the phenyl-based moieties. Combination ligands that have been applied in MOFs for PAHs such as the 1,3,5-triformylphloroglucinol-co-p-phenylenediamine and the octadecyl-3-methylimidazolium-co-methylorange composite have yielded LOD values of 0.24 - 1.01 and 0.1 – 2 ng L^{-1} and good recoveries of 73 – 110 and 80 – 104%, respectively (Figure 4 and Table 1).

Table 1: List of organic functional groups that have been supported on magnetite nanoparticles for application in the isolation of PAHs from solution

Functionalizing agent	Sample	No. of PAHs	LOD (ng L^{-1})	Efficiency (%)	Reference
Dopamine	water	6	0.5 - 1.9	76.4 - 107	[80]
Pyrrole	water	10	0.38 - 5.01	72.4 - 115.7	[81]
Triphenylamine	water	6	0.04 - 3.75	80.21- 108.33	[79]
Aniline supported on alginate beads	water	3	10 - 40	86.0 - 97.8	[82]
Diphenyl	urine	16	0.04–0.39	56 - 112	[83]
Glucose	water	7	0.2 – 0.6	76–110	[84]
Chitosan	wastewater	8	-	50 - 96	[85]
Divinylbenzene	water	7	0.6 – 1.4	-	[86]
Chromium terephthalate (MIL-101(Cr)) on silica	water	6	2.8 - 27.2	81.3 - 102	[87]
Chromium terephthalate (MIL-101(Cr))	water	4	44 – 64	85.8 – 97.3	[88]
Cyanopropyltriethoxysilane (Cyano-ionic liquid)	sludge	5	400 – 590	89.50 - 110.2	[89]
Trimethoxysilylpropyl methacrylate (ionic liquid surface)	Tea/coffee	7	0.1 - 10	87.5 - 104.5	[90]
3-cholorpropyl-triethoxysilane	water	16	11.7 - 61.4	63.2 – 98.9	[91]
1,4,7,10-tetrabenzyl-1,4,7,10-tetraazacyclododecane	water	7	0.03 - 1.2	81.1 - 115.5	[92]
Graphiticcarbonnitride	water	7	50 - 100	80.0 - 99.8	[93]
Palm fatty acid	sludge	4	10 - 50	81.1 - 119.3	[94]
n-octadecylphosphonic acid	water	15	14.1 – 70.0	54.2 - 119.1	[95]
Octadecyl (C_{18})	soil	8	0.5 - 1.0 ng/g	63.2 - 92.8	[96]
Octadecyl (C_{18})	water	16	800 - 36000	46 - 95	[97]
Tetradecanoic acid (hemimicelles) ferrofluids	water	8	0.2 - 0.5 (LOQ)	85 - 94	[98]
Cetyltrimethylammonium	water	15	0.4 – 20.6	19.40 - 102.99	[99]
Hexadecylsilane	water	5	0.14 - 0.31	88 - 96	[100]
1-hexadecyl-3-methylimidazolium (liquid coating)	water	3	0.33 – 8.33	76 - 105	[101]
Nylon 6	water	4	50 - 580	80 - 111	[102]
Phosphatidylcholine	water, milk	6	0.2–0.6	42 - 62	[103]
Styrene-divinylbenzene-co-4-vinylbenzenesulfonic acid	water	6	1.93 – 3.27	81.6 - 98.5	[104]
Octadecyl-3-methylimidazolium-co-methylorange (ionic liquid surface)	water	5	0.1 - 2	80.4 - 104.0	[105]
1,3,5-benzenetricarboxylic acid-co-mercaptoacetic acid-co-$FeCl_3$ (MIL-100(Fe))	water	13	32 – 2110	81.4 - 126.9	[106]
Tert-butyl methacrylate-co-glycidyl methacry-late	water	3	50 - 1200	62.5 - 104	[107]
1,3,5-triformylphloroglucinol-co-phenylenediamine	water	6	0.24 - 1.01	73 - 110	[108]

Both 1,3,5-triformylphloroglucinol and p-phenylenediamine possess the benzene ring rich in π-electrons. The octadecyl and phenyl groups offer enhanced hydrophobic and π-π interactions respectively in the octadecyl-3-methylimidazolium-co-methylorange combination MOF.

Generally, the performance of these organic molecules as functional ligands seems to overlap. The minimum LOD values recorded range from 0.04 to 10 for alkaloids, 0.04 to 44 for ringed moieties, 0.1 to 400 for silanes, 0.14 to 800 for substituted acyclic (silane and alkyl) groups, 0.03 to 50 for macromolecular groups and 0.032 to 50 ng L^{-1} for combination groups. However, it was observed that among alkaloid and cyclic-based groups only aniline which had been entrapped in magnetised alginate, 2-phenylthylamine on magnetized CNPs and terephthalate doped with Cr(III) had LOD values above 1 ng L^{-1}. Probably the choice of supporting material was the limiting factor. Of the silane functional groups, only the cyanated and chlorinated moieties, and tetraethoxysilane supported on graphene had LOD values greater than 1 ng L^{-1}. Cyonation and halogenation have electron-withdrawing effects while the antagonizing effect of graphene organic frameworks can be attributed to the instability of the coating layer. The fused triazine rings in graphiticcarbonnitride destabilize the π-system which might explain its high LOD values compared to other macromolecular coating groups (Figure 3 and Table 1). Comparing the organic groups that polymerize on the surface and those that form ionic liquid coatings on the surface of NPs, it can be observed that performance depends solely on the type of functional group rather than the type of surface formed (Table 1).

3.3.4 Advances in application of MOFs

One noted improvement in the application of MOFs in the analysis of PAHs is the use of metal-doped organic functional groups. The target is to provide an organic coating layer with a spacious framework. The metal acts by increasing the pore sizes to allow for a selective adsorption of very large analytes such as the PAHs. The most commonly used doped MOFs are the MIL-101(Cr) and MIL-100(Fe) MOFs that are made of Cr(III) and Fe(III) organic moieties respectively. MIL-101 and MIL-100

stand for Material of Institute Lavoisier no. 101 and 100, respectively. The functional agent in MIL101(Cr) is chromium terephthalate while the MIL-100(Fe) MOF adopts a co-functional group consisting of 1,3,5-benzenetricarboxylic acid-co-mercaptoacetic acid (Figure 4). Huo and Yan (2012) used a magnetic MIL-101 supported on magnetized silica for 6 PAHs in environmental water samples with LODs of 2.8 – 27.2 ng L^{-1} and recovery values of 81.3 – 102% [87]. A magnetic MOF(MIL-101) recently reported by Zhou et al. (2017) performed less than the silica-supported nanosorbent with LODs and recoveries values of 44 – 64 ng L^{-1} and 85.8 – 97.3% recorded respectively for 4 PAHs in water samples [88]. This observation might be an indication of the potential synergy due to the silica support rather than the doping metal. Du et al. (2016) have applied a magnetic MIL-100(Fe) for adsorption of 13 PAHs in spiked water samples with its LOD values of 32 – 2110 ng L^{-1} and recoveries of 81.4 - 126.9% similar to those of the unsupported MIL101(Cr) [106].

The stabilization of organic functional agents on silica before embedding on metal oxide NPs has yielded better performance of MOFs. This can be related to the covalent bonds that form between the agents and the hydroxyl groups on the surface of the silica. Silica supports for MOFs have also performed better than other supports that have been reported in the analysis of PAHs such as graphene and alginate. The observed impact where metal oxide MOFs perform better compared to metal MOFs can be explained in terms of the stabilizing effect of the bond formed between the oxide and the functional monomers. Generally, the application of moieties with unsubstituted phenyl groups remains a better option for MOF-based nanosorbents for PAHs.

Advances in the application of co-polymeric organic moieties includes the magnetic nanosorbent doped with poly(styrene-divinylbenzene-co-4-vinylbenzenesulfonic acid) [104]. This nanosorbent was described as having both the hydrophilic and hydrophobic properties. The hydrophilic sulfonic acid helps with sorbent dispersion in water while the phenyl groups are responsible for adsorbing the PAHs through

hydrophobic and π-π interactions. These hydrophilic–hydrophobic magnetic MOFs can be an area worthy exploring in the analysis of PAHs.

3.4 Challenges associated with nanoparticles

The physicochemical properties of PAHs especially the lack of functionality has hindered the application of naked NPs. The search for effective functional coating agents is still a challenge considering that such agents have to be nonpolar. Lack of functionality on the potential coating agents also affects their stability on the surface of the metal, the metal oxide or the silica support. The hydrophobic nature of the coating agents also implies that selectivity towards target analytes is reduced as most of the nonpolar matrices will be co-adsorbed.

Another issue that is general with the use of NP is their disposal. With an increased application of NPs in all spheres of science, it is inevitable that these engineered nanomaterials may result in a sudden boom in their presence in the environment. While scientists are enticed by their perfect properties that allow them to be applied in almost any field of science, an unmonitored introduction of NPs into the environment raises concern. There is need for proper management of nanoparticle waste. It is predicted that in the near future, scientists will begin to wary about the presence of NPs in the environment and their potential health and environmental effects. Only scientific evidence can prompt environmental and health agencies to consider NPs under contaminants of emerging concern. The current limitations are that instruments that can be used for detecting NPs in the environment are scarce.

4 Molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) have also gained popularity in search for effective adsorbents in the analysis of PAHs. MIPs are prepared by polymerization of functional monomers with a cross-linking agent in the presence of a template molecule. A simple addition polymerization process initiated by free radicals obtained from thermolysis of azo-based compounds such as azo-bisisobutyronitrile and 4,4-azo(4-cyanovaleric acid) gives a stable solid product with the template

molecules trapped within its three-dimensional structure [109,110]. Once the template molecules have been washed out, cavities with a shape complementary to the template molecule remains. These cavities are very selective towards target analytes and are therefore utilized in the isolation of target analytes of similar topology from complex samples [46,109–114]. The weak interactions formed between the analyte and the cavities are easy to cleave during washing and elution.

4.1 Monomers and cross-linkers for synthesis of polymer adsorbents for PAHs

As shown in Table 2, the common monomers used in synthesis of PAH-imprinted polymers are the acidic methacrylic acid, the basic 4-vinylpyridine and the neutral vinylbenzene. The structures of these compounds are shown in Figure 5(a). All these compounds can interact with PAHs through hydrophobic interactions. The presence of aromatic rings in 4-vinylpyridine and vinylbenzene results in introduction of π-π interactions which further enhance aromatic stacking on the PAH used as a template. The terminal C=C bond found in all the three monomers is involved in addition polymerization with the C=C bond of the cross-linker. Other functional monomers that have been used are given in Table 2.

The most commonly used cross-linkers include ethylene glycol dimethacrylate (EGDMA) and p-divinylbenzene (Figure 5(b) and Table 2). A cross-linker serves as a stabiliser for the imprinted cavities and imparts mechanical stability to the polymer matrix in order to retain its molecular recognition capability [113]. MIPs polymerized with a p-divinylbenzene cross-linker are rather rigid compared to those prepared from EGDMA. EGMA-based MIPs have better cross-selectivity, an essential property in the analysis of multiple analytes that are related in steric and functional components [115,116].

Table 2 List of molecularly imprinted polymers for adsorption of polycyclic hydrocarbons from solution

PAH Template	Target PAHs	Sample	Monomer	Cross linker	Adsorption capacity mg g^{-1}	Application	LOD	Recovery	Elution solvent	Instrument	Reference
Pyr	23 PAHs	spiked water	4-vinylpyridine	di-vinylbenzene		GPC	-	-	DCM/ACN	HPLC-UV/VIS	[111]
Polymer combination	16 US-EPA PAHs	spiked cyclohexane solution	vinylbenzene	EGDMA	5.19	direct	-	40.2-98.8	heptane	GC-MS	[116]
Commercial magnetic MIP	16 US-EPA PAHs	river, tap, lake and mineral water	crosslinked vinylic polymer		-	direct contact	0.0013–0.969	20.0-98.5	acetone	HPLC-DAD	[135]
Commercial MIP	(15+1 EUPAHs)	tea leaves and vegetable oils	vinylbenzene	di-vinylbenzene	-	GPC and MISPE	0.1–0.3 mg kg^{-1} (LOQ)	72 – 99	DCM/ethyl acetate	GC-MS	[136,137]
16 US-EPA PAHs	16 US-EPA PAHs	sea water	phenyltrimethoxysilane	tetra-ethoxysilane	2.7	MISPE	5.2–12.6 ng L^{-1}	83 - 113	DCM/AA	GC-MS	[127]
Commercial MIP	15 PAHs	sesame, peanut, soya, rape and olive oil	-	-	-	MISPE	0.04–0.66 µg kg^{-1}	56.0- 109.1	ethyl acetate	HPLC-FLD	[138]
Multitemplate (16 PAHs)	8 PAHs	sediments, dust and industrial effluent	MAA	EGDMA	6.93	direct	10–30 ng L^{-1}	85 – 96	direct	FLS	[130]
Multitemplate 6 PAHs	6 PAHs used as templates	spiked ground water	MAA	EGDMA	0.685	MISPE	0.3–1.5 mg L^{-1}	58-89	direct	FLS	[129]
Multitemplate 5 PAHs	5 PAHs used as templates	dust	4-vinylpyridine	EGDMA	1.93	MISPE	0.5-0.9 ng L^{-1}	68.5-88.7	ACN	GC-MS	[128]

PAH Template	Target PAHs	Sample	Monomer	Cross linker	Adsorption capacity mg g^{-1}	Application	LOD	Recovery	Elution solvent	Instrument	Reference
Pyr (graphene-based MIP)	5 PAHs	water samples	MAA + 4-vinylpyridine	EGDMA	0.102	MISPE	2.96 - 33 ng L^{-1}	73 - 105.4	DCM	GC-MS	[139]
Ant	5 PAHs	aqueous solution	tetraiodobisphenol A	phloroglucinol	-	optosensor	30 ng L^{-1}	-	toluene	FLS	[118]
Toluene as a pseudo-template	4 PAHs	waste and sea water	4-vinylpyridine	EGDMA	-	thin films	0.054 – 1.5 µg L^{-1}	28.3-47.0	ethyl ether	GC-MS	[140]
Commercial copolymer	3 PAHs	soil, sludge & sediment	vinylbenzene	di-vinylbenzene	-	MISPE	-	63.4-92.6	MeOH/THF	HPLC-DAD	[141]
B[a]P	B[a]P in presence of 4 other PAHs	water samples	HEMA-MAO-PHEN	EGDMA	0.054	cryogel cartridges	24.86 µg L^{-1}	87.4	ACN	HPLC-FLD	[122]
Pyr and Phe co-template	B[a]P	spiked olive oil, sunflower oil, linseed oil	2-vinylpyridine	di-vinylbenzene	0.64	MISPE	-	63 - 114	DCM	enzyme-linked immunosorbent assay (ELISA)	[142]
Pyr	B[a]P (in presence of 3 other PAHs)	cigarette smoke	MAA + 4-vinylpyridine	EGDMA	18.33	smoke filter		63.6	MeOH/AA	GC-MS	[134]
Pyr (mag-MIP)	Pyr in presence of 5 other PAHs	spiked aqueous samples	4-vinylpyridine	di-vinylbenzene	-	optical sensors	20 ng mL^{-1}	-	direct	FLS	[143]
Pyr, Ant & Nap	Pyr, Ant & Nap	spiked solution	2-vinylpyridine	EGDMA		MISPE		92.8–96.7	ACN	GC-MS	
Multi-template	Nap, Ant and Pyr	spiked solution	2-vinylpyridine	EGDMA		MISPE	0.01-09 mg L^{-1}		ACN	GC-FID	[77]

PAH Template	Target PAHs	Sample	Monomer	Cross linker	Adsorption capacity mg g^{-1}	Application	LOD	Recovery	Elution solvent	Instrument	Reference
(Ag/Fe_3O_4-SiO_2)											
B[a]P	B[a]P (in presence of one other PAH)	spiked solution	vinylferrocene as monomer and redox tracer	EGDMA	3.7	e-MIP for sensors	-	-	-	Cyclic Voltammetry	[144]
Phe	Phe (in presence of 13 other PAHs)	aqueous solution	4-vinylpyridine - glycidylmethacrylate co-monomer	-	0.05	MISPE	-	<10-100	-	-	[145]
b-naphthol	Nap	spiked water	TOPC	TEOS	-	direct contact	-	-	DMSO	FLS	[146]
Ant (nanopolymer)	Ant	spiked aqueous samples	MAA	EGDMA	374.3	direct	-	90.3–99.9	toluene	HPLC-DAD	[117]
Methyl-B[a]P	B[a]P (in presence of 15 other US-EPA PAHs)	cigarette smoke	4-vinylpyridine	divinylbenzene	0.021	MISPE	-	96	DCM/hexane	GC-MS	[131]
B(a)P	B[a]P (in presence of 15 other PAHs)	cigarette smoke	MAA	TRIM	0.0078	MISPE	-	87	DCM	GC-MS	[123]
B[a]P	B[a]P	spiked tap water, lake water and instant coffee	4-vinylpyridine	divinylbenzene	0.0163	MISPE	-	72.5-96.5	DCM	HPLC-FLD	[124]
Fln	Fln (in presence of 10 other PAHs)	spiked river water	tetraiodobisphenol A	phloroglucinol	-	optosensor	35 ng L^{-1}	>80	ACN	FLS	[147]

PAH Template	Target PAHs	Sample	Monomer	Cross linker	Adsorption capacity mg g^{-1}	Application	LOD	Recovery	Elution solvent	Instrument	Reference
B(a)P	B[a]P (in presence of 10 other PAHs)	spiked natural water	tetrabromobisphenol A	phloroglucinol	-	optosensor	10 ng L^{-1}	>70	ACN	FLS	[125]
B[a]P	B[a]P (in presence of Chr and Pyr)	marine sediments	MAA	EGDMA	75	microtraps	1 µg g^{-1}	98	TCM/MeOH	FLS	[126]
Phen (magnetic polymers)	Phe (in presence of 3 other PAHs)		vinylbenzene-co-MAA co-polymer		15.57	direct	3.64 ng mL^{-1}	97.1-101.9	acetone	FLS	[133]
Pyr (nanofilms)	Pyr (in presence of Chr and B[a]P	aquatic and air samples	pyrene and terthiophene monomer electropolymerized on gold surface of a QCM crystal		11.35	nanofilm sensors	-	100	DCM/ACN/ EtOH	FLS	[120]
Binary imprinting (Nap and Pyr)	Pyr (in presence of Chr)	spiked solution	4,4′-diisocyanatodiphenylmethane that acts as both monomer and cross-linker		-	sensors	-	-	heating at 90°C	luminescence spectrometer	[148]

AA – acetic acid, ACN – acetonitrile, Ant – anthracene, B[a]P – benzo[a]pyrene, DCM – dichloromethane, DMSO - dimethyl sulfoxide, EGDMA - ethylyne glucol methacralic acid, EtOH – ethanol, Fln – fluoranthene, FLS – fluorescence spectrometer, HEMA-MAO-PHEN - poly(2-hydoxyethyl methacrylate-N-methacryloyl-L-phenylalanine), mag-MIP – magnetic molecularly imprinted polymer, MeOH – methanol, MIP – molecularly imprinted polymer, MMA - methyl methacrylate, Nap – naphthalene, Phe – phenanthrene, Pry – pyrene, TCM, trichloromethane, TEOS – tetraethoxysilane, THF – tetrahydrofuran, TOPC - 3-(Triethoxysilyl)propyl isocyanate, TRIM - trimethylolpropane trimethacrylate

4.2 Templates for creating cavities

Table 2 summarizes the PAHs that have been used as templates. An attempt was made to include all the polymers that have been reported in the analysis of PAHs. The common PAH templates include anthracene [117–119], pyrene [111,120,121] and benzo[a]pyrene [122–126]. Baggiani et al. (2007) observes that pyrene is suitable as an imprinting molecule because it is not carcinogenic or mutagenic [111]. The choice of benzo[a]pyrene is mainly because this PAH is a potent carcinogen which is usually used as a basis for PAH pollution [122–125]. Advancements in MIP fabrication for PAHs include multi-template imprinting in which a mixture of PAHs is used for imprinting [127–130]. In some cases, a compound with structural orientation similar to target analyte (s) referred to as a dummy template has been applied in synthesizing MIPs for PAHs [131,132]. This approach saves to eliminate any possibilities of template bleeding and imprinting effect. Combination polymers have also been reported in which two polymers are physically mixed after being separately imprinted with different PAHs. A benzo[k]fluoranthene-imprinted and indeno[1 2 3-cd]imprinted polymer combination has been applied in the isolation of the 16 US-EPA priority PAHs from solution [116].

