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Development of Selective Sorbent Materials for the Determination of Polychlorinated Biphenyls (PCBs) in the Environment

DISSERTATION

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vorgelegt von
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Dedicated to my family

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List of Abbreviations

2-VP	2-vinylpyridine
4-VP	4-vinylpyridine
AA	Acrylamide
ABCHC	2,2'-azobis-(cyclohexylcarbonitrile)
ADVN	2,2'-azobis-(2,4-dimethylvaleronitrile)
AIBN	2,2'-azobis-(isobutyronitrile)
AMBN	2,2'-azobis-(2-methylbutyronitrile)
ATSDR	Agency for toxic substances and disease registry
BaP	Benzo[a]pyrene
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CNTs	Carbon nanotubes
DAD	Diode array detector
DVB	Divinyl benzene
EGDMA	Ethylene glycol dimethacrylate
FIB	Focused ion beam
FT-IR	Fourier transform infrared
g	gravitational acceleration constant
GC-μECD	Gas chromatography micro electron capture detector
HCB	Hexachlorobenzene
HEMA	2-hydroxyethyl methacrylate
HPLC	High performance liquid chromatography
IF	Imprinting factor
IUPAC	International Union of Pure and Applied Chemistry
K_{oc}	octanol-water partition coefficient

LF	Langmuir-Freundlich
MAA	Methacrylic acid
MAE	Microwave assisted extraction
MDL	Method detection limit
MIP	Molecularly imprinted polymer
MISPE	Molecularly imprinted solid phase extraction
MIT	Molecular imprinting technology
MWCNTs	Multi-walled carbon nanotubes
NIP	Non-imprinted polymer
NMR	Nuclear magnetic resonance
PCBs	Polychlorinated Biphenyls
PLE	Pressurised liquid extraction
POPs	Persistent organic pollutants
$ScCO_2$	Supercritical carbon dioxide
SEM	Scanning electron microscope
SFE	Super-critical fluid extraction
SPE	Solid phase extraction
SWCNTs	Single-walled carbon nanotubes
TFMAA	Trifluoromethylacrylic acid
THF	Tetrahydrofuran
TRIM	Trimethylolpropane trimethacrylate
U.S. EPA	United States Environmental protection agency
UAE	Ultrasonic assisted extraction
ULEX	Ulm Extractor
UV-Vis	Ultraviolet visible spectroscopy
WHO	World health organization
XPS	X-ray Photoelectron Spectroscopy

List of Publications

This thesis is based on the following publications, which are included at the end of the thesis.

Paper I. Elizabeth N. Ndunda, Vincent O. Madadi, Boris Mizaikoff. An alternative clean-up column for the determination of polychlorinated biphenyls in solid matrices. *Environmental science processes & impacts, 2015,* 17: 2101-2109, DOI: 10.1039/C5EM00409H (*Reproduced by permission of the Royal Society of Chemistry*).

Paper II. Elizabeth N. Ndunda, Boris Mizaikoff. Synthesis of stationary phases that provide group recognition for polychlorinated biphenyls by porogenic fragment template imprinting. *J. Sep. Science,* 2016, 39: 939-946 DOI: 10.1002/jssc.201500960 (*Reproduced by permission of the John Wiley and Sons).*

Paper III. Elizabeth N. Ndunda and Boris Mizaikoff. Multi-walled Carbon Nanotubes: Innovative Sorbents for Pre-concentration of Polychlorinated Biphenyls in Aqueous Environments. *Analytical methods,* 2015, 7: 8034-8040, DOI: 10.1039/C5AY01496D) *(Reproduced by permission of the Royal Society of Chemistry)*

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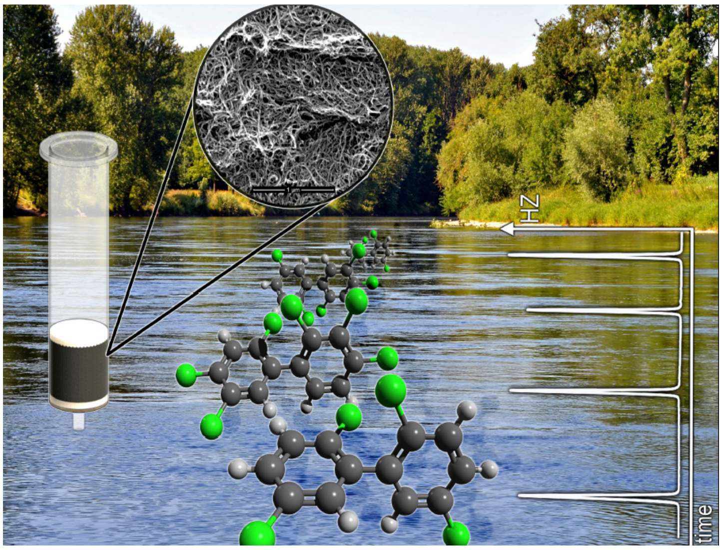


Paper IV. Elizabeth N. Ndunda, Boris Mizaikoff. Molecularly imprinted polymers for the analysis and removal of polychlorinated aromatic compounds in the environment: a review. *Analyst,* 2016, 141: 3141-3156, DOI: 10.1039/C6AN00293E *(Reproduced by permission of the Royal Society of Chemistry).*

Thesis outline

This cumulative thesis aimed at the investigation of alternative sorbents based on molecularly imprinted polymers (MIPs) and multi-walled carbon nanotubes (MWCNTs) for monitoring of polychlorinated biphenyls (PCBs) in various environmental matrices. The first section of the thesis is the general introduction, where the concept of molecular imprinting and the three main imprinting approaches are discussed. The non-covalent imprinting approach owing to its simplicity and availability of functional monomers is the preferred approach, and was exclusively applied in this thesis. The emerging term "class selective MIPs" for simultaneous recognition and detection of a group of similarly structured compounds is also discussed, and was adopted during these studies. The current knowledge on use of MIPs and MWCNTs in sample pre-treatment and the need for validation of affordable analytical protocols for monitoring of PCBs is discussed. Facts about PCBs are presented, and the particular concern about PCBs in the Kenyan environment is discussed with a view of identifying the existing technology gaps, as far as their monitoring is concerned.

The second section contains the 4 papers that make up this thesis. Paper I sought to develop a reliable and affordable column for clean-up of extracts during the determination of PCBs in complex matrices. Thus prepared cartridges based on MIPs were successfully applied for the quantification of PCBs in sediments and soil. Motivated by the performance of the cartridges and their re-usability, further determination of PCBs in soils from various solid waste disposal sites and sediments from a second river was achieved, and the results are included at the end of Paper I. Paper II introduces two different strategies for imprinting of PCBs based on chlorinated benzene and xylenes serving as porogenic templates, whereby porogenic fragments present a particularly attractive option for chlorinated compounds. Paper III discusses the potential of using MWCNTs as sorbents for the quantitative pre-concentration of PCBs from aqueous samples. Thereby, it was established that these sorbents work well with highly chlorinated constituents, and may also be applicable in water purification, yet only in the absence of oxidizing agents. Paper IV is a review on strategies that have been applied to synthesis polymers selective to polychlorinated aromatic compounds (PACs), and subsequent application of these polymers in determination of PACs in the environment.

Zusammenfassung

Die Anwendung molekular geprägter Polymere (MIPs) als Sorptionsmaterial in der Festphasenextraktion (SPE) ist eine interessante Alternative zu herkömmlichen Verfahren der Probenvorbehandlung. Die Selektivität und Stabilität molekular geprägter Polymere, welche von erheblicher Bedeutung in der Ultraspurenanalyse sind, können unter anderem eine gründliche Probenvorbehandlung durchaus vereinfachen. Weitere neuartige Sorptionsmaterialien sind mehrwandige Kohlenstoffnanoröhrchen (MWCNTs), die bereits seit langem Gegenstand intensiver Forschung sind, vor allem wegen ihrer herausragenden chemischen und mechanischen Stabilität, ihrer großen Oberfläche, sowie ihren elektrischen Eigenschaften. Darüber hinaus zeigen MWCNTs besonders ausgeprägte Wechselwirkungen mit aromatischen Verbindungen aufgrund des π-Elektronensystems der Graphenschichten, und wurden daher zusammen mit MIPs als Sorptionsmittel zur Anreicherung von polychlorierten Biphenylen (PCBs) in verschiedenen umweltrelevanten Medien untersucht.