4.3 Performance of molecularly imprinted polymers

The performance of imprinted polymers that have been reported in the analysis of PAHs is summarized in Table 2. A comparison of adsorption capacity values shows that the highest binding capacity ever reported is a nanoparticle polymer synthesized by Hassan et al. (2016). The synthesis approach was through precipitation polymerization in which anthracene was used as an imprinting template. The polymer had a binding capacity of 373.4 mg g^{-1} towards anthracene [117]. The benzo[a]pyrene-imprinted MIP microspheres by Krupadam et al. (2014) also have a high binding capacity value of 75 mg g^{-1} [126]. The microspheres were obtained via an emulsion strategy in which precipitation polymerization was done in a microfluid reactor. The emulsion approach has also been used by Li and Wang (2013) to prepare magnetic MIP nanoparticles with a binding capacity of 15.57 mg g^{-1} towards

phenanthrene [133]. A styrene-methacrylic acid co-polymer and phenanthrene as the imprint were encapsulated on Fe_3O_4 nanoparticles through sonication. The high performance of micro and nano scale polymer particles can be attributed to the enhanced surface area for an effective interaction with the PAHs.

The performance of MIPs for PAHs prepared by bulk precipitation is generally lower compared to those prepared by precipitation polymerization (Table 2). Only the pyrene-imprinted polymer by Xie et al. (2013) has recorded an adsorption capacity (18.33 mg g^{-1}) close to that of a magnetic nanoparticle by Li and Wang (2013) [133,134]. The main disadvantage with bulk polymerization is that the product is a solid mass that needs to be crushed into a fine powder. During grinding, the MIP granules will have a random shape and size distribution while some of the imprinted cavities will collapse. Other MIPs produced through bulk polymerization with a fair adsorption capacity include the polymer combination [116] and the multi-template approaches [127,129,130]. These approaches form part of the advances in the application of MIPs in the analysis of PAHs in environmental samples.

4.4 Challenges in the application of MIPs as adsorbents

The challenges in the synthesis of MIPs for PAHs stem from the lack of functionality and the hydrophobic nature of PAHs. Recognition of PAHs in the MIP cavities is therefore controlled by the shape and size of the cavities through hydrophobic and π–π interactions between template and monomer molecules. Steric recognition reduces selectivity towards target analytes because it implies that any compound sterically related to PAHs will be recognized. This becomes a disadvantage compared to analysis of compounds with functional groups that tend to interact with monomers in the cavity sites through mainly H-bonding at specific sites. It should be noted that imprinting factors reported for PAH-imprinted polymer are generally lower compared to polymers imprinted with compounds with functional groups. An imprinting factor is a ratio of the amounts of analytes adsorbed by the imprinted polymer against the amount adsorbed by a non-imprinted polymer. The higher the imprinting factor, the better the selectivity of the polymer towards target compounds.

The reduced selectivity has led to synthesis of PAH-imprinted polymers mainly to target a single PAH or a portion of the 16 US-EPA priority PAHs except for commercial MIPs [135–137], the multi-template imprinted MIP [127] and the polymer combination [116]. At the same time, both multi-template imprinting and polymer combination approaches have drawbacks. Research into multi-template imprinting lacks data on possibility of preferential imprinting. The polymer combination approach was also done by mixing the polymers at 1:1 (w/w) without further optimization. A number of researchers have reported the use of their MIPs to isolate up to five PAHs from solution as shown in Table 2. Most studies have rather focused on proving the specificity of MIPs in which a polymer is used to selectively adsorb a single PAH in the presence of other PAHs (Table 2). This is unadvisable considering that PAHs exist as mixtures in the environment and a number of these are listed in priority lists by different environmental protection agencies.

However, targeting all the 16 US-EPA PAHs also has issues relating to lack of selectivity which in turn relates to a wide range of recovery values. For example, the methylbenzo[a]pyrene-imprinted polymer by Ho et al. (2010) had recovery values ranging from 15 to 96% [131]. The benzo[a]pyrene-imprinted polymer reported by Krupadam et al. (2014) had binding capacities of 75.9, 6.9 and 7.1 μg g^{-1} for benzo[a]pyrene, pyrene and chrysene respectively [126]. Disregarding the fact that these observations were favourable because the target was to prove specificity of the MIPs towards a single PAH, PAHs cannot be found in isolation in the environment and quantitation of a single PAH should not be promoted.

It is also noted that most of the templates or multiple templates that have been used during the imprinting process are selected without doing cavity-tuning experiments. In this regard, two studies have emphasized the need for MIP-cavity tuning experiments. Dicket et al. (1999) investigated the effects of naphthalene, acenaphthene, phenanthrene, anthracene, pyrene and benzoperylene on the cavity efficiency for the sequestration of these six PAHs [118]. Ncube et al. (2017) did rigorous polymer selection experiments that led to a polymer combination that

displayed a broad affinity towards all the 16 US-EPA PAHs with an average efficiency percentage of 65 ± 13.3 (n = 16, SD) [116]. In both cases, the researchers have argued that the size and shape of the imprinting PAH is essential in enhancing selectivity towards multiple analytes. The MIP cavity-tuning experiments have shown that cavities imprinted using a PAH molecule will favour adsorption of PAHs of larger molecular weight than the imprint itself. An emphasis has therefore been made on the need for cavity-tuning experiments if the aim is to target all PAHs listed by several organizations in their priority lists.

4.5 Recent advances in imprinted polymer fabrication

Synthesizing MIP particles in the nano scale through precipitation polymerization is among the recent advances in MIP fabrication. Hassan et al. (2016), for example, synthesized an NP-PAH-MIP nanosorbent with an adsorption capacity that is over 100 times more than most of the PAH-imprinted MIPs [117]. Embedding MIP particles on NPs to improve surface area has recently gained interest from researchers. The common NPs are the metal oxides especially the paramagnetic Fe_3O_4. In addition to increased surface area, its magnetic property allows the MIP particles to be separated from the matrix solution or target mixture using an external magnetic field. A magnetic MIP has been reported by Li et al. (2013) [107]. Villar-Navarro et al. (2017) have used a commercial PAH-imprinted magnetic MIP for the analysis of 16 PAHs in water samples [135]. Kibechu et al. (2017) have synthesized a graphene oxide and pyrene-imprinted polymer composite for isolation of 5 PAHs in water samples with an adsorption capacity of 101.83 μg g^{-1} [139]. Medina-Castillo et al. (2010) have reported a magnetic pyrene-imprinted polymer for forming sensor spots through magnetic separation [143]. Yang et al. (2016) have reported a MIP embedded on titanium oxide microbeads for determination of OH-Pyr with a recovery of 94.2% in human urine samples [149].

5 Adsorbents for solid phase microextraction

SPME is another isolation technique that has been utilized in the analysis of PAHs from matrix effects. The most common SPME fibers are prepared with fused silica.

Fused silica is fragile and new supports based on metal wires have been developed to provide mechanical stability. Several organic polymers supported on both silica and metal wires have been reported as functional coating materials in the analysis of PAHs. SPME fibres based on both PMOs and MOFs and, advanced forms of MOF such as hybrid co-polymers, polymeric ionic liquids and MIL-53(M) (M = metal) have been reported as viable alternatives to the SPE approach. The advantage is that SPME combines sample extraction or isolation, pre-concentration and injection into chromatographic techniques into a single step. It is also solvent-free when the analysis is performed on GC making it a better technique than SPE-GC methods [150–153]. Some of the SPME fibres that have been reported in the analysis of PAHs followed by GC-MS determination since 2007 are summarized in Table 3. Those followed by HPLC determination [5,153,154] and Raman spectroscopy [155] have also been reported.

Generally, SPME hybrid fibres seem to perform better in the presence of matrix effects compared to SPE-based sorbents. While the recovery values are comparable, the LOD values for aniline as an SPME adsorbent (Table 3) are better than those when used as an SPE-based sorbent (Table 1). These observations may also be explained in terms of the supporting material, that is, alginate versus fused silica. The performance of the metal-doped MIL53(Al) supported on steel wire as an SPME sorbent is almost 30-times and 320-times higher than the SPE-based MIL101(Cr) and MIL100(Fe) respectively (Section 3.3.4). While the two approaches were applied in the analysis of different PAHs in different sample types, the results still give a snap-view of the capability of the SPME approach in isolating PAHs from complex samples. Comparing the divinylbenzene-based SPME fibres (Table 3) to MIP particles used as MISPE in which divinylbenzene was used as a cross-linker (Table 2), it can be concluded that MIPs perform better than the SMPE approach. This is an indication of the specificity of MIPs due to imprinted cavities.

Table 3 Adsorbents for SPME followed by gas chromatographic determination

Adsorbent	Support	PAHs	Sample	LODs (ng L^{-1})	Recovery(%)	Reference
Aniline	Fused silica	4	Dam water	1 - 3	76 - 109	[152]
Aniline	Platinum wire	5	Spiked water	0.1 - 6	82 -111	[156]
MIL-53(Al)	Steel wire	16	wastewater	0.10 – 0.73	76 - 126	[157]
C18-TMS/TEOS	TiO_2/Ti wire	6	water	3 - 25	85 - 102	[151]
Polypyrrole/SBA-15	Steel wire	8	wastewater	3 - 20	-	[150]
Divinylbenzene	Polydimethylsiloxane (commercial)	16	water	27 - 128	71 - 106	[158]
Divinylbenzene	Polydimethylsiloxane (commercial)	27	water	0.07 – 0.76	62 - 102	[159]
Divinylbenzene	Polydimethylsiloxane (commercial)	9	Smoked meat	16 - 75	-	[160]
Gold nanoparticles	Fused silica	6	seawater	10000 – 200000	92 – 105	[161]
Poly(VBHDIm$^+$NTf$_2^-$) (ionic liquid)	Fused silica	6	Drinking water	6 - 83	64 - 106	[162]
Poly(VBHDIm$^+$NTf$_2^-$) (ionic liquid)	Fused silica	12	Spiked water	3 - 70	-	[163]
Poly(VHDIm$^+$NTf$_2^-$) (ionic liquid)	Fused silica	5	Well water	5 - 60	88 - 110	[164]
Phenyltrimethoxysilane/ SBA-15	Copper wire	8	River and wastewater	5 - 37	93 - 130	[165]
Graphene oxide	Fused silica	6	water	5 – 80	84 – 118	[166]
Graphene	Fused silica	8	Water and soil	1.5 – 2.7	72 - 102	[167]
MMA–POSS hybrid	Titanium wire	8	water	6 - 50	79 - 104	[168]

Poly(VBHDIm$^+$NTf$_2$)$^-$ - poly(1-(4-vinylbenzyl)-3-hexadecylimidazolium bis[(trifluoromethyl) sulfonyl]imide, MIL-53(Al) - Material of Institute Lavoisier no. 53 (Aluminium), TEOS - tetraethyl orthosilicate, SBA-15 - Santa Barbara Amorphous No.15, TMS – tetramethylsilane, Poly(VHDIm$^+$NTf$_2$)$^-$ - poly(1-vinyl-3 hexadecylimidazolium)bis[(trifluoromethyl)sulfonyl]imide, MMA–POSS - methyl methacrylate- polyhedral oligomeric silsesquioxane

The LOD values in Table 3 also indicate that both the type of support and type of functional monomer are important in SPME. Aniline has given better values when supported on a platinum wire than on fused silica. Graphene as a functional agent has performed better than graphene oxide when both are supported on fused silica. Advancements in the SPME approach are focussed on producing robust fibres with a longer lifespan. Current fibres still have a disadvantage of low thermal stability, ease of breakage, swelling in organic solvents and lack of stability of functional moieties on the surface.

6 Other novel adsorbents for PAHs

Other SPE-based adsorbents that have been used during isolation of PAHs in the last decade include octadecyl (C_{18}) particles mainly for isolating PAHs from aqueous samples [169–175]. Zhang et al. (2007) used a double SPE sheet made of C_{18} and SDB-XC adsorbent for the 16 US-EPA priority PAHs in water samples [176]. Magnetized C_{18} has also been reported [96,177]. Zhang et al. (2010) caged the magnetic C_{18} adsorbent on the hydrophilic barium alginate to enhance its dispersibility in aqueous solutions [177]. Wang et al. (2014) have used cotton fiber as an SPE sorbent for 7 PAHs (LOD of 0.1 – 2 ng L^{-1} and recoveries of 70.69 – 110.04%) without any chemical modifications [178]. A nanoporous calcium carbonate vateritic polymorph prepared from eggshell waste for determination of PAHs from water samples has been reported with extraction efficiencies of 85 – 110% [179]. Recently, Saini et al. (2017) reported a novel sol gel-C_{18} coated fabric phase sorptive extraction media in which an octadecyl cellulose trimethoxysilane sol-gel was coated onto an activated cellulose substrate. When applied in water samples for isolation of 4 PAHs, the LOD values ranged from 0.1 to 1 ng L^{-1} [180].

An automated microextraction by packed sorbents (MEPS) coupled with GC-MS in which 8 PAHs were analysed from spiked water samples has been reported by Fu et al. (2012). This approach was compared with SPE and its LODs were 0.8 – 8.2 ng L^{-1} compared to the SPE's 4.8 – 39.5 ng L^{-1} [181]. Its advantages over SPE were described as better sensitivity and less organic solvent for elution. A silica aerogel has

recently been developed by Filho et al. (2017) with an adsorption capacity towards aromatics above 25 mg g^{-1} [182]. However, low removal efficiencies of 16 - 18% were reported. A synthetic fibre coating from polyaniline as an SPE sorbent by Bagheri et al. (2007) had detection limits as low as 0.1 – 6 ng L^{-1} for PAHs in spiked water samples [156]. The application of organo-bentonite for adsorption of 4 PAHs has been reported with removal percentages of 49.6 - 69.4% [183]. Khalili-Fard et al. (2012) have reported sulfur microparticles as SPE sorbents for PAHs with LODs and recoveries recorded at 7 – 48 ng L^{-1} and 78 - 108% [184]. A magnetic nanoparticle-phenanthrene hybrid probe based on indirect competitive chemiluminescence enzyme immunoassay (MNPs-icCLEIA) has been reported by Sun et al. (2017) with an LOD value of 850 ng L^{-1} towards phenanthrene [185] .

7 General challenges and possible areas for improvement in adsorbents for isolating PAHs

The main challenge has been that most of these adsorbents, natural and synthetic, are used in SPE as an extra step after extraction from samples. Once isolation has been achieved there is still an elution step and finally concentrating the analytes into levels detectable in chromatographic quantitation instruments. There is still need for new advancements that can eliminate these multiple step procedures. While the SPME approach eliminates these disadvantages, the technique still lacks robustness and reusability. With most of the current sample preparation techniques addressing environmental friendliness, low cost and simplicity, focus needs to turn towards miniaturization and automation of SPE techniques for PAHs. Recently automation and miniaturization approaches have been introduced in the analysis of PAHs and alkylated PAHs [8,186]. While disks have been reported in the analysis of PAHs [49], other miniaturized SPE techniques that can be explored include the pipette tip-based SPE and the multi-well SPE plates that are commonly used for automated SPE approaches [187]. The advantage of miniaturized approaches is that the amount of adsorbent and eluting solvent is greatly reduced while automation also offers better control over the analysis procedure giving results that are more accurate and precise.

Most optimization experiments do not investigate the effect of the presence of environmental parameters such as dissolved organic and inorganic matter. Another challenge is the observation that most of these adsorbents have been applied in either aqueous samples or spiked water compared to solid samples which harbour PAHs. PAHs are lipophilic and tend to preferentially accumulate on solid particles. While quantitation in water samples provides an immediate snap view of the current quantities directly linked to drinking water, considerations for potential leaching from soil, sediments and sludge into surface and ground water cannot be ignored.

The application of PMO and MOF nanomaterials has gained massive interest from analytical chemists. The introduction of doped functional ligands for PMOs and MOFs to provide tunable mesopores of specific size is an interesting research area as shown by the application of MIL101(Cr) and MIL100(Fe) ligands. The area that still needs improvement is the stability of the functional coating material on the surface on NPs. The search for stable supports that can be incorporated into the magnetic MOF composites remains a priority for researchers.

8 Conclusions

This review has attempted to address the adsorbents that have been applied in the analysis of PAHs from solutions and environmental samples. Generally, satisfactory progress has been made in the last decade considering that physicochemical properties of PAHs make them a difficult analyte to analyze. The traditional adsorbents like silica remain important while nanoparticles and molecularly imprinted polymers have gained ground. The synthesis of nanosorbents coated with functional organic moieties and the fabrication of molecular imprinted polymers for PAHs forms part of the progress in the analysis of PAHs. SPE remains a promising isolation approach and more research that include possibility of online coupling with chromatographic instruments is needed that will allow for easy application in analysis of PAHs from complex environmental samples. New trends and advancement of current ones are needed in finding better periodic mesoporous organic silica, metal

organic frameworks functionalization and molecularly imprinted polymer fabrication approaches in the analysis of PAHs.

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4.2 PAPER 2

This paper entitled “Synthesis and characterization of a molecularly imprinted polymer for the isolation of the 16 US-EPA priority polycyclic aromatic hydrocarbons (PAHs) in solution” has been published in *Journal of Environmental Management*. The article reports rigorous MIP-cavity tuning experiments and batch adsorption studies that led to a smart sorbent consisting of benzo[k]fluoranthene-imprinted and indeno[123-cd]pyrene-imprinted polymers mixed at 1:1 (w/w) with a high affinity for sixteen polycyclic aromatic hydrocarbons.

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Synthesis and characterization of a molecularly imprinted polymer for the isolation of the 16 US-EPA priority polycyclic aromatic hydrocarbons (PAHs) in solution

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Abstract

A smart sorbent consisting of benzo[k]fluoranthene-imprinted and indeno[1 2 3-cd]pyrene-imprinted polymers mixed at 1:1 (w/w) was successfully screened from several cavity-tuning experiments and used in the isolation of polycyclic aromatic hydrocarbons from spiked solution. The polymer mixture showed high cross selectivity and affinity towards all the 16 US-EPA priority polycyclic aromatic hydrocarbons. The average extraction efficiency from a cyclohexane solution was 65 ± 13.3% (n = 16, SD). Batch adsorption and kinetic studies confirmed that the binding of polycyclic aromatic hydrocarbons onto the polymer particles resulted in formation of a monolayer and that the binding process was the rate limiting step. The imprinted polymer performance studies confirmed that the synthesized polymer had an imprinting efficiency of 103.9 ± 3.91% (n = 3, SD). A comparison of the theoretical number of cavities and the experimental binding capacity showed that the overall extent of occupation of the imprinted cavities in the presence of excess polycyclic aromatic hydrocarbons was 128 ± 6.45% (n = 3, SD). The loss of selectivity was estimated at 2.9% with every elution cycle indicating that the polymer can be re-used several times with limited loss of selectivity and sensitivity. The polymer combination has shown to be an effective adsorbent that can be used to isolate all the 16 US-EPA priority polycyclic aromatic hydrocarbons in solution.

Keywords:
Polycyclic aromatic hydrocarbons; Molecular imprinting technology; Molecularly imprinted polymers; Benzo[k]fluoranthene; Indeno[1 2 3-cd]pyrene

1 Literature review

Polycyclic aromatic hydrocarbons (PAHs) belong to an important class of persistent organic pollutants that are commonly found in the environment at low concentrations. 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 like roads, parking areas and construction sites, industrial effluents from the manufacture of chemicals and carbonaceous waste incineration. They then enter the wastewater treatment plants through the sewerage (Zorpas et al., 2011; Cai et al., 2007; Singh and Agrawal, 2008). The general adverse effects of PAHs to the environment and humans is widely documented (Abdel-Shafy and Mansour, 2015;Maliszewska-Kordybach, 1999; Dean and Suess, 1985). Environmental and potential carcinogenic health concerns of PAHs have necessitated their inclusion in lists of priority pollutants by several regulatory agencies such as the US Environmental Protection Agency (US-EPA), European Union 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).

Most of the techniques used in the extraction of PAHs are followed by a clean-up step in which the PAHs are isolated from the matrix effects and finally pre-concentrated for detectability in chromatographic instruments (Rawa-Adkonis et al., 2006). This step is almost always through solid phase extraction (SPE) or gel permeation chromatography (GPC) with various adsorbent phases such as octadecyl (C_{18}) and molecularly imprinted polymers (MIPs) while membrane-based extractions like membrane assisted solid extraction and liquid phase micro extractions have been used (Chimuka et al., 2011; Hussen et al., 2007; Cacho et

al., 2003; Ferrer and Barcelo, 1999). The use of MIPs as sorbents for SPE is currently trendy because of their high selectivity towards target analytes. This technique involves fabrication of polymer material with specific recognition sites for the target analyte. The ability of MIPs to selectively bind the target analyte is based on the theory that after removal of the template molecule the interactions between complementary functionalities present in the imprint molecule and the monomer(s) prior to the initiation of polymerization are conserved in the product polymer. The technique of using these polymers as SPE adsorbents for analyte isolation is referred to as molecularly imprinted solid phase extraction (MI-SPE).

The molecular imprinting technology occurs by polymerization of a functional monomer and a cross-linker in the presence of a template. The functional monomer interacts with the template molecule through non-covalent interactions like van der Waals and hydrogen bonding while the cross-linker controls the morphology of the polymer by imparting mechanical stability to the polymer formed (Augusto et al., 2010; Spivak, 2005; Sarafraz-Yazdi and Razavi, 2015). During polymerization, 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 synthesis of MIPs for PAHs offers a challenge because PAHs are lipophilic with no pronounced functional groups. Thus steric cavities are created through hydrophobic and p-p interactions. The common PAHs that have been used are the three-ringed anthracene (Ant) and phenanthrene (Phen), the four-ringed pyrene (Pyr) and fluoranthene (Fln) and the five-ringed benzo[a]pyrene (B[a]P) (Baggiani et al., 2007; Çorman et al., 2017; Dickert et al., 1999; Hassan et al., 2016; Ho et al., 2011; Lai et al., 2004; Li and Wang, 2013). The use of Pry and B[a]P as the most preferred imprinting choices is rather contrasting because B[a]P is highly

carcinogenic and serves as a marker for PAH pollution while the suitability of Pyr as an imprinting molecule is because it is not considered carcinogenic or mutagenic. Cavities imprinted with three-ringed PAHs are too small for sequestration of the high molecular weight (HMW) PAHs. Other researchers have used a mix of different PAHs as templates for one MIP (Song et al., 2012; Krupadam et al., 2010a). This type of MIP fabrication is called multi-template MIP imprinting. Dummy template imprinting has also been reported where a compound with a structural orientation close to the target analyte (s) is used (Ho et al., 2010; Kirsch et al., 2001).

The specificity of the imprinted polymer becomes a disadvantage especially where several analytes such as the 16 US-EPA priority PAHs are targeted. The search for a good polymer must focus on maximizing the number of target analytes that can be extracted from solution. There is need for MIP-cavity tuning experiments in the search for an efficient MIP that could effectively extract all the 16 US-EPA priority PAHs. Most of the reported template or mixed template choices in the fabrication of MIPs for PAHs are unoptimized with researchers using a trial-and-error and/or experttemplate selection approach. Most of the PAH-imprinted polymers that have been reported were mainly used to target a single PAH or a portion of the 16 US-EPA priority PAHs. Multi-template imprinting in which all the 16 US-EPA priority PAHs were used as a template is rather uneconomic and not green. The premise of this study was that there is that one PAH that can give cavity imprints with a high affinity for all the 16 US-EPA priority PAHs. The purpose of this work was therefore to do MIP cavity tuning experiments using several potential PAH imprints to synthesize a polymer that can efficiently extract all the 16 US-EPA priority PAHs from solution.

2 Research methodology

2.1 Chemicals and reagents

A 2000 mg L^{-1} PAH mixture with 99.9% purity was obtained from Supelco (Bellefonte, PA, USA). Pyrene, fluoranthene, benzo[a] pyrene, benzo[k]fluoranthene, benzo[ghi]perylene and indeno[1 2 3-cd]pyrene were all obtained in powder form from Sigma-Aldrich (Johannesburg, South Africa). Hexane, heptane, methanol, dichloromethane and cyclohexane were purchased from SigmaAldrich (Johannesburg, South Africa).

2.2 Instrumentation

For temperature controlled shaking, a WiseCube WIG-105 Precise Shaking Incubator by Wisd Laboratory Instruments, Wertheim, Germany was used. A Pegasus 4D two-dimensional gas chromatograph and time-of-flight mass spectrometer (GC-TOF/MS) (LECO Corp., St. Joseph, MI, USA) was used for quantitation of PAHs. The chromatograms were obtained on a 7890B GC System with a 7683B Series Injector (Agilent Technologies, DE, USA). Separation of analytes was achieved on a 30 m × 0.32 mm x 25 mm RXI - 5SIL MS capillary column (Restek Corp., Bellefonte, PA, USA). The temperature program was set at an initial value of for 0.5 min. It was then ramped in the 5 - 20°C range and hold times between 0.5 and 3 min from 80 to 310 °C. The total run time was 32 min. The ionization mode was electron impact (E þ70 eV). Data processing was done using ChromaTOF software (LECO Corp, St. Joseph, MI, USA) version 4.5.1.

2.3 Selection of an efficient PAH-imprinted MIP

The approach was to investigate and select a PAH that could be used as a template to synthesize a MIP that would quantitatively extract all the 16 US-EPA priority PAHs. Six different PAHs were chosen for the investigation based on their steric properties and/or structural orientation of the rings. Of the six PAHs, three of them were pyrene-based and the other three were fluoranthene-based with a 5-membered ring in their structure. The pyrene-based PAHs were Pyr (4 rings),

B[a]P (5 rings) and the 6-ringed indeno [1 2 3-cd]pyrene (Indeno). The fluoranthene-based were Fln (4 rings), benzo[k]fluoranthene (B[k]F) with 5 rings and benzo[ghi] perylene (B[ghi]P) which has 6 rings. In all studies, p-vinylbenzene was used as the functional monomer. Styrene is more complementary to the PAHs than the vinylpyridine and methylmethacrylate monomers. Ethylene glycol dimethacrylate (EGDMA) was used as a cross-linker because of its flexibility compared to p-divinylbenzene (Spivak, 2005).

Some of the MIPs synthesized from individual imprints were physically combined at 1:1 (w/w) and investigated. The investigated combinations were based on structural orientation and number of rings of the imprints. For example, a Pyr-imprinted MIP was combined with a Fln-imprinted MIP (both are LMW and have 4 rings), the Pyr-imprinted MIP was also combined with a B[a]P imprinted MIP (sterically related 4 and 5-ringed imprints) and with a B[ghi]P-imprinted MIP (sterically related 4 and 6-ringed imprints). Eventually, the B[a]P-imprinted MIP would also be combined with a B[ghi]P-imprinted MIP (sterically related 5 and 6-ringed imprints). The same reasoning was used in combining the Fln-based imprinted MIPs. There were nine possible MIP combinations investigated. Thus, a total of 15 MIP and MIP combinations were screened as potential adsorbents.

2.3.1 Synthesis of the MIPs

In a round bottom flask, a mixture of 3 mL of dimethyl sulfoxide and 0.03618 mmol (equivalent to 7 - 10 mg) of the PAH template was added. It was then heated to 60°C whilst stirring at 500 rpm for 5 min to completely dissolve the template. The pvinylbenzene functional monomer (20 mL) was then added into the dissolved mixture whilst stirring and heating for a further 30 min. The EGDMA cross-linker (400 mL) was added into the mixture. Heating and stirring were continued for another 60 min. The system was purged with nitrogen gas for 5 min to remove oxygen from the reactor. The initiator (10 mg of 4,4-azo(4-cyanovaleric acid)) was then added and the temperature was slowly increased to 80°C. Polymerization was achieved within an hour. The system was allowed to

cool down to room temperature. The MIP was then transferred into a petri dish and dried in an oven at 70°C. The dry MIP was then ground into a fine powder and transferred into a 50 mL vial. A total of six polymers imprinted with the PAHs chosen for investigation as potential imprints were synthesized. A 50 mL solution of hexane/dichloromethane (4:1 v/v) was then added into the six vials. Dichloromethane was used to remove the porogen molecules trapped within the MIP 3D structure. While removal of the imprint molecule creates cavities, the removal of porogen molecules creates some diffusion pathways that become essential during re-adsorption of analytes and their subsequent elution from the cavities. The vials were then placed on a WiseCube shaking incubator. Shaking was done at 150 rpm for 4 h with temperature maintained at 45°C. The supernatants were then analyzed for the imprint molecule using GC-TOF/MS. This was then repeated every 2 h until the imprint PAH was no longer detected. A non-imprinted polymer (NIP) was also synthesized in a similar way as the MIP but in the absence of a template. Synthesis and washing of NIPs and MIPs was done simultaneously.

2.3.2 MIP screening studies

A 5 mL cyclohexane solvent was spiked at 2 mg mL-1 PAH mixture. A mass of 20 mg of the MIP was then dropped into the solution and allowed to adsorb PAHs for 3 h. The mixture was then filtered through 0.45 mm frits. The filtrate was analyzed for PAHs using a GC-TOF/MS. The same procedure was done simultaneously for all the synthesized MIPs individually and as combinations. A PAH that was favoured by a particular MIP or MIP combination would have a low peak area in the supernatant. The MIP favoured by most of the PAHs was chosen as the best one for the extraction of the 16 US-EPA priority PAHs and was used in subsequent studies.

2.4 Batch studies

2.4.1 Effect of initial concentration

The effect of initial concentration of PAHs in solution was investigated by placing 10 mg of the selected MIP directly into six 2 mL cyclohexane solutions spiked in the 0.1 - 5 mg mL-1 range with the 16 PAHs. Binding was allowed to occur at ambient conditions for 60 min. The amount of each PAH extracted by the MIP 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 Eqn (1). The total adsorption capacity at each spiking concentration was then calculated as the sum of the individual capacity values using Eqn (2). The imprinting factor was calculated as the ratio of equilibrium adsorption capacity values of the MIP and the NIP using Eqn (3). Calculating the binding capacity value was essential in determining the amount of MIPs that can be used for a routine PAH. The procedure used in calculating the MIP-binding capacity was also used to determine the NIP-binding capacity. The imprinting factor (IF) was then calculated as a ratio of the binding capacity of the MIP to that of the NIP (Eqn (3)). The IF value helps in evaluating the adsorption effectiveness of the MIP.

$$q_{PAH} = (C_i - C_f)V/m \quad (1)$$

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)

$$q_e = \sum q_{PAH} \quad (2)$$

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

$$IF = q_{MIP}/q_{NIP} \quad (3)$$

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 in order to explain the extent of adsorption. The linearized Langmuir and Freundlich adsorption isotherm models are described using Eqns. (4) and (5) respectively. The linearity of the plotted graphs was evaluated as the coefficient of determination (r^2). The acceptable model was the one with the highest r^2 value.

$$C_e/q_e = 1/bq_m + C_e/q_m \quad (4)$$

where C_e is equilibrium concentration (mg L^{-1}), q_e is the amount adsorbed at equilibrium (mg g^{-1}), q_m is maximum adsorption capacity (mg g^{-1}) and b is constant related to energy of adsorption (L mg^{-1}).

$$logq_e = logK_f + (1/n)logC_e \quad (5)$$

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

2.4.2 Effect of contact time

The effect of contact time on the attainment of the equilibrium binding capacity was investigated from 20 - 300 min. A mass of 10 mg of the MIP was placed in a 5 mL cyclohexane solution containing 2 mg mL^{-1} of PAHs. Aliquots (10 mL) of the solution were collected at 20, 40, 60, 80, 100, 120, 150, 200, 250 and 300 min. The aliquots were injected manually into the GC system. The data obtained was used in plotting the linearized Lagergren pseudo first and second order models using Eqns (6) and (7) respectively. The mechanism of adsorption was then explained using the model with the highest r^2 value.

$$log(q_e - q_t) = logq_e - k_i t/2.303 \quad (6)$$

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

$$t/q_e = 1/k_2 q_e{}^2 + t/q_e \quad (7)$$

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

2.5 MIP-PAH elution solvent

PAHs are non-polar compounds and interact with MIP cavities through van der Waals and π-π interactions. Thus heptane, hexane, toluene and dichloromethane were investigated. Polar solvents like methanol and acetonitrile were not investigated because they are mainly used in MIP-template elution where the target is to break H-bonding (Martín-Esteban, 2016; Martin-Esteban, 2001). In addition, polar solvents like methanol are not compatible with GC analysis and their use as eluent would require reconstitution with a nonpolar solvent for injection into the GC system. The order of ascending polarity of the chosen solvents is heptane < hexane < toluene < dichloromethane.

The selected polymer (120 mg) was directly placed in 10 mL of cyclohexane spiked at 500 mg mL^{-1} with the 16 PAHs. Extraction was done over 3 h at 40°C. The MIP was then divided into 10 mg portions. Each of the four solvents (3 mL) was then used to elute the PAHs from the MIP cavities. The eluting solvents were added in 3 x 1 mL aliquots. Hot solvents maintained at 40°C were also tested in order 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 GC system. Peak areas were then 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.

2.6 Performance assessment studies

2.6.1 Harvesting of MIP

The amount of imprint used and the capability of the imprinted polymer were compared in order to explain and validate the feasibility of synthesizing the MIP and applying it as a viable alternative in quantifying PAHs from environmental samples. The imprinting ability was calculated as the amount of the imprinting PAH needed to synthesize a unit mass of the MIP using Eqn (8). An imprinting efficiency was evaluated in order to understand the relationship between the amount of the imprint PAH used and the maximum amount of the PAHs that can be adsorbed by the synthesized MIP. The imprinting efficiency was calculated as the ratio of the imprinting ability versus the experimental adsorption capacity using Eqn (9).

$$q_i = m_i/m_{mip} \quad (8)$$

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 synthesized MIP in g.

$$IE = q_m/q_i \times 100 \quad (9)$$

where IE is the imprinting efficiency and q_i is the imprinting capacity per unit mass of the MIP (mg g^{-1}).

2.6.2 Imprinted cavity efficiency

The theoretical number of imprinted cavities per unit mass of the MIP was calculated using Eqn (10). This value was compared with the experimental number of cavities which was itself determined using Eqn (11). Finally, the cavity efficiency was calculated as the fraction of MIP cavities that are available for sequestrating PAHs and Eqn (12) was used.

$$n_{PC} = q_i N_A/M \quad (10)$$

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})

$$n_{EC} = \sum_{i=1}^{16} (q_m N_A / M) \quad (11)$$

where n_{EC} is the number of experimental cavities per unit mass of the MIP (g^{-1}), q_m is the maximum adsorption capacity per unit mass of the MIP (g g^{-1}) and M is the relative molecular weight of the adsorbed PAH (g mol^{-1})

$$CE = n_{EC}/n_{PC} \times 100 \quad (12)$$

where CE is the cavity efficiency.

2.6.3 Regeneration of the MIP

The prospects of engineering a polymer that can be re-used in its original state are part of green chemistry technology. The efficiency of the synthesized MIP after repeated application in adsorbing PAHs from solution was investigated. A mass of 10 mg of the MIP was placed in a 5 mL cyclohexane solution spiked at 1000 mg mL-1. After a 2 h extraction time, the MIP was removed and the final concentration of the solution used in calculating the equilibrium adsorption capacity using Eqns (1) and (2). The MIP was then washed at least five times with hexane or until no PAH was identified in the eluent. The same MIP was then re-used on a fresh spiked solution of the same concentration and volume. The procedure was repeated seven times. The re-usability of the MIP was evaluated by comparing the equilibrium adsorption capacity at each extraction cycle.

3 Results and discussion

3.1 Synthesis and selection of suitable MIP

3.1.1 Selecting the best PAH-imprinted MIP

To ensure that MIP that maximizes extraction of PAHs was selected, the three-top performing imprinted polymers and polymer combinations in the extraction of each of the 16 US-EPA priority PAHs were selected. The idea was that the best imprinted MIP should appear in the top 20% performing polymers and polymer combinations for the extraction of each PAH. A summary of the preferred MIPs in the extraction of individual PAHs is given in Table 1. Naphthalene, acenaphthylene, acenaphthene and fluorene were excluded from the results analysis because they have large peak areas which made it almost impossible to analyze the results of the other PAHs. It was observed that almost all the PAHs did not favour themselves as imprints with fluoranthene being an exception. Previous studies have reported that MIP imprinted by a particular PAH would favour the sequestration of that PAH and other PAHs closely related to it (Baggiani et al., 2007).

Our observation might be related to possibility of competition for binding sites considering that selection of the MIP was done by extracting all the 16 PAHs simultaneously. The MIPs synthesized from the 5-ringed PAH templates appear prominently in their binding preference for 6-ringed PAHs. For example, the B[k]F and B[a]P imprinted MIPs were most effective in the sequestration of the three 6-ringed PAHs. This scenario has also been reported elsewhere in which 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 5 and 6-ringed PAHs and the higher hydrophobicity of the 6-ringed PAHs, allow for a stabilized interaction between a HMW PAH and the cavities of a MIP imprinted with a 5-ringed PAH (Hafidi et al., 2008). This observation implies that the imprinting

PAH and other sterically related PAHs have to seek alternative binding sites. For the 5-ringed PAH, a preference towards MIPs imprinted with the HMW PAHs was observed. Likewise, the 4-ringed benzo[a]anthracene and chrysene seemed to be favoured by MIPs imprinted with 5-ringed PAHs. Only the MIPs imprinted with pyrene and fluoranthene 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 of the PAHs except anthracene, fluoranthene and benzo[b]fluoranthene (Table 1). The low MW PAHs (with 3 - 4 rings) mostly preferred the fluoranthene/ pyrene combination.