Die Synthese von MIPs zur Erkennung von PCBs wurde nach dem sogenannten 'Dummy-Templat' Ansatz durchgeführt, wobei das angewandte Template nicht unbedingt dem Zielanalyten entspricht, der anschließend in der realen Anwendung selektiv erkannt werden soll. PCB 15 wurde als Dummy-Templat eingesetzt und dient in diesem Fall als Modell für die folgenden Zielanalyten: PCB 28, 52, 101, 138, 153 und 180. Synthetisiert wurde mit Hilfe der Suspensionspolymerisation in wässrigem Medium. Dabei wurden sphärischen Mikropartikel im Größenbereich von 6-60 µm erhalten. Die elektronenstrahlmikroskopisch beobachteten rauen Oberflächen stimmen mit den hohen spezifischen Oberflächen im Bereich von 134.45 bis 285.56 m^2 g^{-1} gut überein. Bindungsstudien führten zu einem Prägefaktor von 1.09, wobei die geringe molekulare Erkennung auf schwache Wechselwirkungskräfte (d.h., π-π Stapelung) in der Synthese zurückgeführt werden kann. Die eingehende Charakterisierung der Polymeren unter Anwendung von Langmuir und Freundlich Adsorptionsisothermen zeigte, dass MIPs geringfügig höhere maximale Bindungskapazitäten gegenüber den nichtgeprägten Polymern (NIP) (60. 2 *vs.* 54. 9 µg g^{-1}) aufwiesen. Das optimierte MIP zeigte auch ein heterogeneres Bindungsverhalten als das NIP, welches charakteristisch für geprägte

Polymere aufgrund der heterogenen Verteilung der Bindungsaffinitäten ist. Des Weiteren wurde ein Referenzpolymer in Abwesenheit des Templats sowie des funktionellen Monomers synthetisiert, welches die niedrigste Bindungskapazität (23.5 μg g^{-1}) und eine homogene Verteilung an Bindungsaffinitäten aufwies.

Näher an der beabsichtigten Anwendung zur Anreicherung von PCBs in organischen Medien führte eine weitergehende Optimierung zu Wiederfindungsraten von 94.9 bis 99.0% im Vergleich zu konventionellen C18 Sorbentien, die eine Wiederfindung zwischen 69.2 und 78.9% ergab und zu hohen Verlusten bei der Probenaufgabe führte. C18 zeigte daher wenig vorteilhafte Wechselwirkung mit unpolaren PCBs im Vergleich zu den entwickelten MIPs. Andererseits zeigte kommerziell verfügbares Sulfoxid-modifiziertes Kieselgel eine verbesserte Wiederfindung (83.8 bis 90.2%). Eine weitere Optimierung der Materialien zur Anwendung in wässrigen Medien führte zu einer verringerten Wiederfindung (<70%), sowohl C18 sowie das Sulfoxid-modifizierte Kieselgel, während die molekular geprägte Festphasenextraktion (MISPE) eine Wiederfindung >70% ergab. Die beiden kommerziell erhältlichen Sorptionsmittel wurden daher zur Anreichung von PCB in wässriger Umgebung ausgeschlossen, und mittels MISPE die Anreichung von PCBs in realen Wasserproben mit einer Wiederfindungsrate >80% gezeigt. Die realisierten Nachweisgrenzen im Bereich von 0.002 bis 0.01 ng mL^{-1} waren niedriger als der festgelegte maximale Belastungswert von 0.5 ng mL^{-1} für ΣPCBs im Trinkwasser. Dies bedeutet, dass die im Rahmen dieser Dissertation entwickelte Anreicherungsmethodemethode zur quantitativen Überwachung von PCBs geeignet ist.

Zur Bestimmung von PCB in komplexen Matrizes wurde MISPE entsprechend weiterentwickelt. Organische Stoffe konzentrieren sich speziell in geologischen Senken auf, weshalb im Rahmen der kontinuierlichen Überwachung von PCBs in der Umwelt die Analyse von Boden- und Sedimentproben von besonderem Interesse sind. Aufgrund der Komplexität dieser beiden Matrizes wurde eine verbesserte MISPE-Kartusche zur Aufreinigung/Anreicherung von Boden- und Sedimentextrakten entwickelt, wobei die MIP Partikel und angesäuertem Kieselgel kombiniert wurden. Dieses Verfahren wurde anschließend zur Extraktion und Quantifizierung von PCBs aus Boden- und Sedimentproben eingesetzt. Eine Wiederfindungsrate von >70% wurde für alle Analyten realisiert. Das Verfahren wurde weiters zur Bestimmung der PCBs in realen Boden- und Sedimentproben aus Kenia getestet. Neben der Möglichkeit PCBs sowohl im Boden als auch im Sediment zu quantifizieren, bot die entwickelte Methode saubere Extrakte, die wenig bis gar keine Störungen in der anschließenden chromatographischen Analyse verursacht haben und niedrige Nachweisgrenzen erzielten. Das Volumen des Lösungsmittels konnte im Vergleich zu konventionellen Probenaufbereitungs- und Probenaufreinigungsverfahren ebenfalls signifikant reduziert werden. Molekular geprägte Polymere haben bereits in der Vergangenheit eine außerordentliche Materialbeständigkeit bewiesen. In der vorliegenden Studie wurden dementsprechend hohe Wiederfindungsraten von mehr als 80% selbst nach 30 Anwendungszyklen und Regeneration bestätigt.