This was because fluoranthene and pyrene are both four membered rings resulting in imprint cavities that were just big enough for the three and four 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 like the benzo[a]anthracene and chrysene.

For the HMW PAHs (with 5 - 6 rings), there was a high affinity for the B[k]F/Indeno combination and other combinations that contain cavities imprinted by these two PAHs. The possible explanation for this observation could be the hydrophobicity (Log K_{ow}) of the PAHs, the size and shape of the cavities. When the number of rings of the PAHs increases, the polarity decreases making them 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 easily bind. 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 the 16 PAHs. This combination was chosen because it was the most appearing combination as given in Table 1. Where this combination is not present, they were preferred as either B[k]F or Indeno or their combinations with another MIP.

Table 1

Summary of PAHs and their MIP template preferences; only the top 3 preferred imprinted MIPs and imprinted MIP combinations out of 15 polymers and polymer combinations are shown. Polymer combination marked in RED was selected as the best. The polymers marked in BLUE show where the polymer combination performed better alone or in combination with other polymers.

PAH	Rings	[a]Preferred imprinting PAH and/or PAH combinations
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
Benzo[b]fluoranthene	5	Indeno > B[k]F > B[k]F/B[a]P
Benzo[k]fluoranthene	5	B[a]P/B[ghi]P > Indeno/B[ghi]P≈ B[k]F > B[k]F/B[a]P
Benzo[a]pyrene	5	Py/B[a]P≈ Indeno > B[a]P/B[ghi]P≈ B[k]F/B[a]P
Indeno(1,2,3-cd) pyrene	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

3.1.2 Implications of the selected MIP combination

The B[k]F and Indeno-imprinted MIP combination was selected after performing rigorous optimization 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 Pyr and Fln-based PAHs investigated as potential imprints. The observations made are an indication that cavity tuning experiments are essential if the target is to synthesize a polymer with a high affinity for a number of structurally related analytes. Most of the reported tuning

experiments have mainly considered changing the monomer and the cross-linker in order to maximize interaction between the analyte and the cavity active groups. Tuning experiments using various template molecules becomes important if the target is to maximize the number of analyzed PAHs.

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 analyze 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 (6) different PAHs and targeting the PAHs used as the imprints (Dickert et al., 1999).

3.1.3 Characterization

The BET surface area characterization 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. The leached MIP exhibited more surface area (386.6 $m^2 g^{-1}$) when compared to NIP (360.6 $m^2 g^{-1}$) and the unleached MIP (332.6 $m^2 g^{-1}$). 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.

The TGA results in Fig. 1 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 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.

3.2 Batch adsorption

3.2.1 Effect of initial concentration

The Langmuir isotherm was the dominant mechanism of adsorption with an r2 value of 0.9961 compared to the 0.9661 value for the linearized Freundlich isotherm model. 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 as the mechanism of adsorption and its assumptions were used in further explaining the extent of adsorption of PAHs on to the MIP surface.

3.2.2 Effect of contact time

The effect of contact on the binding capacity of the MIP is summarized in Fig. 2. Generally, the graph shows a strong dependence of adsorption on contact time. The MIP attained its maximum binding capacity within 80 min. When a polynomial of 5^{th} order with a 0.9348 coefficient of determination was inserted through the data points (Fig. 2), the estimated maximum binding capacity was 5.19 ± 0.392 mg g^{-1}. The non-imprinted polymer had a maximum capacity of 2.49 ± 0.271 mg g^{-1}. The binding preferences summarized in Table 2 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 stabilized 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 delocalized electrons that participate in the π - π interactions with the functional monomers. However, the synthesized MIP still displayed a broad selectivity towards the 16 PAHs with no imprinting effect observed (Table 2). The individual binding capacities ranged from 200.8 - 493.9 mg g^{-1} with fluoranthene and benzo[ghi]perylene recording the lowest and highest capacities respectively. This was related to the extraction efficiencies for individual PAH which ranged from 40.2 - 98.8%. The average binding capacity

was 317 ± 45.1 mg g^{-1} (n = 16, SD). The average extraction efficiency of the MIP in extracting all the 16 PAHs from a spiked solution was 65 ± 13.3% (n = 16, SD).

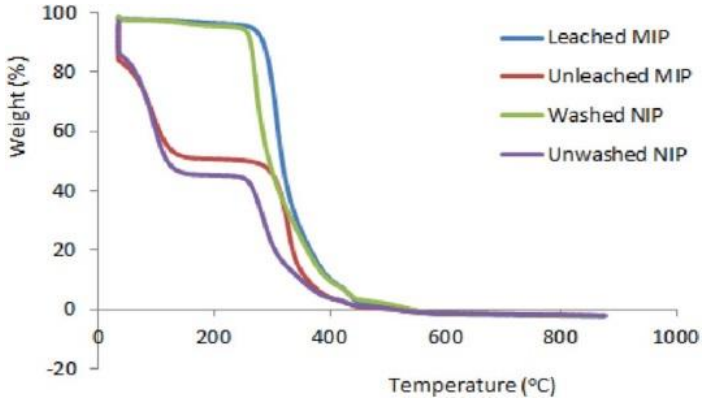


Fig. 1. Thermal stability of the synthesized polymer combination.

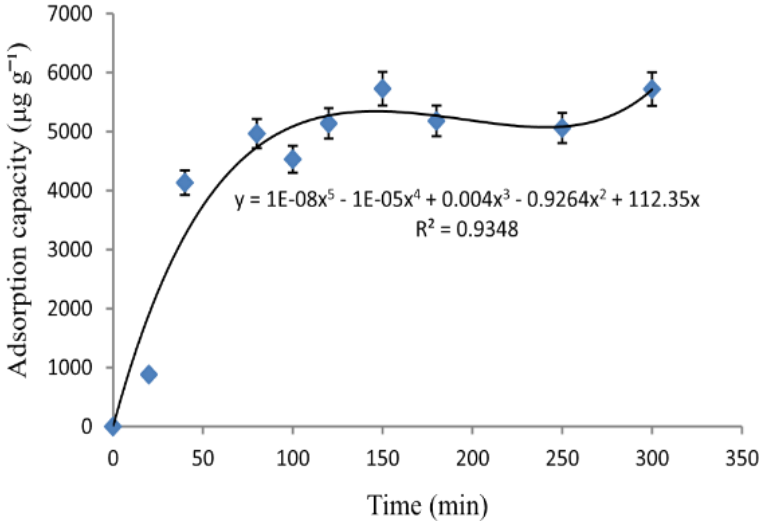


Fig. 2. Effect of contact time on adsorption capacity (n = 3, SD).

3.2.3 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

linearized Lagergren pseudo second order equation is presented in Fig. 3. The implication of the pseudo second order model is that 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 Fig. 3 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 in describing the extent of adsorption of PAHs in MIP cavities. The rate constant (k_2) predicted from the intercept of the model in Fig. 3 was 0.784 min^{-1}. This value implies that there was fast mass transfer of PAHs into the imprinted cavities. The Lagergren pseudo first order rate constant was 0.049 min^{-1}.

Several articles have reported the performance of PAH-imprinted MIPs. Song et al. (2012) have synthesized a multi-template MIP for PAHs with a binding capacity of 2.7 mg g^{-1}. In this rare approach, all the 16 US-EPA priority PAHs were used for imprinting the MIP. The imprinting factor was 2.25. A similar multi- template MIP synthesized using all the 16 PAHs by Krupadam et al. (2009) had a binding capacity of 6.93 mg g^{-1} which is comparable with the 5.19 mg g^{-1} obtained in this study. When the same authors used a 5-template MIP, the binding capacity was 1.93 mg g^{-1}. When a sixth PAH was added to produce a 6-template MIP, the binding capacity dropped to 0.69 mg g^{-1} (Krupadam et al., 2010a, 2010b). The imprinting factors were 4.5, 2.4 and 5.8 respectively. Ho et al. (2010) reported 0.02 mg g^{-1} for a B[a]P imprinted MIP synthesized for the extraction of B[a]P.

Table 2
MIP adsorption preferences at maximum binding capacity (n = 3). Extraction was done from a spiked cyclohexane solution.

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	16	5188.8	317.6	100.0

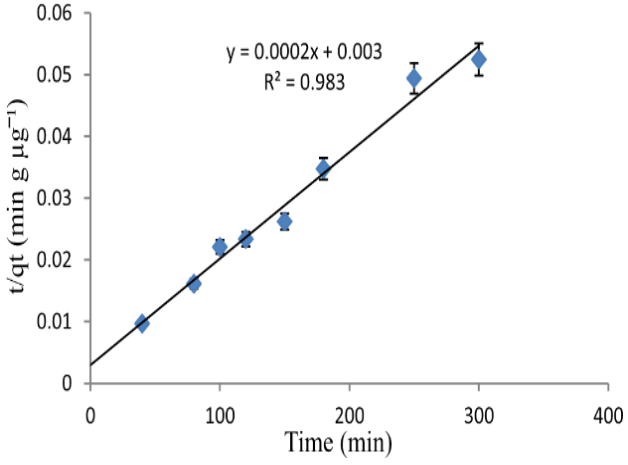


Fig. 3. Pseudo second-order kinetic adsorption of PAHs on the surface of the MIP (n = 3, SD).

Adsorption capacities higher than that of the MIP synthesized during this study have been reported. In most cases, such binding capacities were tested on the imprinting molecule only. Hassan et al. (2016) got a binding capacity towards anthracene of 374.3 mg g^{-1} using an anthracene-imprinted MIP. In another study, the same authors reported a relatively lower adsorption capacity of 0.0078 mg g^{-1} for the 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 g^{-1}. Li and Wang (2013) reported 15.57 mg g^{-1} for phenanthrene using a phenanthrene-

imprinted MIP while Tiu et al. (2016) recorded a binding capacity of 11.37 mg 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.

3.3 MIP-PAH elution solvent

A good choice of eluting solvent is that which maximizes 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 Fig. 4. However, the 5 and 6 ringed PAHs were eluted better by the least polar solvent, heptane. When both hexane and heptane were heated to 40°C and investigated as potential elution solvent, their eluting power was greatly enhanced as shown in Fig. 5. Hot hexane still performed well for naphthalene, acenaphthylene, acenaphthene and fluorene. Hot heptane was more effective in the elution of most of the PAHs from MIP cavities. Other advancements of the choice of eluting solvent in MI-SPE analyte elution include the use of dichloromethane in combination with other solvents like acetic acid and hexane followed by GC determination have been reported in elution of PAHs from MIP cavities (Ho et al., 2011; Song et al., 2012). However, dichloromethane has been used without comparing its extracting power with other theoretically relevant non-polar solvents like hexane.

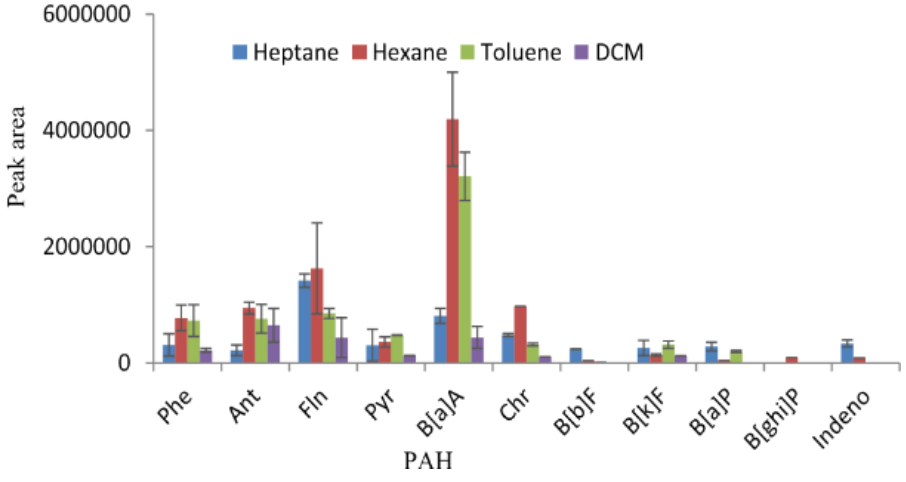


Fig. 4. Performance of four solvents in eluting PAHs from MIP cavities (n = 3, SD). Phe (phenanthrene), Ant (anthracene), Fln (fluoranthene), Pyr (pyrene), B[a]A (benzo[a] anthracene), Chr (chrysene), B[b]F (benzo[b]fluoranthene), B[k]F (benzo[k]fluoranthene), B[a]P (benzo[a]pyrene), B[ghi]P (benzo[ghi]perylene, Indeno (indeno[1 2 3-cd]pyrene).

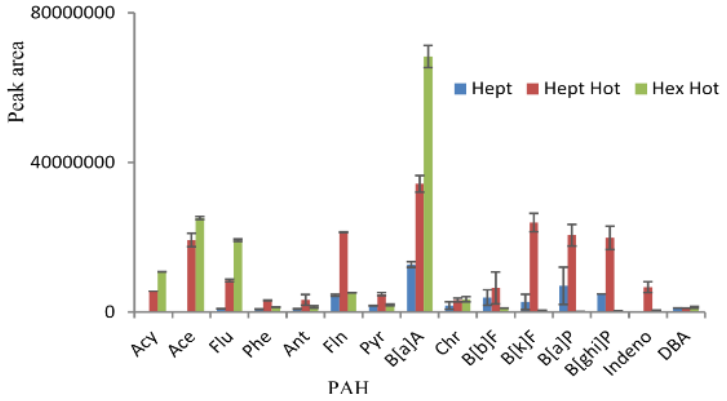


Fig. 5. Acy (acenaphthylene), Ace (acenaphthene),Flu (fluorene), Ant (anthracene), Fln (fluoranthene), Pyr (pyrene), B[a]A (benzo[a]anthracene), Chr (chrysene), B[b]F (benzo[b]fluoranthene), B[k]F (benzo[k]fluoranthene), B[a]P (benzo [a]pyrene), B[ghi]P (benzo[ghi]perylene, Indeno (indeno[1 2 3-cd]pyrene), DBA (dibenzo[ah]anthracene).

3.4 MIP performance results

3.4.1 Imprinted cavity efficiency

The results in Table 3 show that 10 mg of imprinting PAH resulted in synthesis of an average of 2.002 g of the polymer. This mass was obtained after the MIP had been washed to remove the template. This result equates to 5 mg of an imprinting PAH being needed to synthesize 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. This gives an imprinting efficiency of 103.9%. This level of efficiency sounds impractical and was not expected. The possible explanation is

that the experimental adsorption capacity does not eliminate the effect due to those PAHs that adsorb on the hydrophobic backbone of the MIP.

The theoretical background of attempting to calculate the imprinting efficiency is based on the assumption that one imprint molecule results in formation of a single cavity and each cavity can only adsorb one molecule. This idea is also supported by batch adsorption results in which a monolayer adsorption mechanism was predicted. Based on this assumption, a 10 mg mass of the imprinting PAH would give a total of 1.19×10^{19} cavities per gram of MIP. This value was estimated using Eqn (11). The experimental number of cavities calculated from the maximum adsorption capacity using Eqn (12) was 1.53×10^{19} per gram of the synthesized 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 3
Imprinting efficiency results (n = 3)

	Average	%RSD
Mass of MIP (g)	2.002	3.76
Mass of imprint (mg)	10.00	-
Imprinting ability (mg g^{-1})	5.002	3.79
Experimental adsorption capacity (mg 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	[a]5.04

[a]Calculated as a propagation of errors.

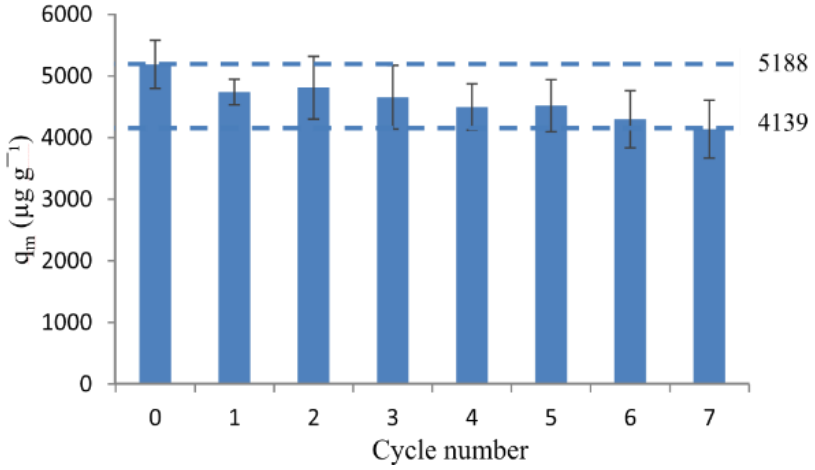


Fig. 6. Effect of MIP re-use on its binding capacity (n = 3, SD).

3.4.2 Regeneration and re-use of the MIP

When the polymer combination was repeatedly used to extract PAHs at maximum conditions, a general decrease in the maximum adsorption capacity was observed (Fig. 6). 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% loss in the binding capacity with every washing cycle. After the MIP had been regenerated seven times, the maximum binding capacity had dropped to 4.139 from the initial 5.188 mg 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 331.1 mg of PAHs. The results of the regeneration studies have shown that the MIP can be re-used with minimal loss in its selectivity.

4 Conclusions

An adsorbent combination that consists of a benzo[k] fluoranthene-imprinted and indeno[123-cd]pyrene-imprinted polymer mixed at 1:1 (w/w) was successfully identified for the effective extraction of the 16 US-EPA priority PAHs from solutions. The polymer combination displayed a broad selectivity towards the 16

PAHs with no imprinting effect observed. The adsorption and kinetic studies showed that the adsorption process results in formation of a monolayer that attains a maximum capacity with 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. However, cavity efficiency studies showed that a small amount of the PAHs was adsorbing on the hydrophobic backbone of the polymer. The selected polymer combination can be used as an effective adsorbent in MI-SPE clean-up steps for the determination of priority PAHs. The results of this study are an indication that selecting an imprint molecule is essential in tuning MIP cavities if the target is to extract several analytes of related structural and sterical orientation.

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4.1 PAPER 3

This paper entitled "Development of a single step membrane assisted solvent extraction-molecularly imprinted polymer technique for extraction of PAHs in wastewater followed by gas chromatography mass spectrometry determination" has been submitted for peer review in *Journal of Chromatography A*. The article reports a membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) technique that was developed, optimized and applied in the analysis of PAHs in effluents from a wastewater treatment plant.

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Development of a single step membrane assisted solvent extraction-molecularly imprinted polymer technique for extraction of PAHs in wastewater followed by gas chromatography mass spectrometry determination

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Abstract

A technique that combines membrane assisted solvent extraction and molecularly imprinted polymer into a single step format has been successfully developed and evaluated for the extraction of the 16 US-EPA priority polycyclic aromatic hydrocarbons in wastewater samples followed by determination using gas chromatography coupled with time of flight mass spectrometry. The technique involves placing the polymer particles into a hydrophobic polypropylene membrane bag which is in contact with an aqueous donor phase. The technique was optimized for various parameters that include presence of an organic modifier in the donor phase, stirring rate and extraction time. The limits of detection using the technique were in the 0.01 – 0.45 ng mL^{-1} range. Linearity values determined in the 10 – 1 000 ng mL^{-1} calibration range were all above 0.9912. Extraction efficiencies of the 16 analytes calculated as averages for three spiking concentrations ranged from 66.8 – 96.8% (RSD = 3.1 – 12.3%). The optimized method was tested for the extraction of polycyclic aromatic hydrocarbons in wastewater samples in the presence of surrogate standards. The developed method showed great reproducibility with inter day repeatability values ranging from 0.6 – 24.9%. The analytes were quantified in the nanogram level showing that the developed technique is a viable alternative in the analysis of trace organic compounds in complex aqueous samples.