Zur Optimierung des Prägefaktors sowie des Templats wurden zwei weitere Dummy-Template auf Basis der Fragment-Templat Prägung untersucht. Hexachlorbenzol (HCB), sowie eine Mischung der Konstitutionsisomere orto-, meta- und para-Xylol, wurden als porogenes Fragment-Templat eingesetzt. Die resultierenden, porösen und sphärischen Mikropartikel waren durch spezifische Oberflächen im Bereich von 4.03 bzw. 329.7 $m^2 g^{-1}$ für Xylol-nichtgeprägtem (NIPs) und Xylol-geprägte Polymere (XIP) gekennzeichnet. Polymere die mit einem Mischungsverhältnis von 1:16:80 (HCB als Templat: funktionelles Monomer : Vernetzer) synthetisiert wurden ergaben einen Prägefaktor von 1.2, im Gegensatz zu jenen mit einem Verhältnis von 1:8:40. Das gleiche Phänomen wurde bei der anschließenden chromatographischen Retention beobachtet, wobei das MIP die höchsten Retentionsfaktoren unter allen synthetisierten Polymeren ergab. Diese Beobachtungen bestätigen die Notwendigkeit einer erhöhten Konzentration an funktionellem Monomer für das nicht-kovalente Prägen, das die Bildung stabiler Template-Monomer Komplexe begünstigt. Die Ergebnisse der chromatographischen Trennungen, sowie der SPE Bindungsstudien legen nahe, dass Xylol zur Prägung von PCBs hervorragend geeignet ist.

Für eine mögliche Anwendung von MWCNTs als Sorptionsmittel zur Anreichung während der Analyse von PCBs in wässriger Umgebung wurden im Vergleich unbehandelte MWCNTs und oxidierte MWCNTs untersucht. Verschiedene Einflußparameter, wie die CNT Dimension, die Durchflussrate, das Elutionsmittel, die

Oberflächenoxide und der pH-Wert wurden untersucht, vor allem in Hinblick auf die Wiederfindungsrate. MWCNTs mit kleineren Außendurchmessern (10 nm) ergaben höhere Wiederfindungsraten im Vergleich zu MWCNTs mit großen (110-170 nm) Durchmessern. Ein starkes Elutionssmittel (*n*-hexan:dichlormethan, 1:1) bei einer mittleren Durchflussrate von 5 mL min^{-1} ergab die beste Wiederfindungsrate. Die Anwendung von MWCNTs zur Anreichung von PCBs aus realen Gewässerproben führte zu Wiederfindungsraten von 46.0 bis 92.5%. PCB 28 ergab die niedrigste Wiederfindung, welche auf die hohe Löslichkeit und den niedrigen Oktanol-Wasser-Verteilungskoeffizienten (K_{ow}) im Vergleich zu den anderen PCBs zurückgeführt werden kann. Die Oxidation von MWCNTs erhöht ihre Dispersion in Wasser, jedoch mit negativen Auswirkungen in Bezug auf eine verminderten Wiederfindungsrate. Sauerstoffhaltige funktionelle Gruppen führen zur Bildung von Wasserclustern und verringern somit die Anzahl möglicher Bindungsstellen für die Adsorption von PCBs. Da im Rahmen der Untersuchungen an Realproben nur 100 mg Material erforderlich waren, um die gewünschten Ergebnisse zu erzielen, eignen sich MWCNTs auch als kommerziell sinnvolles, alternatives Sorptionsmittel im Vergleich zu C18 zur quantitativen Anreichung aromatischer Verbindungen in der Umweltanalytik.

Summary

The application of molecularly imprinted polymers (MIPs) as sorbents in solid phase extraction (SPE) leading to molecularly imprinted SPE (MISPE) is among the most advanced application scenarios for molecular imprints, and is nowadays considered a viable alternative to conventional procedures of sample pre-treatment. Furthermore, the selectivity and stability that MIPs provide evidently facilitates thorough sample pre-treatment, which is important for ultra-trace level detection. Alternative promising sorbents are multi-walled carbon nanotubes (MWCNTs), which have been the subject of extensive research mainly due to their outstanding chemical and mechanical stability, electrical properties, and high surface area. In addition, owing to the π-electron system of the graphene sheets, MWCNTs strongly interact with aromatic compounds, and were therefore together with MIPs investigated during the present thesis research as potential sorbents for the pre-concentration of polychlorinated biphenyls (PCBs) from various environmental matrices.