Keywords

Membrane assisted solvent extraction, molecularly imprinted polymer, wastewater, polycyclic aromatic hydrocarbon

Abbreviations

PAHs - polycyclic aromatic hydrocarbons, SPE, solid phase extraction, MASE - membrane assisted solvent extraction, SLM - supported liquid membrane, HF-LPME - hollow fibre liquid phase micro-extraction, MASE-MIP - membrane assisted solvent extraction-molecularly imprinted polymer, GC-TOF/MS - gas chromatograph in one-dimensional mode and time-of-flight mass spectrometer, DMSO - dimethyl sulfoxide, HMW - high molecular weight

1 Introduction

The analysis of complex samples still offers researchers a challenge due to the presence of various forms of matrix effects which eventually affect extraction efficiency of sample preparation techniques. The search for effective and selective extraction techniques for complex samples of both solid and aqueous origin remains a priority. The situation is exacerbated by the fact that some targeted analytes exist in trace amounts and have physicochemical properties similar to their matrix counterparts. One such group of pollutants with unfavourable properties are the polycyclic aromatic hydrocarbons. These compounds are ubiquitous in the environment with several studies reporting quantitation in solid and aqueous environmental samples, as well as in air and food samples [1–4].

Traditionally, the liquid-liquid techniques have been used in which the polycyclic aromatic hydrocarbons (PAHs) are transferred from the aqueous sample into an organic solvent followed by direct injection into chromatographic instruments or injection after reconstitution. This approach extracts a considerable amount of matrix effects. The liquid-solid approach has since been adopted to counteract the drawbacks associated with the traditional liquid-liquid techniques. A solid-liquid approach involves passing an aqueous sample through an adsorbent material packed in solid phase extraction (SPE) cartridges. The target analytes become isolated from matrix effects by binding on the adsorbent. For the analysis of PAHs in environmental samples, several adsorbents have been used as packing material for SPE cartridges and gel permeation chromatography columns. The major drawback in the analysis of PAHs is that PAHs are highly hydrophobic and lack functionality in their structure. The search for adsorbents specific for them becomes a challenge

because the impact of matrix effects remains pronounced leading to reduced recoveries and high limits of detection especially in samples such as wastewater.

Attempts to eliminate matrix effects in the analysis of complex samples has led to introduction of microporous membrane-based liquid-liquid extraction. These include the membrane assisted solvent extraction (MASE), a technique first used by Hauser and Popp (2001) [5–8] and the supported liquid membranes (SLMs) such as the miniaturized hollow fibre liquid phase micro-extraction (HF-LPME) first reported by Jönsson and Mathiasson (1999) [9–12] while emulsion liquid membranes [13] and ionic liquid membranes [14] have also been reported. Selectivity in MASE depends on polarity and size of the compounds which is similar to SLM in a two phase system unless a carrier or a three phase system is used. The hydrophobic and size-exclusive properties of the micromembranes used during these techniques are responsible for eliminating both hydrophilic compounds and higher molecular weight hydrophobic matrix compounds. Other advantages include high enrichment power and the potential for automation through online interfacing with chromatographic instruments [11,15–17]. The main drawback of membrane-assisted extraction techniques is that extraction is not exhaustive [16]. Membrane memory effects are also possible due to clogging of the surface and the pores of the membrane by high molecular weight compounds and other hydrophobic compounds [18].

Recent advances in the application of membranes has been to incorporate nanosorbents and immunoassays on the SLM in order to enhance further selectivity [13,19,20]. Electro-membranes have also been reported as a way of facilitating transfer across SLMs [21–26]. While the extract is free of matrix effects, the electro-membrane approach has a low sample throughput. Other studies have reported a non-porous polymeric membrane in combination with pressurized liquid extraction [27,28]. Another strategy has been to follow up the membrane technique with isolating the target analytes on a solid adsorbent. This is common with the MASE technique where the sorbent material can be placed in the lumen of the membrane or used as an SPE packing material [15,29–32].

The MASE technique for PAHs from aqueous samples has been reported before [6,33–36]. This technique alone is not a favourable approach in the analysis of PAHs

with March et al. (2011) recording recoveries of 12.5 - 23% for 7 PAHs from sea water [35], Zuin et al. (2006) recording 13.6% for benzo[a]pyrene in sugarcane solution samples [33] while Alcudia-Leon et al. (2009) did not report recoveries for their analysis of 5 PAHs in water samples [34]. However, Rodil et al. (2007) recorded recoveries above 65% for all the 16 US-EPA priority PAHs using the MASE method coupled with large volume injection programmed temperature vaporisation [6]. The same technique was reported by Prieto et al. (2008) and obtained recoveries of 81 – 116% in the analysis of the same PAHs in estuarine water samples [36]. Amdany et al. (2014) have used a semipermeable membrane device as a passive sampler for PAHs in dam water with recoveries of 55 - 123% [37]. The HF-LPME technique has been reported by Charalabaki et al. (2005) for the extraction of PAHs from wastewater effluent samples [38]. An adsorbent-assisted MASE technique in the analysis of six PAHs from water samples has been reported by Ge et al. (2012) with recoveries recorded at 88.1 – 110.9% [39].

The MIP-assisted membrane approaches have been reported within our research group for the analysis of triazines and 17β-estradiol in aqueous solutions. Both membrane bags [29] and flat membranes [40,41] in combination with MIPs were investigated. A membrane bag containing MIPs has been recently reported in combination with Soxhlet extraction for analysis of PAHs in wastewater sludge samples [42]. Other studies that have reported membranes and MIPs include Barahona et al. (2016) who used an HF-LPME coated with MIPs for extraction of triazines from aqueous samples [43]. Takeda et al. (2005) used a molecularly imprinted nylon membrane for enhanced selective extraction of L-phenylalanine [44].

This study aimed at investigating the potential of combining membrane assisted solvent extraction and a molecularly imprinted adsorbent into a single step format for analysis of PAHs in wastewater. The approach is herein referred to as the membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) technique. The premise is that when the analytes cross the membrane, they are selectively isolated by binding onto the MIP cavities. The bound PAHs can then be eluted using an appropriate solvent. MIPs were preferred as the adsorbent because of their

specificity towards target analytes and the ease of synthesis under laboratory conditions. Further, MIPs work best in non-polar organic solvents where their selectivity is retained. The single entity MASE-MIP technique eliminates the need for a separate clean-up of the membrane extract and forms part of the search for modern technologies aimed at giving high selectivity and minimizing the number of steps during analysis.

2 Methodology

2.1 Chemicals and reagents

A 10 μg mL^{-1} PAH calibration mix in acetonitrile (CRM 47940) with 99.0% purity was obtained from Supelco (Bellefonte, PA, USA). HPLC-grade dimethylformamide, acetonitrile, methanol and dichloromethane were purchased from Sigma-Aldrich (Johannesburg, South Africa). All chemicals used in preparation of the polymer were purchased from Sigma Aldrich (Johannesburg, South Africa).

2.2 Instrumentation and apparatus

Quantitation of PAHs was done on a Pegasus 4D gas chromatograph in one-dimensional mode and time-of-flight mass spectrometer (GC-TOF/MS) (LECO Corp., St. Joseph, MI, USA) with a 7683B Series auto-injector (Agilent Technologies, DE, USA). The PAHs were separated on a 30 m x 0.32 mm x 25 mm RXI-5SIL MS capillary column by Restek Corporation (Bellefonte, PA, USA). The temperature program was increased from 80 to 310°C over 32 min. Data processing was done using ChromaTOF software version 4.5.1. (LECO Corp, St. Joseph, MI, USA). An MS-MP8 magnetic stirrer by Daihan Scientific Co.,Ltd, Seoul, South Korea was used for stirring. Microporous polypropylene membrane bags of dimensions 4 cm long, 6 mm internal diameter, 0.22 μm pore size and 160 μm thickness were from Gerstel GmbH & Co. KG (Mülheim, Germany).

2.3 Synthesis of the molecularly imprinted polymer

The MIP used in this study was prepared using the method reported by Ncube et al. (2017) [45]. The adsorbent consisted of a benzo[k]fluoranthene and an indeno[1 2 3-cd]pyrene-imprinted polymer prepared separately and then mixed at 1:1 (w/w). This polymer combination is reported to have a high cross selectivity towards the 16 US-

EPA priority PAHs. It has a binding capacity of 5.188 mg g^{-1} with only 2.9% loss in selectivity when re-used [45].

2.4 General MASE-MIP extraction procedure

The MASE-MIP system was set up as shown in Fig. 1. The membrane extraction cell consisted of a 20 mL headspace vial filled with 15 mL of deionized water spiked with PAHs. The deionized water was spiked at 0.5 µg mL^{-1} individual PAH concentration. The membrane bag was attached to a metal funnel and fixed with a PTFE ring. The membrane bag was filled with 80 mg of the MIP particles and 1 mL of an organic acceptor phase. The membrane bag with MIP particles dispersed in the acceptor phase was placed inside the extraction cell containing the donor solution and stirred for pre-set 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. A steady vacuum pump was then applied for 1 min to dry off the MIP particles completely. The PAHs were eluted from the MIP cavities using 3 × 1 mL of an organic solvent. The solvent extract would then be reduced to 1 mL before being injected directly by GC-TOF/MS for identification and quantitation of PAHs.

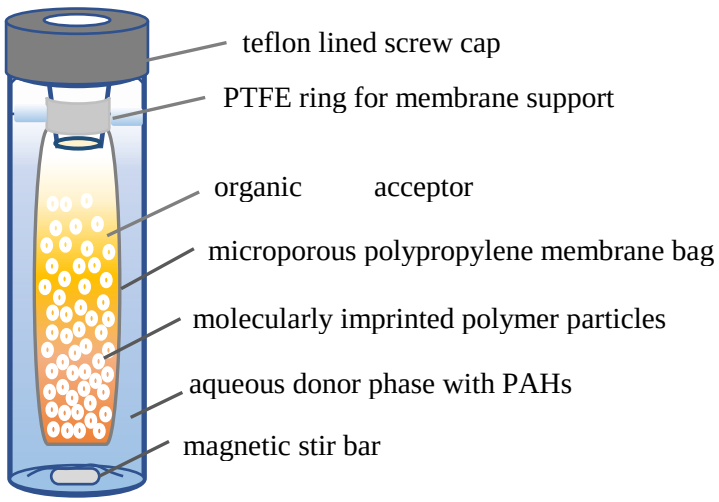


Fig 1 Experimental set-up of the membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) technique

2.5 Optimization of the membrane assisted solvent extraction combination-molecularly imprinted polymer technique

Parameters adopted from the MIP optimization experiments by Ncube et al. (2017) were the use of heptane as the acceptor phase in the membrane bag and also as the eluting solvent for GC-TOF/MS analysis [45]. Heptane is non-polar and immiscible with water making it a good acceptor phase for PAHs. The amount of the MIP placed in the membrane bag was also maintained at 80 mg [45]. Spiked deionized water was used as the donor phase.

2.5.1 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 [6,35,36]. Polar organic solvents that dissolve in water are used in minimizing this effect. Ethanol, acetonitrile, methanol and dimethyl sulfoxide (DMSO) were investigated as potential organic modifiers. Each organic modifier was added at 15% of the aqueous donor solution containing 0.5 μg mL^{-1} of individual PAHs. Stirring was done at 420 rpm for 60 min. Once the best modifier had been identified, its composition in the donor phase was also optimized. The composition was investigated in the 10 – 50% (v/v) range. The optimized amount of the organic modifier was then used in subsequent experiments.

2.5.2 Effect of stirring rate

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. The effect of stirring was investigated from 420 – 1000 rpm. Extraction was done for 20 min from a 0.5 μg mL^{-1} aqueous donor phase modified with 25% DMSO. The optimum stirring rate was used in subsequent optimization studies.

2.5.3 Effect of contact time

The effect of contact time on the extent of transfer of PAHs across the membrane and their eventual binding onto the MIP cavities was investigated up to 4 h. Extraction was done from a donor phase spiked at 0.5 μg mL^{-1} and modified with 25% DMSO. Stirring was done at 1000 rpm.

2.6 Transfer kinetics

The extent of transfer of individual PAHs from the donor solution across the membrane and their eventual binding on the MIP cavities under optimum conditions was evaluated as extraction efficiency using Eqn. 1. The efficiencies were given as an average for extracting deionized water spiked at 300, 600 and 900 ng mL^{-1} concentration levels.

$$E = C_A V_A / C_D V_D \times 100 \quad (1)$$

where E is the enrichment factor, C_D and V_D are the concentration of a PAH in the donor phase before extraction in ng mL^{-1} and the volume of the donor phase respectively in mL. C_A and V_A are the concentration of a PAH in the acceptor solvent in ng mL^{-1} and the volume of the acceptor solvent in mL used to elute PAHs from the MIP cavities respectively.

2.7 Application of the method in wastewater samples

The developed technique was then used to extract PAHs from real wastewater samples obtained from Goudkoppies wastewater treatment plant. In our previous studies, we quantified PAHs that accumulate on sludge during wastewater treatment at the same plant. This study therefore used the optimized MASE-MIP technique coupled to GC-TOF/MS to test the performance of the method in effluent wastewater. This was important because the effluent wastewater is pumped into river streams that eventually leads supplies for drinking water. Three deuterated PAHs were added to wastewater samples as surrogate standards before extraction to monitor the efficiency of the developed MASE-MIP technique considering that optimization was done on spiked de-ionized water samples.

3 Results and Discussion

3.1 Theoretical basis of the membrane assisted solvent extraction combination-molecularly imprinted polymer technique

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 concentration gradient. The transferred target

compounds will in turn be 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 hydrophobic semi-permeable membrane is used to minimize matrix effects by preventing the larger molecular weight compounds and also polar compounds from reaching the acceptor phase. The inclusion of MIPs with cavities specific for the binding of 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, optimized and evaluated for the quantitation of total PAHs from wastewater samples.

3.2 Optimization of the MASE-MIP for PAHs in wastewater

3.2.1 Influence of addition of the organic modifier in the sample

Of the four potential organic modifiers, DMSO gave better extraction values indicating that it performed well in keeping the PAHs in solution. The results for varying the composition of DMSO in the donor phase are shown in Fig. 2.

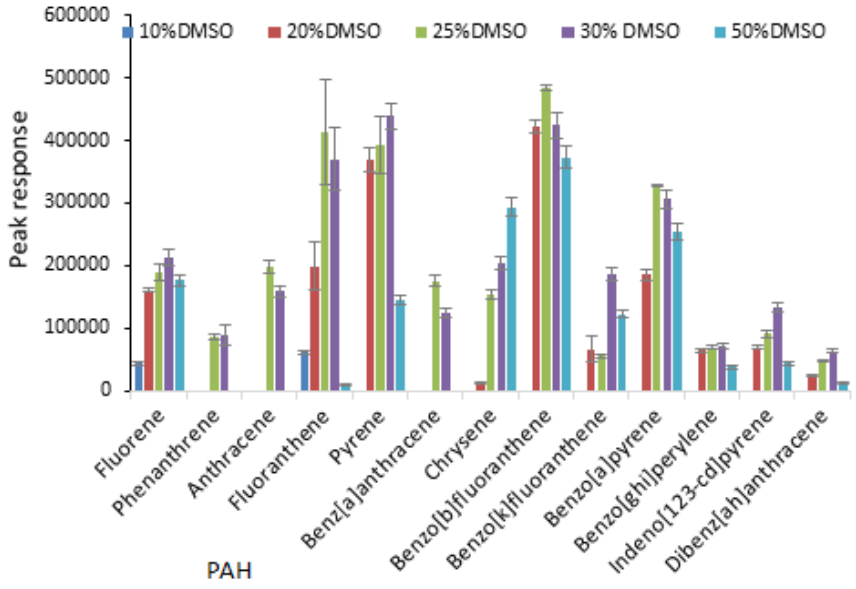


Fig 2 Effect of varying composition of dimethyl sulfoxide(DMSO) in the donor phase

Generally, most of the PAHs were extracted better at 25% DMSO composition except for acenaphthylene (not shown in Fig 2) that peaked at 20% DMSO composition while benzo[k]fluoranthene, indeno[123-cd]pyrene and dibenz[ah]anthracene had maximum extraction when DMSO composition was 30%. For fluorene, phenanthrene, pyrene and benzo[ghi]perylene that also seemed to peak at 30% composition, an ANOVA analysis showed that peak areas were not significantly different from those for 25% at 95% confidence interval. The 25% DMSO composition was therefore accepted as a universal optimum. The observed transfer efficiencies at optimum modifier composition might be due to a compromise between dissolving PAHs from the glass walls of the MASE apparatus while allowing for their transfer across the membrane into a non-polar acceptor phase. At modifier compositions higher than optimum, the analytes become too soluble in the donor phase and/or the donor phase becomes too viscous to allow for free mobility. Other organic modifier solvents in MASE-based techniques reported for PAHs include 5% and 10% ethanol [6,35] and 20% methanol [36]. In all these studies, the performance of the modifier against other potential modifiers was not done.

3.2.2 Effect of the stirring rate and time

There was a general continous increase in the amount of extracted PAHs as the stirring speed was increased from 420 to 1000 rpm. The range of the stirring rate investigated was limited by maximum stirring rate of the magnetic stirrer used. 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 1 100 rpm limit hence 1000 rpm was used for further experiments.

The effect of time on the extraction of PAHs from an aqueous solution across the membrane is summarized in Fig 3. The results show that most of the LMW PAHs reached equilibrium within 60 min. This might suggest that partitioning into the membrane is the limiting factor since MIPs inside the membrane have enough binding capacity. The high molecular weight PAHs attained optimum when extraction was done for 120 min beyond which there was no significant increase in the amount extracted. high molecular weight (HMW) PAHs generally have low mobility which might delay their transfer to the donor phase-membrane interface.

This contributes to their slow transfer across the membrane even though their partitioning across the membrane into the acceptor phase is fast. The optimum extraction time was taken as 120 min and was used in further experiments.