Therefore, following the dummy template approach - whereby the template used is not necessarily the target analyte in the real-life application - MIPs for recognition of PCBs were synthesized. PCB 15 was used as the dummy template to model the compounds targeted for analysis in the present study, *i.e.,* PCB 28, 52, 101, 138, 153, and 180. Since the synthetic protocol was aqueous suspension polymerization, the resulting particles were microspheres in the size range of 6 to 6 μm, as determined by scanning electron microscopy (SEM). The observed rough surfaces were in agreement with the recorded high specific surface areas ranging from 134.45 to 285.56 m^2 g^{-1}. Batch rebinding studies resulted in an imprinting factor of 1.09, whereby the low molecular recognition was attributed to the weak forces of interaction (*i.e.,* π-π stacking) during the synthesis. In depth characterization of the polymers using Langmuir and Freundlich adsorption isotherms showed that the MIP had slightly higher maximum binding capacities than the non-imprinted polymer (NIP) (60.2 *vs.* 54.9 μg g^{-1}). The MIP was also more heterogeneous than the NIP, which is characteristic of imprinted polymers because of the heterogeneous distribution of the binding site affinities. In addition, a blank prepared in the absence of both the template and the functional monomer gave the lowest binding capacity (23.5 μg g^{-1}) and revealed a homogenous distribution of binding site affinities.

Closer to the intended application, optimization of the MISPE for application in cases where the analytes are in organic medium resulted in recoveries of 94.9-99.0% compared to conventional C18 materials, which gave recoveries between 69.2 and 78.9%, with significant losses in the loading step. C18 therefore demonstrated poor interactions with non-polar PCBs, as compared to MIPs. On the other hand, commercially available sulfoxide-modified silica showed enhanced recoveries (83.8-90.2%). Further optimization for application in aqueous environment resulted in decreased recoveries for both C18 and sulfoxide-modified silica (<70%), while the MISPE maintained recoveries >70%. Consequently, the two commercial sorbents were ruled out as possible sorbents for pre-concentration of PCBs in aqueous environment; therefore, MISPE was applied in pre-concentration of spiked real-world water samples giving recoveries >80%. The realized detection limits ranging from 0.002 to 0.01 ng mL^{-1} were lower than the set maximum contaminant level of 0.5 ng mL^{-1} for ∑PCBs in drinking water; implying that the developed protocol may be applied for monitoring of PCBs in environmental contamination scenarios.

Towards the determination of PCBs in complex matrices, the MISPE was then modified and optimized for this purpose. Soil and sediment were the matrices of interest, as they are considered geological sinks for organics, thus serving as important matrices for monitoring of PCBs. Due to the complexity of these two matrices, a specific column for clean-up of extracts from the two matrices was designed using MIP particles and acidified silica gel. The cartridge was optimized and applied in validation of a full method from extraction to quantification using spiked soil and sediment samples. Recoveries >70% for all the analytes were realized. The method was then applied for the determination and quantification of PCBs in real-world soil and sediment samples, which had been collected in Kenya. Besides being able to quantify PCBs in both soil and sediment, the developed method offered clean extracts showing little or no interferences during the subsequent chromatographic analysis, and achieved low detection limits. Furthermore, the volume of extraction solvent used was reduced significantly, as compared to conventional clean-up methods. Since MIPs have in the past proved to be stable and robust materials, in the present study these polymers have confirmed their unequalled stability by maintaining high recoveries (>80%) even after 30 cycles of use and regeneration.

To improve on the imprinting factor and avail more templates for imprinting of PCBs, two other dummy templates based on fragment template imprinting were investigated. Hexachlorobenzene (HCB) was used as the fragment template, and a mixture of otho-, meta-, and para-xylene as porogenic fragment templates. Thus resulting polymers were porous microspheres with specific surface areas ranging from 4.03 to 329.7 m^2 g^{-1} for xylene non-imprinted (NIPs), and xylene imprinted polymers (XIP), respectively. Polymers synthesized using HCB at a template: functional monomer: cross-linking ratio of 1:16:80 gave an imprinting factor of 1.2, in contrast to those prepared with a ratio of 1:8:40. The same phenomenon was observed in the subsequent chromatographic retention, where the MIP gave the highest retention factors among all the polymers synthesized. These observations corroborate with the need for increased functional monomer in non-covalent imprinting, which favours the formation of stable template-monomer complexes. From the rebinding, chromatographic, and SPE results using XIP, it was clear that xylenes are apparently highly suitable dummies for imprinting PCBs.