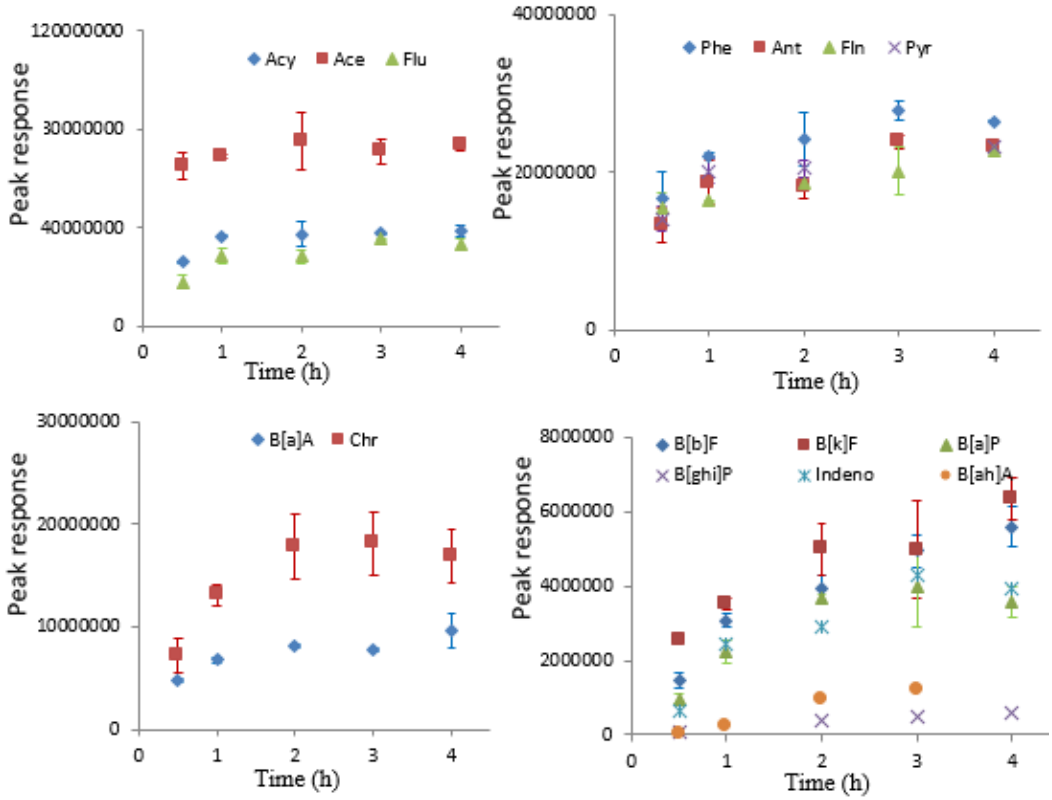


Figure 3 Effect of contact time on the extraction of acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr), benz[a]anthracene, chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), benzo[ghi]perylene (B[ghi]P), indeno[123-cd]pyrene (Indeno) and dibenz[ah]anthracene (B[ah]A) (n = 3, SD)

Compared to the current optimum conditions (1000 rpm for 120 min), other researchers have reported 750 rpm for 30 mins [33,46], 500 rpm for 80 min [36] and 750 rpm for 60 min [6]. In all these studies, the 750 rpm was accepted even though it was the maximum rate investigated. The extraction times are better compared to the

current study because these studies were done in the 40 - 55°C heating range and using the MASE technique only. In addition to the donor phase-acceptor phase interface, the current MASE-MIP technique has a further acceptor phase-polymer interface that involves transfer of analytes to the MIP cavities. The diffusion and binding phases are both time-dependent. Optimum conditions reported by techniques that involve the combination of a membrane and an adsorbent include 750 rpm for 60 min using a MIP-coated HF-LPME technique [43], 10 min of sonication combined with vortex assisted SPE [39] and 440 rpm for 15 min using a stir poly-membrane technique [34]. In view of the above extraction conditions, the stirring rate of 1000 rpm for 120 min optimized in our study was accepted and used in further studies. The multiple point magnetic stirrer allowed for extractions to be done in parallel thus increasing sample through put.

3.3 Method performance and validation

3.3.1 Extraction efficiency

A visual analysis of the performance of the MASE-MIP technique against the MISPE technique when PAHs were extracted from spiked wastewater samples is given in Fig 4. The chromatograms show that even though a more pronounced baseline drift was observed for the MASE-MIP compared to the MISPE approach, more PAHs were extracted using the MASE-MIP technique. This observation implies that more PAHs were bound on the MIP cavities in the MASE-MIP technique than when the wastewater was loaded directly onto the MIP particles. The hydrophobic semi-permeable membrane acts by preventing larger molecular weight compounds and also polar compounds from reaching the acceptor phase. This implies that the extent of matrix interferences is minimal compared to when the MIP is used directly as SPE adsorbents.

Another advantage of the MASE-MIP technique lies in its design (Fig 1). Suspending MIPs in a water immiscible organic solvent inside a membrane bag shifts the equilibrium towards transfer of analytes from the water sample to the organic acceptor phase. The inclusion of MIP with cavities specific for the binding of target analytes eliminates the need for a further clean-up step as is the norm with most extraction techniques for complex samples. The main advantage lies in the

combination of extraction and clean-up procedures into a single format. Thus, even though the extraction process is slower compared to MISPE, the technique is highly selective and specific towards target analytes. The MISPE technique usually involves a washing step that removes matrices from the adsorbent surface before eluting the bound analytes resulting in cleaner chromatograms. However, there is possibility of analyte loss during this MISPE washing step leading to concentrations lower than the true value being reported.

The extraction efficiency for individual PAHs from a spiked water sample using the optimized MASE-MIP technique are presented in Table 1. The results show that extraction efficiency for individual PAHs calculated as averages for three spiking concentrations ranged from 66.8 – 96.8%. Good repeatability was observed with RSD values ranging from 3.11 – 12.3%. Most of the extraction efficiency values for HMW PAHs were higher than those for LMW ones. HMW PAHs are more hydrophobicity which enhances their transfer from the aqueous solution into the heptane acceptor phase where they in turn bind to the polymer cavities. The average extraction efficiency was 77.4 % with an RSD value of 11.5% which is in total agreement with Ncube et al. (2017) that the polymer cavities have an affinity for all the 16 US-EPA priority PAHs [45]. An effective adsorbent is one that has cavities selective towards a class of sterically related analytes. This is important where a large number of analytes is targeted.

The limits of detection for the 16 US-EPA priority PAHs using the MASE-MIP technique were in the 0.01 – 0.45 ng mL^{-1} as summarized in Table 1. All the values were in the nano-gram scale with the LMW PAHs having better limit values than HMW PAHs. This might be related to the low response of the mass spectrometry towards these PAHs. Also, the calibration ranges for LMW PAHs started at 10 ng mL^{-1} while HMW PAHs were quantifiable from 50 ng mL^{-1}. Linearity (R^2) values were all above 0.9972.

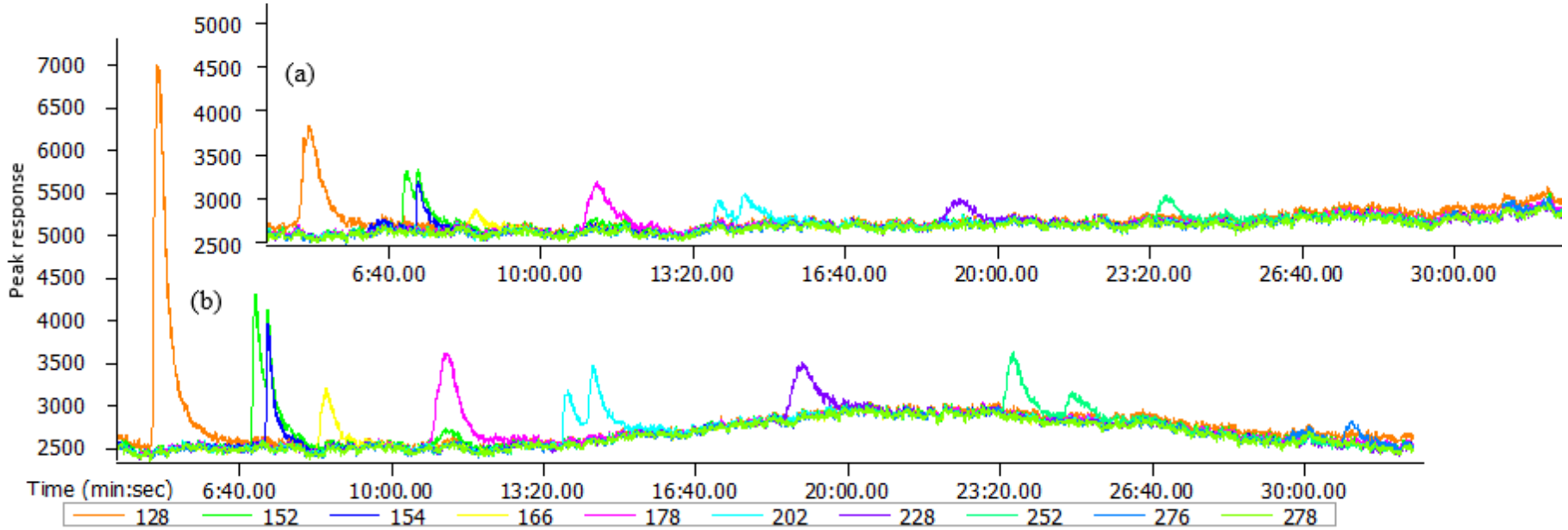


Fig 4 Chromatograms for analytes extracted from spiked wastewater effluent samples using (a) the MISPE only and (b) the MASE-MIP technique. Quantitation ions relate to naphthalene (128), acenaphthylene (152), acenaphthene (154), fluorene (166), phenanthrene and anthracene (178), fluoranthene and pyrene (202), benz[a]anthracene and chrysene (228), benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene (252), benzo[ghi]perylene and indeno[123-cd]pyrene (276) and dibenz[ah]anthracene (278)

Table 1 Identification parameters and calibration results of the 16 US-EPA priority PAHs (n = 3, RSD)

PAH	Retention time (s)	Quantitation ion (confirmation ions)	Linearity (R^2)	Spiking level recoveries 300 ng mL^{-1}	600 ng mL^{-1}	900 ng mL^{-1}	Average recovery (%)	MDL (ng mL^{-1})
Naphthalene	04:54	128 (127, 129)	0.9998	62.6	69.4	68.5	66.8 ± 0.0455	0.01
Acenaphthylene	06:58	152 (153, 151)	0.9992	66.6	75.8	81.3	74.6 ± 0.0814	0.02
Acenaphthene	07:16	154 (153, 152)	0.9982	71.3	78.0	85.8	78.3 ± 0.0758	0.02
Fluorene	08:27	166 (165, 167)	0.9973	70.4	78.6	81.9	77.0 ± 0.0632	0.03
Phenanthrene	11:01	178 (179, 176)	0.9996	70.3	80.9	78.1	76.4 ± 0.0588	0.04
Anthracene	11:19	178 (176, 179)	0.9983	64.8	82.9	87.0	78.2 ± 0.123	0.05
Fluoranthene	13:56	202 (101, 203)	0.9990	62.6	60.4	65.3	62.8 ± 0.0316	0.26
Pyrene	14:26	202 (200, 203)	0.9972	67.2	80.6	80.0	75.9 ± 0.0815	0.06
Benz[a]anthracene	18:58	228 (229, 226)	0.9981	67.3	61.2	79.1	69.2 ± 0.108	0.15
Chrysene	19:23	228 (226, 229)	0.9996	65.8	73.1	74.5	71.1 ± 0.0540	0.14
Benzo[b]fluoranthene	23:29	252 (253, 125)	0.9975	76.3	84.8	98.1	86.4 ± 0.104	0.14
Benzo[k]fluoranthene	24:21	252 (253, 125)	0.9979	66.1	67.2	74.2	69.2 ± 0.0519	0.18
Benzo[a]pyrene	24:48	252 (253, 125)	0.9998	79.6	85.7	83.9	83.1 ± 0.0311	0.45
Indeno[123-cd]pyrene	29:32	276 (138, 277)	0.9996	82.0	79.5	91.1	84.4 ± 0.0594	0.30

PAH	Retention time (s)	Quantitation ion (confirmation ions)	Linearity (R^2)	Spiking level recoveries			Average recovery (%)	MDL (ng mL^{-1})
				300 ng mL^{-1}	600 ng mL^{-1}	900 ng mL^{-1}		
Benzo[ghi]perylene	30:56	276 (138, 277)	0.9997	86.1	87.3	91.7	88.2 ± 0.0270	0.22
Dibenz[ah]anthracene	30:05	278 (139, 279)	0.9979	96.0	97.0	97.6	96.8 ± 0.0069	0.23

3.3.2 Application in wastewater samples

The developed MASE-MIP technique was tested in the quantitation of PAHs in effluent wastewater obtained from Goudkoppies WWTP, Johannesburg, South Africa. The deuterated surrogate standards used were naphthalene-D8, acenaphthene-D10 and phenanthrene-D10. The recovery values for the three surrogate standards were 68.1, 79.8 and 82.2, respectively. Repeatability was between 8.6 and 14.1% for triplicate extraction from spiked wastewater. A statistical analysis using a Student's t-test showed that the recoveries for naphthalene, acenaphthene and phenanthrene given in Table 1 were comparable with those of their deuterated forms at 95% confidence interval. The recoveries for individual PAHs summarized in Table 1 were therefore accepted and used in quantitation calculations. The retention times, quantitation and confirmation ions used to identify specific PAHs in wastewater samples are also shown in Table 1.

Generally, only the LMW PAHs (2, 3 and 4-ringed) were quantified in wastewater samples over a concentration range of 3.4 – 1829.8 μg L^{-1} as shown in Table 2. The total concentration of the quantifiable PAHs in the wastewater effluent was 3230.3 μg L^{-1}. The highest amount recorded was for acenaphthene. Acenaphthene has one of the highest solubility values in water (3.4 mg L^{-1}) which might explain its levels in wastewater effluents [47]. Of the quantified PAHs, pyrene and anthracene have the lowest solubility at 0.14 and 0.07 mg L^{-1}, respectively [47]. Naphthalene and acenaphthene contributed 67% of the total PAH concentration. This implies that the WWTP under investigation is releasing insignificant amounts of the toxic HMW PAHs in its effluent. The HMW PAHs have low solubility in water and tend to accumulate on sludge during wastewater treatment thus limiting their bioavailability.

In a related study, up to 10.75 μg g^{-1} total PAH content in dry sludge was quantified in sludge samples obtained from the same WWTP [42]. The MASE-MIP extraction of PAHs from the same wastewater samples was repeated in the second day with RSD values ranging from 0.6 – 24.9%. These inter-day results are an indication that the developed technique is reproducible.

Table 2 Analysis PAHs in wastewater effluent samples (n = 3, RSD)

PAH	Concentration in wastewater effluent		Inter-day
	Day 1 (ng mL^{-1})	Day 2 (ng mL^{-1})	%RSD
Naphthalene	343.1 ± 0.121	459.9 ± 0.126	11.9
Acenaphthylene	NQ	NQ	-
Acenaphthene	1829.8 ± 0.044	1804.7 ± 0.050	0.6
Fluorene	679.6 ± 0.101	641.8 ± 0.109	2.3
Phenanthrene	80.0 ± 0.068	62.7 ± 0.122	9.9
Anthracene	9.8 ± 0.053	5.2 ± 0.004	24.9
Fluoranthene	284.6 ± 0.132	418.3 ± 0.069	15.5
Pyrene	3.4 ± 0.201	3.3 ± 0.134	1.6
Benz[a]anthracene	NQ	NQ	-
Chrysene	NQ	NQ	-
Benzo[b]fluoranthene	NQ	NQ	-
Benzo[k]fluoranthene	ND	ND	-
Benzo[a]pyrene	ND	ND	-
Indeno[123-cd]pyrene	ND	ND	-
Benzo[ghi]perylene	ND	ND	-
Dibenz[ah]anthracene	ND	ND	-

NQ -not quantified; ND – not detected

This study is the first in which PAHs are quantified in wastewater effluents in South Africa. The predominance of LMW PAHs in wastewater effluent samples has been observed elsewhere with Blanchard et al. (2004) reporting that the 2 – 4 ringed PAHs contributed 98% of the 10 521 ng L^{-1} total PAH concentration in effluents from the Seine Aval plant, Spain [48]. Results by Manoli and Samara (1999) showed that the 2 -4 ring PAHs contributed 95% of total PAH concentration in a Thessaloniki plant, Greece [49]. The HL-LPME technique by Charalabaki et al. (2005) reported the total concentration of 5 PAHs (naphthalene, acenaphthene, phenanthrene, fluoranthene and pyrene) in effluents from 8 different WWTPs between 0.03 and

0.503 µg L^{-1} [38]. Sánchez-Avila et al. (2009) recorded 3.9 µg L^{-1} at Mataro WWTP, Spain of which the naphthalene content contributed 89%. The only other PAHs detected were fluorene, fluoranthene and pyrene [50].

4 Conclusions

A membrane assisted solvent extraction technique was successfully combined with a molecularly imprinted polymer to produce a novel technique for the extraction of organic pollutants in complex aqueous samples. The technique has the advantage of combining a size-selective membrane and a polymer that is specific for its target analyte into a single step format. This results in reduced matrix effects and sample turnaround time both of which are essential in analysis of trace compounds from complex samples. Validation parameters that include recoveries, limits of detection, relative standard deviations and linearity were satisfactory. The developed method was coupled with GC-TOF/MS and successfully applied in the analysis of trace quantities of PAHs in wastewater samples.

Funding

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4.2 PAPER 4

This paper entitled "Development and optimisation of a novel three-way extraction technique based on combination of Soxhlet extraction, membrane assisted solvent extraction and molecularly imprinted polymer using sludge polycyclic aromatic hydrocarbons as model compounds has been published online in *Journal of Separation Science*. The article reports a novel Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique that was developed, optimized and applied in the analysis of PAHs in wastewater sludge samples.

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Development and optimisation of a novel three-way extraction technique based on combination of Soxhlet extraction, membrane assisted solvent extraction and molecularly imprinted polymer using sludge polycyclic aromatic hydrocarbons as model compounds

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ABSTRACT

A novel technique that integrates extraction and clean-up into a single step format is reported as part of the search for new sample preparation techniques in the analysis of persistent organic pollutants from complex samples. This was achieved by combining the extraction efficiency of the Soxhlet extractor, the selectivity of a size exclusion membrane and the specificity of a molecularly imprinted polymer for the extraction of polycyclic aromatic hydrocarbons from wastewater sludge followed by quantitation using GC-TOF/MS. The approach is described as the Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique. This technique was optimised for various parameters such as extraction solvent, reflux time and membrane acceptor phase. The applicability of the developed technique was optimised using a wastewater sludge certified reference material and then tested on real wastewater sludge samples. The method detection limits ranged from 0.14 to 12.86 ng g^{-1} with RSD values for extraction of the 16 US-EPA priority polycyclic aromatic hydrocarbons from wastewater sludge samples ranging from 0.78 to 18%. The extraction process was therefore reproducible and showed remarkable selectivity. The developed technique is a promising prospect that can be applied in the analysis organic pollutants from complex solid samples.

Abbreviations

Membrane assisted solvent extraction-molecularly imprinted polymer, MASE-MIP; molecularly imprinted polymer, MIP; persistent organic pollutant, POP;

polycyclic aromatic hydrocarbons, PAHs; Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer, SE-MASE-MIP; wastewater treatment plant, WWTP

Keywords
Polycyclic aromatic hydrocarbon; molecularly imprinted polymer; Soxhlet extraction; membrane assisted solvent extraction; wastewater sludge.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise a large and diverse group of organic compounds characterised by at least two benzene rings fused in linear, cluster, or angular arrangements. The introduction of PAHs into the environment is mainly via natural and anthropogenic combustion of bituminous products. The anthropogenic sources are the major contributors of PAHs and reach wastewater treatment plants (WWTPs) through surface runoff and sewerage. During wastewater treatment, sludge becomes an important sink for PAHs owing to their hydrophobicity [1,2]. PAHs are known to have potential human health effects and several agencies have prioritized some of the PAHs as potential carcinogens [3] while several countries have set maximum permitted quantities in solid samples [4].

Analysis of PAHs in solid samples generally consists of a solvent extraction step followed by clean-up and concentration using an adsorbent. For solid samples, the conventional solid–liquid extraction techniques like Soxhlet extraction and ultra-sonication extraction are still used extensively in present analytical procedures [5–9]. New modern sample preparation techniques such as supercritical fluid extraction, pressurized liquid extraction and microwave-assisted extraction have been developed as an alternative to these classical techniques for analysis of PAHs [6,10–15] while advancements in the Soxhlet extractor have also been reported [16]. The main disadvantage with current extraction techniques for complex samples such as sludge is that most of them still require an additional clean-up step. Multiple steps during sample preparation are tedious and prone to errors due to analyte loss or contamination. Attempts to eliminate this step have led to techniques like the single-step matrix solid-phase dispersion-gel permeation

chromatography technique reported for the analysis of PAHs in wastewater sludge [17] and mussel samples [18]. This simultaneous approach has the advantage of reducing amount of solvents used, sample handling and analysis time. A recent review by Fumes et al. (2017) emphasizes the need for miniaturization and automation techniques as a way of counteracting problems associated with multiple step approaches [19]. A few online and miniaturized extraction techniques have been applied in the analysis of PAHs [20–23]. However, availability and accessibility of compatible instruments in addition to extreme matrix effects has limited the applicability of miniaturized and online techniques in complex solid samples. Reduction of analysis steps therefore remains a relevant area of study that can be explored in order to reduce disadvantages associated with multiple step analysis.

Previously, our research group has demonstrated the combination of membrane assisted solvent extraction with molecularly imprinted polymers as a two way extraction of compounds from complex aqueous samples giving very selective extraction [24–26]. In this study, the goal was to develop and optimize into a single-step a three way Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique for the selective extraction of complex solid samples with PAHs in wastewater sludge as model compounds. The selective nature of the membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) technique in the extraction of organic compounds from complex aqueous samples has been reported before. The proposed SE-MASE-MIP technique combines three extraction devices into a single entity to eliminate the need for a further clean-up step. Soxhlet extraction was targeted for its high efficiency and reproducibility, the membrane for its size-selectivity and the molecularly imprinted polymer (MIP) for its specificity towards target compounds. The potential selectivity of the developed SE-MASE-MIP technique was tested during extraction of PAHs in wastewater sludge samples. The quantitation of PAHs in wastewater sludge is critical because the capability of most wastewater treatment plants (WWTPs) to remove these compounds during treatment is unknown. In South Africa, about 80% of wastewater treatment facilities use their sewage sludge for agronomic purposes

[27]. Unmonitored introduction of PAHs to the environment without fully understanding the chemical composition of the wastewater sludge can result in adverse consequences in the near future.

2 Materials and Methods

2.1 Chemicals and reagents

A 10 μg mL^{-1} PAH calibration mix in ACN (CRM 47940) with 99.0% purity was from Supelco (Bellefonte, USA). HPLC-grade hexane, heptane, toluene, dichloromethane, ACN and cyclohexane were purchased from Sigma-Aldrich (Johannesburg, South Africa). Empty 3 mL cartridges with 10 μm frits were from Sorbent AB (Frölunda, Sweden). Benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene used in the synthesis of the polymer were all from Sigma-Aldrich (Johannesburg, South Africa). Sewage sludge-PAHs reference material (LGC 6182) was from Industrial Analytical Pty Ltd (Kyalami, South Africa).

2.2 Instrumentation

A Pegasus 4D 1-D GC-TOF/MS (LECO Corp., St. Joseph, USA) was used for quantitation of PAHs. The separation and identification conditions were adopted without moderation from previous work by Ncube et al. (2017). The calibration range was in the 10 - 1000 ng mL^{-1} with coefficient of determination values for the 16 PAHs all above 0.9972. The Soxhlet apparatus was prepared in the School of Chemistry, University of the Witwatersrand, Johannesburg, South Africa. Thimbles were purchased from GE Healthcare UK Limited (Buckinghamshire, UK). PTFE hydrophobic membrane bags of 0.22 μm pore size, 160 μm thickness and 15 mL internal volume were from Gerstel GmbH & Co. (Mülheim, Germany).

2.3 Sample collection and storage

Anaerobically digested and sun-dried sludge samples were collected into 250 mL brown bottles with Teflon caps. The samples were collected during the spring season with permission from the Goudkoppies and Northern Works WWTPs. The samples were loaded in cooler boxes and transported to University of the

Witwatersrand where they were stored at 4°C. Before analysis, the samples were placed in darkness at ambient conditions for 3 h and then oven-dried at 70°C for 2 days. The dried sludge was finally crushed and a powder was collected through a 100 μm sieve.

2.4 Synthesis of the molecularly imprinted polymer

The procedure and conditions of synthesis of the benzo[k]fluoranthene-imprinted and indeno[1,2,3-cd]pyrene-imprinted polymers were adopted from our previous MIP-cavity tuning studies [28]. This polymer combination has a high affinity for all the 16 US-EPA priority PAHs and attains its maximum adsorption capacity of 5.19 mg g^{-1} within 80 min [28].

2.5 Development of the Soxhlet extractor-membrane-polymer technique

The procedure for the SE-MASE-MIP technique involved pouring 30 mL of an extracting solvent into a 50 mL Soxhlet round-bottomed flask. A total of 80 mg of a benzo[k]fluoranthene-imprinted and indeno[1,2,3-cd]pyrene-imprinted polymers mixed at 1:1 (w/w) was weighed into a membrane bag. An organic acceptor phase (1 mL) was transferred into the membrane bag. The membrane bag was sealed and dropped into the flask containing the solvent. A sewage sludge sample would then be placed in the extraction thimble. The set-up was heated at the boiling point of the extracting solvent. The solvent was allowed to reflux every time extracting the PAHs and depositing them into the flask. The PAHs would instantly cross the membrane and eventually adsorb into the MIP cavities. The technical setup of the single entity SE-MASE-MIP technique is presented in Fig. 1. After extraction, the MIP-membrane sachet was removed from the flask and the MIP transferred into an empty 3 mL cartridge. The cartridge was then mounted onto an SPE unit. The trapped PAHs were eluted gravitationally using a heptane solvent recommended by Ncube et al. (2017) followed by analysis using a GC-TOF/MS instrument.

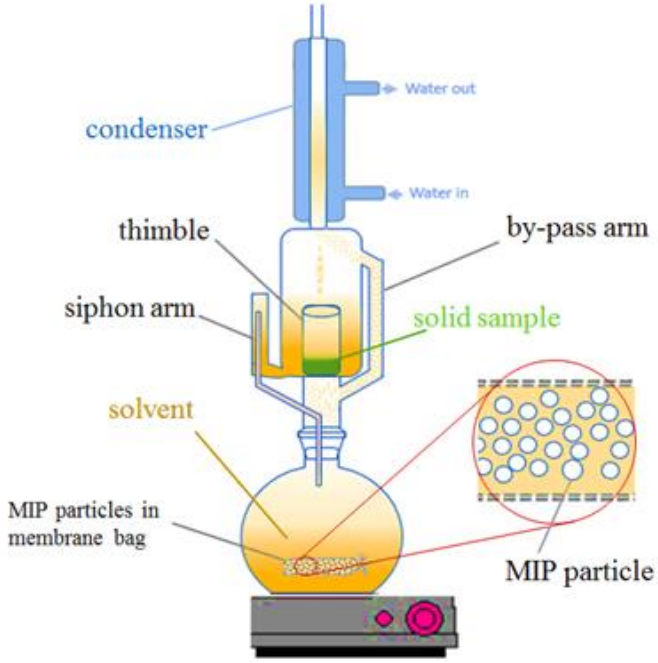


Fig. 1. The single entity apparatus for the Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique.

2.6 Optimization of the Soxhlet extractor-membrane-polymer technique

2.6.1 Effect of temperature

A volume of 30 mL cyclohexane solution spiked at 500 ng mL^{-1} with PAHs was transferred into the flask of the Soxhlet apparatus. A membrane bag containing MIP particles was then dropped into the spiked solution. The Soxhlet setup was then completed but without the thimble and the sludge sample. The MIP-solvent solution was heated and its temperature maintained at room temperature (about 20°C), 40°C, 60°C and 80°C for 3 h. The membrane bag containing MIP particles was then separated from the solvent and the PAHs eluted from the MIP cavities and finally quantified on a GC-TOF/MS instrument.

2.6.2 Selecting an extracting solvent and extraction time

The aim was to identify a solvent that will effectively extract PAHs from sludge contained in the thimble and allow for their consequent sequestration by the MIP placed inside a membrane. Boiling point, polarity and water solubility were

considered in choosing potential extraction solvents. Thus hexane, acetone, dichloromethane, and cyclohexane were investigated. In addition to the five chosen solvents, a combination of hexane and polar solvents (acetone and tetrahydrofuran) was investigated. The PAHs were extracted from 1 g of the sludge certified reference material (CRM) of 100 µm particle size. Each solvent and solvent mixture was allowed to reflux at its boiling point for 12 h. The extraction was performed with the Soxhlet system only and the PAHs were quantified directly from the extract following filtering through 0.45 µm pore size filters. Extraction time was investigated by refluxing the optimized solvent for 4, 8, 12, 16 and 20 h.

2.6.3 Acceptor phase organic solvent

The acceptor phase is expected to aid the transfer of analytes across the membrane into the cavities of the polymer inside the membrane bag. Hexane, heptane and toluene were chosen as potential organic solvents to be used as the membrane acceptor phase. Hexane had been identified earlier as the best extraction solvent during experiments to select an efficient solvent for Soxhlet extraction. 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 [26]. The hexane solvent in the Soxhlet flask was spiked at 2 µg mL^{-1} with PAHs. The system was heated at 40°C for 3 h.

2.7 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 MIP enclosed in a membrane bag and their eventual enrichment into the elution solvent. The kinetic transfer was evaluated as extraction efficiency.

2.7.1 Extraction efficiency across the membrane

The extraction efficiency of the MASE-MIP section of the SE-MASE-MIP technique was investigated by extracting PAHs from both aqueous solution and organic solvent. The idea was to understand the extent of transfer of PAHs across the membrane into the acceptor phase and their eventual binding into the MIP cavities. Hexane previously optimized as the best extraction solvent was used as

the organic solvent. Each solution (20 mL) was spiked in the 500 – 1000 ng mL^{-1} range. Extraction was performed over an optimized equilibrium time of 180 min. The purpose of this procedure was to determine the extraction efficiency of PAHs from solution across the membrane into the cavities of the MIP. The difference in concentration of the PAHs in solution before and after extraction was used to calculate the transfer efficiency using Eq. (1).

$$E_m = (C_i - C_f)/C_i \times 100 \quad (1)$$

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

2.7.1.1 Soxhlet extractor-membrane-polymer extraction efficiency

The extraction efficiency of the optimized 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 in the thimble of the Soxhlet system and extracting PAHs using hexane for 16 h. The extraction efficiency of the SE-MASE-MIP system was then calculated as the percentage ratio of the extracted amounts versus the initial amounts of PAHs in the sludge CRM using Eq. (2).

$$E_S = (C_e V/C_s m_s) \times 100 \quad (2)$$

where E_s is the Soxhlet-membrane-MIP extraction efficiency, C_e is the concentration of a PAH in the eluting solvent after extraction (μg mL^{-1}), V is the volume of the eluting solvent (mL), C_s is the concentration of a PAH in the solid CRM sample before extraction (μg g^{-1}) and m_s is the mass of the CRM sample (g).

3 Results and discussion

3.1 Theoretical basis

The theoretical basis of the SE-MASE-MIP technique is that as the analytes are being extracted from a solid sample using Soxhlet extraction, they are

immediately transferred across the membrane into a non-polar acceptor phase and eventually into the cavities of the polymer. The membrane is size-selective and helps eliminate large molecular weight matrices which have a tendency of clogging the cavities of the polymer thereby reducing its efficiency. The performance of the MASE-MIP technique has been demonstrated in other studies [24]. The integrated three-way extraction using the SE-MASE-MIP technique eliminates the need for a further clean-up step which is common with Soxhlet extraction and most other solid-liquid extraction techniques by introducing an in-situ clean-up procedure.

3.2 Effect of temperature

The results in Fig. 2 show that there was an increase in the amount of PAHs extracted with increase in temperature. However, above 60^0C there was a decrease for low molecular weight (LMW) PAHs owing to their high kinetic energy. Interesting to note was that the transfer of high molecular weight (HMW) PAHs was minimal at low temperatures. Benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[1 2 3-cd]pyrene could not be detected from elution of the MIP at 20°C and 40°C. HMW PAHs have limited mobility compared to LMW PAHs [29–31]. This might have affected the diffusion kinetics and could explain the MIP's preference for LMW PAHs at lower temperatures. The enhanced transfer of PAHs across the membrane and their subsequent binding into the MIP cavities at higher temperatures can be attributed to the kinetic energy of the PAHs that aid their transfer across the membrane.

3.3 Choice of extracting solvent

The results of the top three performing extraction solvents and solvent mixtures are shown in Fig. 3. Generally, the results show the extracting power of hexane for all PAHs except for naphthalene (not shown in Fig. 3), phenanthrene, chrysene and benzo[b]fluoranthene which were extracted better by the hexane/tetrahydrofuran mixture.

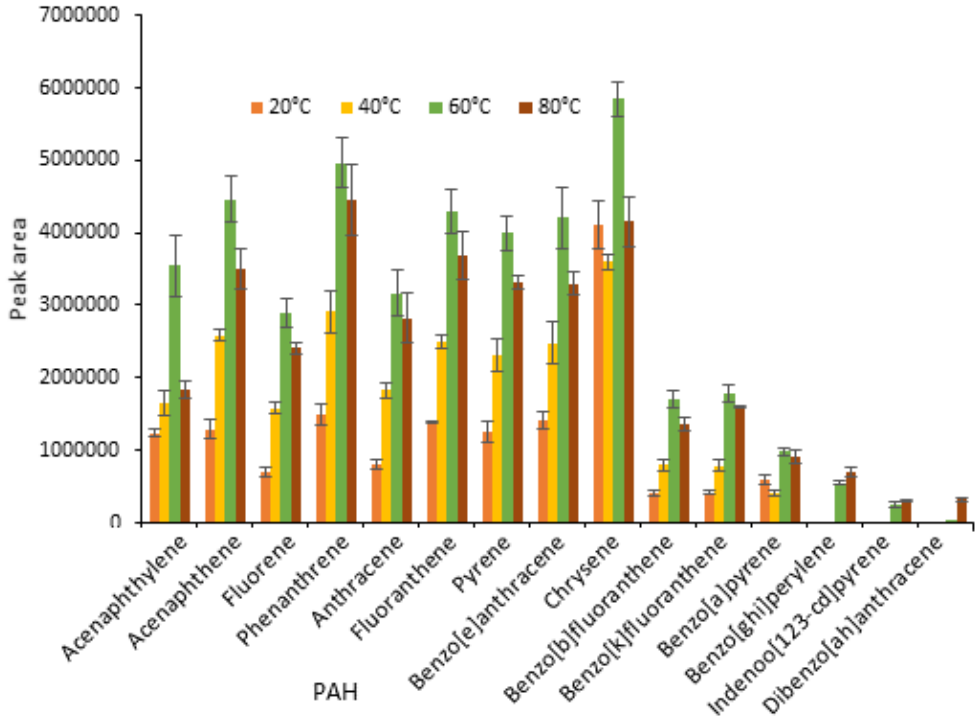


Fig. 2. Effect of temperature on the transfer and sequestration of PAHs (n=3, SD)

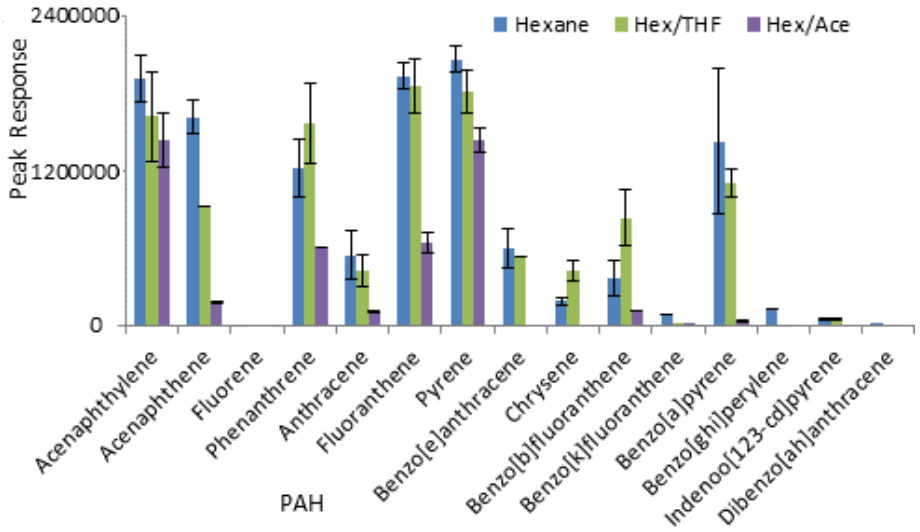


Fig. 3. Peak areas representing the amounts of the 16 PAHs extracted by different solvents (n = 3, SD)

This behaviour of the hexane/tetrahydrofuran 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 analysis carried out on the peak areas of PAHs extracted using hexane and the hexane/tetrahydrofuran mixture showed that only naphthalene, acenaphthene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[g,h,i]perylene were significantly different at 95% confidence level. The boiling temperature difference between hexane and tetrahydrofuran is only 3 units and the mixture refluxed at 60°C. This might explain the comparability in the performance of hexane and hexane/tetrahydrofuran mixture. Some researchers have observed that the use of hexane mixed with a relatively polar solvent like acetone performs better than when the solvents are used individually owing to the de-wetting ability of the polar solvent component which becomes essential in the extraction of wet samples [32]. In this study, hexane performed better mainly because the samples had been properly dried. Hexane was then used in further studies. Soxhlet extraction of the analytes reached optimum at 12 h. However, during further studies extraction was done for 16 h based on convenience where the set-up could be left to reflux overnight unmonitored.

3.4 Selection of the membrane acceptor solvent

Hexane performed better than heptane and toluene in that order as an acceptor phase for LMW PAHs specifically naphthalene, acenaphthylene, acenaphthene, phenanthrene and anthracene as shown in Fig. 4. For all the other PAHs having 4 or more rings, heptane became dominant as an acceptor phase followed by hexane. An ANOVA analysis was used to confirm that the difference in performance of the heptane and hexane acceptor phases was significant at 95% confidence level. Heptane is more hydrophobic than both hexane and toluene. This could have led to heptane remaining in the pores of the MIP through hydrophobic interactions with the functional monomers while at the same time acting as a better acceptor of the PAHs despite its solubility with the donor solvent. The performance of the heptane as the acceptor phase might also be related to its boiling point (98°C) compared to that of the hexane (68°C) used as the Soxhlet extracting solvent. The effect could have enhanced the transfer of

PAHs from the extraction solvent into the MIP cavities. Heptane was then accepted as the accepting solvent and used for further studies

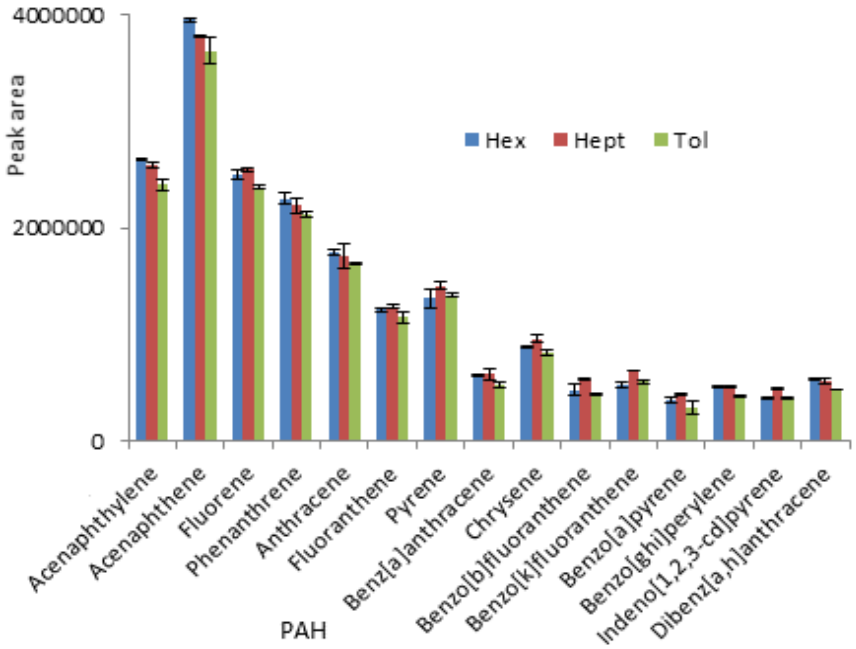


Fig. 4. Peak areas representing the amounts of the 16 PAHs transferred when different solvents were used as membrane acceptor phase (n = 3, SD)

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3.5 Extraction efficiency of the Soxhlet extractor-membrane-polymer technique

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 (Fig. 5). Severe baseline drift is observed for the Soxhlet extract chromatogram as shown in Fig. 5 (a). 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 Fig. 5 (d) where the PAHs were identified in the heptane eluent after applying the SE-MASE-MIP technique, it can be observed that most of the interferents occurred for acenaphthene (*m/z* = 154) with several peaks representing the 154-quantitation ion appearing.