For investigating the potential utility of MWCNTs as pre-concentration sorbents during the analysis of PCBs in aqueous environments, both pristine MWCNTs (as-received) and oxidized MWCNTs were studied. Various parameters that may affect PCBs recovery including CNT dimension, flow rate, eluting solvent, surface oxides, and pH were investigated. MWCNTs with small outside diameters (10 nm) were found to give higher recoveries, as compared to those with larger diameters (110-170 nm). A strong eluting solvent (*n*-hexane: dichloromethane, 1:1) at a medium flow rate (5 mL min^{-1}) gave the best recoveries. Application of MWCNTs for pre-concentration of PCBs from real-world water samples resulted in recoveries of 46.0-92.5%. PCB 28 gave the lowest recovery in all instances, which was attributed to the high solubility and low octanol-water partitioning coefficient (K_{ow}), as compared to the other investigated PCBs. Consequently, it was concluded that the developed method was only applicable for sufficiently chlorinated compounds. Oxidation of MWCNTs evidently increased their dispersion in water, however, with the negative effect of decreased recoveries. Oxygen-containing functional groups lead to the formation of water clusters, thus decreasing the number of surface sites available for the adsorption of PCBs. As only 100 mg of MWCNT material was required to achieve the desired results, MWCNTs presented commercially viable alternative sorbents vs. C18 for the pre-concentration of aromatic compounds in environmental monitoring scenarios.

1. Introduction and scope of this work

For decades, scientists have focused on search for materials that can mimic naturally occurring molecular recognition functions, a topic which is nowadays referred to as biomimetics.[1] It draws inspiration from the fact that nature has developed systems that are well-adapted and self-sustaining, and imitations of the basic principles *via* appropriate technologies would ensure various human problems are solved sustainably. Molecular imprinting technology (MIT) is one of the topics within biomimetics, which has attracted substantial attention in the scientific community.[2,3] MIT entails the design of polymers with tailor made binding cavities that demonstrate selectivity likened to that of natural receptors. Thus resulting molecularly imprinted polymers (MIPs) are frequently referred to as artificial receptors,[4] antibody mimics,[5] biomimetic receptors,[6] and plastic antibodies.[7] Their additional property of stability has contributed to notable applications, such as antibody mimics in immunoassays (referred to as molecularly imprinted sorbent assays (MIAs)),[8] stationary phases in column chromatography (achieving baseline separation of enantiomers),[9] recognition elements in sensors,[10] and adsorbents in solid phase extraction (SPE).[11,12]

So far, the application of MIPs in SPE (so-called molecularly imprinted solid phase extraction (MISPE)) is among the most advanced strategies, and ongoing commercialization of MISPE cartridges is clear evidence of their potential, especially in sample pre-treatment. This critical step in sample analysis has remained open for improvement, as it seeks to simultaneously fulfil four key objectives, *i.e.,* i) pre-concentration of the analyte to enhance its detection, ii) extraction of the analyte to an appropriate form for detection, iii) removal of matrices which may interfere with detection, and iv) optimization of a procedure that is reproducible irrespective of the sample matrix.[13] Incorporation of MIPs in sample pre-treatment is therefore able to revolutionize this step, as these material offer additional properties of stability and selectivity. Selectivity ensures that only the target compounds or similarly structured compounds are retained, while the interfering compounds are eliminated. This is important especially during the determination of environmental contaminants, which occur at ultra-trace levels and in complex matrices, and only exhaustive sample pre-treatment would ensure detection at these levels is achieved.

Polychlorinated biphenyls (PCBs) are among the environmental contaminants of global concern. Their chemical and thermal stability made them major components in electrical equipment and additives in various open systems, thus making them ubiquitous environmental contaminants. Though further production and use was banned in many countries since 1970s, and are currently regulated under the Stockholm convention on persistent organic pollutants (POPs),[14] they continue to be detected in the environment because of past usage, incomplete combustion of chlorine containing compounds, and continued use of PCBs containing equipment, especially in developing countries. There is therefore a great need for development of techniques that are affordable, selective and sensitive to ensure their continuous monitoring in order to protect the environment and human health. It is also the goal of the Stockholm convention on POPs that these chemicals are eventually eliminated from the environment, which can only be tracked through monitoring. Coupled with robust and affordable detection techniques such as gas chromatography electron capture detector (GC-ECD),[15] selective sorbents that achieve thorough sample pre-treatment at a minimal cost will ensure that continuous monitoring is sustainable.