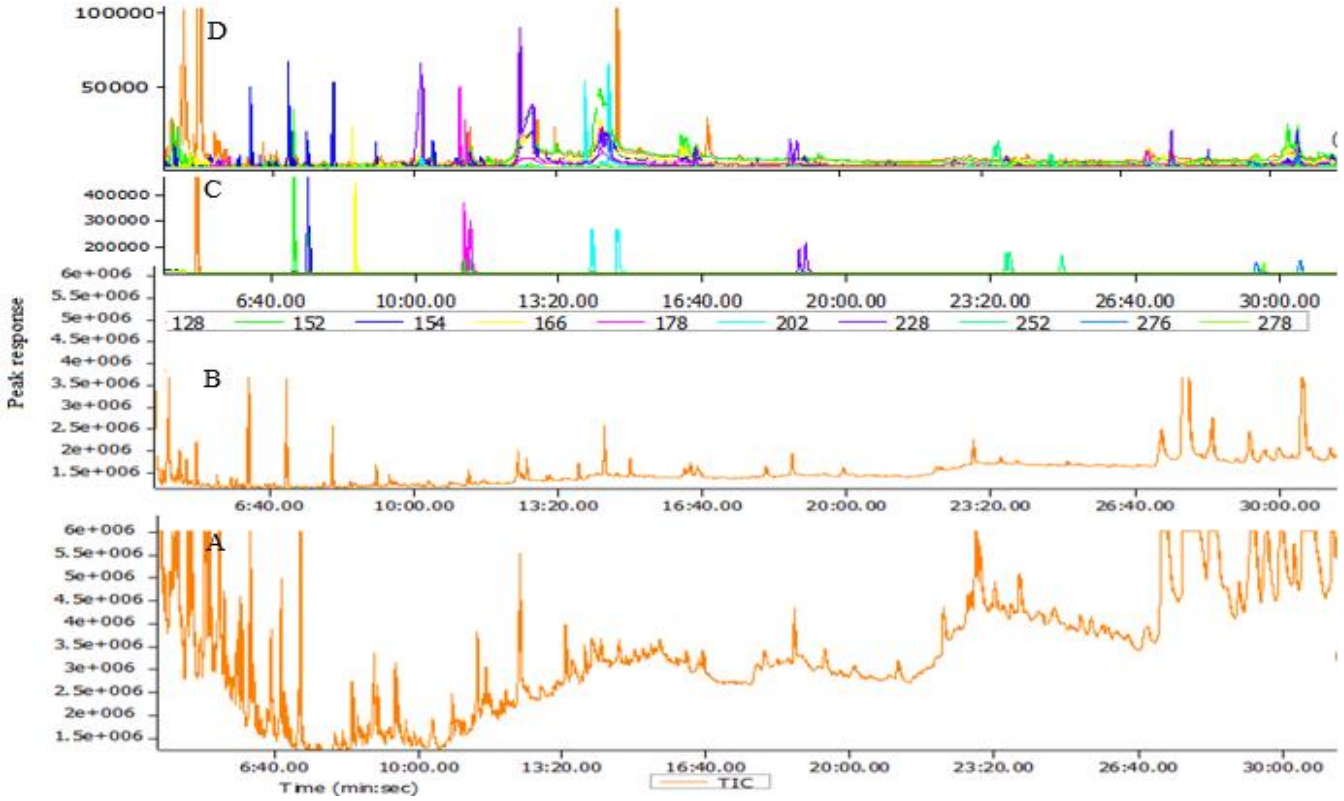


Fig. 5. Chromatograms displaying efficiency of the SE-MASE-MIP technique in the analysis of sludge PAHs. (A) Total ion chromatogram (TIC) showing direct injection of the hexane Soxhlet extract, (B) TIC for injecting heptane after eluting PAHs from MIP cavities, (C) 50 ng mL^{-1} calibration chromatogram showing peaks of PAH quantitation ions and (D) quantitation ion peaks for a sludge sample from Northern Works WWTP

The 128 *m/z* ion which represents the presence of naphthalene also represented interferents in the 13 – 17 min retention time range. The presence of matrix effects was also observed after 30 min where the indeno[1 2 3-cd]pyrene (276 *m/z*) peak appears. While these observations might imply that the developed SE-MASE-MIP technique is not entirely effective, most of the non-target peaks did not interfere with the analyte peaks. Each quantitation ion could be selected individually and the PAH identified and quantified independent of other quantitation ions.

The extraction efficiency of the MASE-MIP section of the SE-MASE-MIP technique in which the PAHs are transferred across the membrane into the MIP-cavities ranged from 73 to 97% from spiked water samples while it was 55 – 88% from spiked hexane solution (Table 1). However, the extraction efficiency of the entire SE-MASE-MIP system in which PAHs were extracted from sludge CRM samples dropped significantly to 17 - 48% as shown in Table 1. The reduction in extraction efficiencies could be attributed to several factors associated with the design and the complexity of wastewater sludge samples. These may include the slow extraction process of the Soxhlet process and the possibility of larger matrix compounds blocking the membrane pores and the polymer cavities. However, the system was very reproducible and showed remarkable selectivity.

3.6 Preliminary application in wastewater sludge

The developed SE-MASE-MIP method was used under optimized conditions to determine the concentration of the 16 US-EPA priority PAHs in wastewater sludge samples obtained from Goudkoppies and Northern WWTPs, Johannesburg, South Africa. The quantitation results are presented in Table 1. Generally, the HMW PAHs appeared in larger quantities than the LMW PAHs in both WWTPs. Northern Works WWTP recorded the highest amount of indeno[1 2 3-cd]pyrene (2.82 mg kg^{-1} dw) while the highest molecular weight dibenz[a,h]anthracene was the prominent PAH in Goudkoppies WWTP sludge samples with a value of 5.40 mg kg^{-1}.

Table 1 Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer combination (SE-MASE-MIP) quality assurance parameters and preliminary application in the extraction of PAHs from wastewater sludge.

PAH	MASE-MIP section extraction efficiency (n = 3, RSD)		SE-MASE-MIP extraction efficiency from sludge CRM (n = 3, RSD)	SE-MASE-MIP MDL (ng g^{-1})	Amount of PAHs in sludge (µg g^{-1} d.w) (n = 3, RSD < 18%)	
	Spiked water	Spiked hexane			Northern Works	Goudkoppies
Naphthalene	66.2 ± 0.152	67.8 ± 0.148	34.6 ± 0.012	0.14	3.04	0.81
Acenaphthylene	74.6 ± 0.081	64.2 ± 0.093	29.7 ± 0.047	0.60	0.003	0.03
Acenaphthene	78.3 ± 0.076	60.5 ± 0.074	22.1 ± 0.029	1.21	0.59	1.75
Fluorene	77.0 ± 0.063	66.4 ± 0.091	37.2 ± 0.039	0.90	0.03	0.52
Phenanthrene	76.4 ± 0.154	60.9 ± 0.108	36.4 ± 0.026	1.15	ND	ND
Anthracene	78.2 ± 0.123	61.7 ± 0.076	16.9 ± 0.020	3.21	0.01	0.04
Fluoranthene	62.8 ± 0.035	77.3 ± 0.080	28.9 ± 0.048	7.88	0.003	0.43
Pyrene	75.9 ± 0.182	54.5 ± 0.101	18.3 ± 0.025	3.29	0.02	0.44
Benz[a]anthracene	69.2 ± 0.224	72.6 ± 0.084	34.5 ± 2.95	4.06	0.36	0.36
Chrysene	71.1 ± 0.036	63.1 ± 0.187	34.5 ± 0.008	9.56	0.19	0.01
Benzo[b]fluoranthene	86.4 ± 0.104	62.1 ± 0.194	34.4 ± 0.059	4.89	ND	0.03
Benzo[k]fluoranthene	69.2 ± 0.035	57.0 ± 0.075	39.9 ± 0.027	4.28	0.02	0.02
Benzo[a]pyrene	83.1 ± 0.122	82.0 ± 0.055	39.9 ± 0.060	12.9	0.61	0.11
Benzo[ghi]perylene	84.2 ± 0.059	88.3 ± 0.164	38.6 ± 0.074	6.64	0.26	0.07
Indeno[1 2 3-cd]pyrene	88.4 ± 0.027	65.2 ± 0.029	32.5 ± 0.159	11.3	2.82	0.73
Dibenz[a h]anthracene	96.8 ± 0.007	86.4 ± 0.058	47.9 ± 0.141	6.49	1.09	5.40

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 [33]. These PAHs have very low solubility and are expected to concentrate in sludge during sewage treatment.

The total content of the 16 US-EPA priority PAHs for the sludge samples from the two WWTPs were above the maximum total concentrations permitted by the EU, the USA and, Denmark and Sweden (6, 4.5 and 3 mg kg^{-1}, respectively). The implication of these results is that the PAH content of the two WWTPs has exceeded the international standards and will continue to rise as long as there is no legislature or sludge treatment processes that aim to reduce release of PAHs back into the environment. The potential risks associated with the use of sludge from these WWTPs as a soil amendment strategy is therefore a cause of concern in South Africa and the concentration of PAHs in wastewater sludge reported in this study cannot be ignored. In addition, the total concentrations of the seven PAHs with known carcinogenicity (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene) were found to be 4.48 and 6.73 mg kg^{-1} for Northern Works and Goudkoppies WWTPs respectively. It should however be noted that the recorded amounts of the most carcinogenic benzo[a]pyrene in the two WWTP sludge samples (0.61 and 0.11 mg kg^{-1}, respectively) were below the maximum permissible limits for land sludge application. China, the EU, France and the USA, for example, have set 3, 2.5, 2 and 1 mg kg^{-1} as their limits respectively. The diagnostic ratio calculations involving PAH isomers with *m/z* of 178, 202, 228 and 276 indicated that these PAHs are from pyrogenic sources. This was confirmation that the quantified PAHs in sludge samples originate from combustion of carboneous products such as wood, rubber and fossil fuels. These observations might imply that the chemical, food and transport industries rather than petroleum and oil spills are the major contributors of PAHs in Johannesburg.

3.7 Comparative studies

The application and performance of the SE-MASE-MIP compared to other techniques that have been applied in the quantitation of PAHs in municipal wastewater sludge is shown in Table 2. The SE-MASE-MIP technique has displayed lower LODs compared to most techniques except for the USEPA 3540C Soxhlet extraction approach [34] and the matrix solid-phase dispersion (MSPD) technique [17,35]. The MSPD by Pena et al. (2008) combined extraction and clean-up steps into a single process like our SE-MASE-MIP technique [17].

The MSPD technique was reported to be advantageous compared to other methods with the need for careful optimization as its only drawback. Generally, it is observed that studies in China and Poland have revealed high levels of PAHs in sludge samples. The South African sludge-PAH 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. It should also be emphasized that the maximum permissible levels set by different countries differ in the number and the PAHs listed. For example, the EU maximum permissible value refers to six of the 16 US-EPA priority PAHs. The USA value lists seven PAHs of which two (chrysene and dibenz[a,h]anthracene) are not listed in the EU list and the other two (benz[a]anthracene and dibenz[a,h]anthracene) are not in the Swedish list. The Swedish list also has six PAHs in the list for its maximum permissible value. Most countries are generally lagging in the evaluation of PAHs and other persistent organic pollutants (POPs) in wastewater sludge from municipal wastewater treatment plants. South Africa, for example, has no legislation concerning POPs in its sludge disposal guidelines. This is mainly due to lack of data on the contamination of wastewater sludge by POPs. Studies in the northern hemisphere have also observed a similar level of ignorance of potential impacts of unmonitored sludge disposal [1,2]. More quantitative scientific evidence is needed to serve as a baseline for the development of improved wastewater sludge treatment methods, disposal options and monitoring protocols that will assist in protecting the environment and minimizing human exposure to POPs.

Table 2 Comparison of techniques that have been used in the extraction of the 16 US-EPA priority PAHs in sludge from municipal wastewater treatment plants from 2001

Technique	Clean-up	No. of PAHs	LOD (ng g^{-1} d.m)	Reproducibility (%RSD)	Instrument	No. of WWTPs	∑(PAH) (mg kg^{-1} d.m)	Country	Reference
SE-MASE-MIP	Combined	16	0.14 – 12.86	<18	GC-MS	2	9.03 - 10.75	South Africa	Current
SE	Reconstitution	16	-	-	HPLC-FLD	1	14.6 – 30.9	France	[36]
USE	SPE	16	4.1 – 12.1	-	HPLC-FLD	1	1.44 – 1.26	Italy	[37]
US-EPA 3540C	SPE	16	0.21 – 1.3	-	GC-MS	11	1.4 - 33	China	[34]
SE	SPE	16	-	-	GC-MS	6	2.5 – 25.9	China	[38]
USE	SPE	16	<50		HPLC-UV	4	2.83 – 9.95	Poland	[39]
USE	SPE	16	90 - 600	9 - 22	HPLC-UV	3	5.755 – 36.440	Poland	[40]
USE	SPE	16	1 - 10	-	GC-MS	6	1.13 – 5.52	Spain	[41]
MSPD	Combined	16 (17)	0.05 – 2	6	HPLC-FLD	5	1.1 – 10.3[a]	Spain	[17]
MSPD	SPE	16 (27)	0.03 – 0.27	-	GC-MS	19	0.323 – 6.066	Spain	[35]
MAE	SPE	16	0.1 – 4	0.1 - 6	HPLC-FLD	1	10.075	Spain	[4]
MAE	direct	16	4 - 12	<10	HPLC-FLD	1	2.666 – 3.176	Spain	[42]
SE, Soxtec, PLE	GPC	16	-	0.29 – 16.5	GC-MS/MS	4	1.56 – 6.18	Kuwait	[43]
USE	SPE	16	0.2 - 45	0.6 - 17	HPLC-FLD	4	0.21 – 2.67	Spain	[44]
Shaking	direct	16	-	-	GC-MS	1	0.48	Morocco	[33]

USE	GPC	16	-	-	GC-MS	12	33.73 – 82.58	China	[45]

[a]Includes amount of dibenzo[a,l]pyrene.

GPC - gel permeation chromatography, MAE - microwave assisted extraction, MSPD - matrix solid-phase dispersion, PAH – polycyclic aromatic hydrocarbon; PLE - pressurized liquid extraction, SE - Soxhlet extraction, SE-MASE-MIP – Soxhlet extraction-membrane assisted extraction-molecularly imprinted polymer, USE - ultrasonic extraction, WWTP – wastewater treatment plant

4 Concluding remarks

A novel and selective SE-MASE-MIP technique has been proposed for analysis of hydrophobic organic compounds in complex solid samples using polycyclic aromatic hydrocarbons in wastewater sludge samples as model compounds. The developed technique is a promising innovative prospect that can be explored in the study of PAHs and other organic pollutants in complex solid samples. Despite the three-way extraction occurring in single format, the technique is reproducible with Soxhlet extraction process being the rate limiting step. Improvements in the technical set-up of the technique especially the Soxhlet extractor is currently being explored by the authors to increase the mass transfer of analytes. The current design has been applied in the determination of PAHs from wastewater sludge samples taken from two wastewater treatment plants.

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Conflict of interest statement

There are no financial or other relations that could lead to a conflict of interest.

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CHAPTER 5

5 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

A smart polymer that consists of a benzo[k]fluoranthene and indeno[123-cd]pyrene mixed at 1:1 (w/w) was successfully selected from several cavity tuning experiments for the effective extraction of 16 US-EPA priority PAHs from solutions. The polymer combination displayed a broad selectivity towards the 16 PAHs with no imprinting effect observed. The adsorption and kinetic studies showed that the adsorption process results in formation of a monolayer that attains a maximum capacity with 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. However, cavity efficiency studies showed that a small amount of the PAHs was adsorbing on the hydrophobic backbone of the polymer. Tuning of MIP-cavities was emphasized as an essential approach in finding smart polymers that can target several analytes of related structural and sterical orientation from matrix effects.

The combination of membrane assisted solvent extraction and molecularly imprinted polymer technique gave high recoveries for all the 16 US-EPA priority PAHs. Clean chromatograms were obtained compared to when the membrane assisted solvent extraction or the molecularly imprinted solid phase extraction techniques were applied to wastewater separately. This was an indication of the effectiveness of the combination technique with detection limits of the technique towards the 16 PAHs ranging from 0.1 – 0.45 ng mL^{-1}. The optimized technique was successfully applied in the quantitation of PAHs in effluent wastewater samples from a wastewater treatment plant. PAHs were detected in the concentration range of 3.4 – 1829.8 ng mL^{-1}.

The Soxhlet extraction-membrane assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique that combines Soxhlet extraction, membrane assisted solvent extraction and the polymer into a single step extraction procedure was also successfully developed and optimized for the extraction of PAHs from complex solid samples. This technique also displayed high selectivity and reproducibility in analysis of PAHs from wastewater sludge samples. The

developed technique is a promising innovative prospect that can be explored in the study of PAHs and other persistent organic pollutants in complex solid samples. Despite the three-way extraction occurring in single format, the technique is reproducible with Soxhlet extraction process being the rate limiting step. Improvements in the technical set-up of the technique especially the Soxhlet extractor and using membranes with smaller pore sizes can be explored to increase the mass transfer of analytes. The current design has been applied in the determination of PAHs from wastewater sludge samples taken from two wastewater treatment plants. The total PAH content for the two WWTPs under study were found to be higher than the maximum total concentration of 6 mg kg^{-1} permitted by the EU. The calculated ratios of the PAH pairs with molecular weight 128, 202, 228 and 276 were indicative that these sludge PAHs are from pyrogenic sources such as burning of coal and wood as well as incineration of solid waste material.

The two developed techniques were considered novel in that they both eliminate the need for a separate clean-up step. A single step format also has the advantage of reducing the possibility of contamination or loss of the analyte during multistep procedures. The sample turnaround time is also greatly reduced. The synthesized smart polymer for the PAHs also increased the selectivity and effectiveness of the techniques in isolating PAHs from complex samples where the adversity of matrix effects is most pronounced. The two extraction techniques can therefore be considered as viable extraction techniques that can be used in the analysis of PAHs and other persistent organic pollutants from complex environmental samples.