The present research thesis therefore focused on the synthesis of imprinted polymers that recognize PCBs for applications as pre-concentration and clean-up sorbents during their determination in aqueous environments and complex matrices, such as soil and sediment. Water is one of the primary channels through which humans and animals can be exposed to these compounds, while soil and sediments are considered geological sinks that contribute to environmental recycling of PCBs. Multi-walled carbon nanotubes (MWCNTs) are other materials which were studied. Besides the π-electron system on the graphene sheets, which contributes to the observed strong interaction with aromatic compounds, their chemical and mechanical stability makes MWCNTs promising materials for application in sample pre-treatment. In fact, MWCNTs are seen as the new generation of sorbents in SPE for analysis of environmental pollutants.[16]

2. Molecular imprinting technology

2.1 Background on molecular imprinting

Extensive research in the field of molecular imprinting was only witnessed from 1980s, but the concept existed long in the 1930s, whereby different drying solvents were observed to leave a 'memory effect' on silica gel pore structure.[17] Later in the 1940s, Linus Pauling argued that the high specificity demonstrated by antigen-antibody interaction is attributed to the formation of an antibody molecule in the presence of an antigen, by so doing dictating the configuration/orientation of the antibody.[18] This theory of antibody formation was later proved to be incorrect, but contributed to the understanding of molecular imprinting. The concept was further shaped in the 1970s and 1980s by the research groups of Wulff[19] and Mosbach,[20] who introduced the covalent and non-covalent imprinting approaches, respectively. The latter approach proved to be straightforward and offered more opportunities for further molecular imprinting exploitation, and continues to be the preferred synthetic approach to date. Combining both approaches is the semi-covalent approach reported in 1995 by the group of Whitcombe.[21] Since then, molecular imprinting has been explored towards achieving the design of materials with near or equal properties as the natural antibodies.

2.2 The molecular imprinting concept

Molecular imprinting entails the creation of ideally molecule-specific binding moieties within or at the surface of a polymer matrix by co-polymerizing functional monomers in the presence of a target molecule. Subsequent removal of the template provides binding sites ideally complementary to the template in shape, size, and orientation of the functionalities. Thus, preferential rebinding of the template with high specificity is ensured. Generally the procedure involves three steps, as outlined below and illustrated in Fig.1.

I. Formation of a pre-polymerization complex between the template and functional monomer *via* covalent or non-covalent interactions.

II. Polymerization of the complex in the presence of cross-linking agents, which is usually activated *via* a radical initiator.

III. Extraction of template leaving behind binding motifs with specific recognition properties for the template.

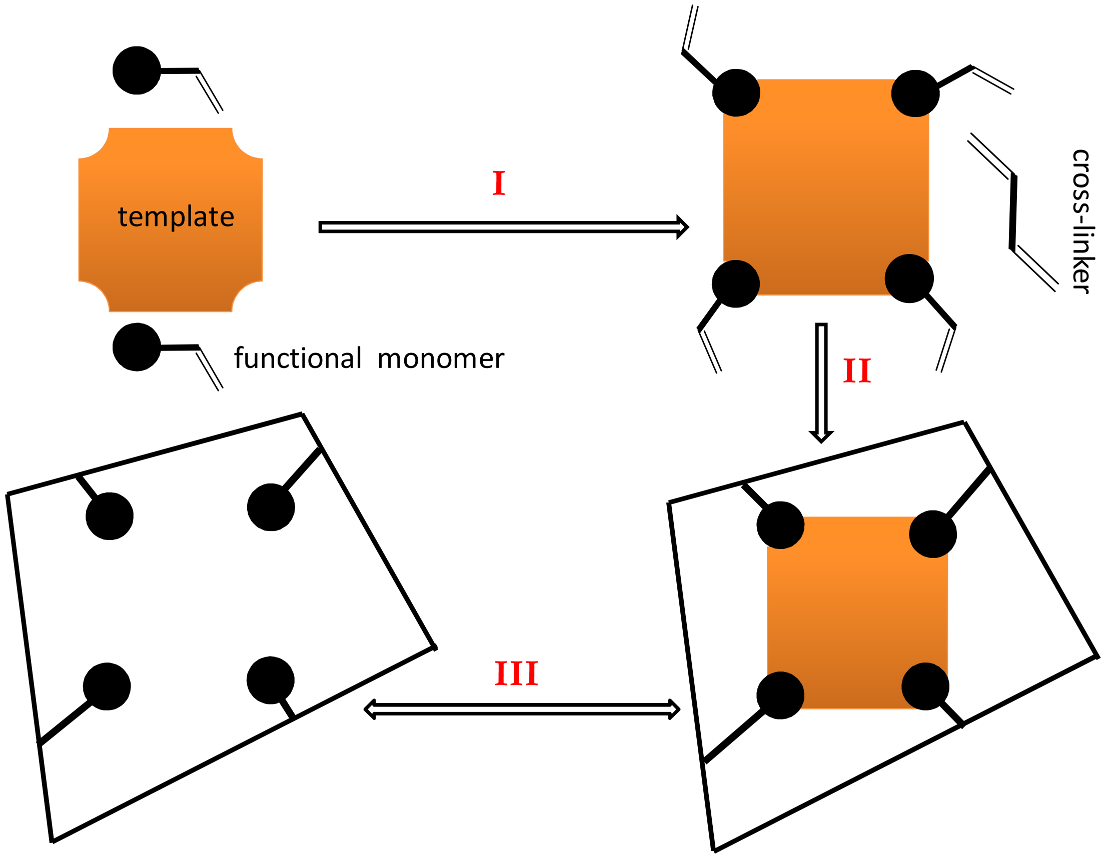


Figure 1: Schematic illustration of the imprinting process, (I) the formation of the pre-polymerization complex, (II) polymerization in the presence of a cross-linker, and (III) removal and rebinding of the template

Depending on the nature of interaction between the functional monomer and the template in step (I), molecular imprinting can be divided into covalent and non-covalent imprinting approaches. There exists a third imprinting approach (i.e., semi-covalent), which is an intermediary between the two main approaches, and involves covalent bonding during the complex formation and non-covalent interactions during rebinding.

2.2.1 Covalent imprinting (pre-organized) approach

In covalent imprinting approach, the template is covalently bound to the functional monomer to form a polymerizable template-monomer complex. Polymerization of the complex in the presence of a cross-linker is then followed by cleavage of the covalent bonds through acid hydrolysis to free the template and create room for rebinding *via* reformation of covalent bonds.[19,22] Since covalent bonds are associated with high binding energy, thus resulting polymers are highly selective, however, this very high binding energy limits their application, especially in separation chromatography because of slow binding kinetics.[2,23] Polymers synthesized *via* this approach offer homogeneous binding sites because of the homogeneous nature of the pre-polymerization complex. Also the likelihood of non-selective binding sites which results from excess functional monomer is eliminated since the complex formation is based on a stoichiometric ratio. The need for prior design/synthesis of a stable template–monomer capable of undergoing covalent bond formation and cleavage, however, limits this approach to ketones, aldehydes, esters of carboxylic/boronic acids and amines.[2,22]

2.2.2 Non-covalent imprinting (self-assembly) approach

Self-assembly by formation of non-covalent bonds (*i.e.,* hydrogen bonding, π-π interactions, van der Walls, and electrostatic forces)[20,23] is more prevalent due to its synthetic simplicity, and the fact that biological associations including antibody-antigen, receptor-ligand, and enzyme-substrate interactions follow similar principles. Polymer synthesis is simply done by solubilizing the template and functional monomers in a

suitable porogen then polymerization initiated in the presence of a cross-linking agent. After synthesis, the template is removed from the resultant polymer by washing with a solvent or a mixture of solvents. Rebinding of the template to the MIP exploits non-covalent interactions. The attractive adoption of this approach is also due to the availability of large number of functional monomers, which can form non-covalent bonds.[24] However, in this approach, due to the weak interaction forces (Table 1), the formation of a stable template-monomer complex is governed by an equilibrium process, which demands for high amount of either functional monomer or template in order to displace the equilibrium towards the formation of a stable complex.[25] Excess monomers results in different combinations ratios of the template-functional monomer complex, which is attributed to the heterogeneous binding sites distribution that MIPs display. Polymers synthesized *via* non-covalent imprinting approach are also characterized by non-selective binding sites due to the random incorporation of the excess monomers.[23,26]
Though non-covalent imprinting appears straightforward, the first step during MIP synthesis facilitating the formation of thermodynamically favorable and stable pre-polymerization complexes is crucial for successful imprinting procedures.[27] Optimizing this complex has in the past predominantly relied on a top-down approach, *i.e.,* trial-and-error based on synthesizing polymers with variations of the template: functional monomer: cross-linker ratio until a final MIP with favorable selectivity is obtained. Nowadays, combinatorial or high throughput screening approaches have largely replaced such efforts by synthesizing a range of MIPs at small scale (also called mini-MIPs) in order to establish the ones with the highest selectivity and binding capacity for subsequent up-scaling.[28–31] In contrast to such top-down strategies, the recent decade has also increasingly seen bottom-up approaches, which entail detailed studies of the chemical interactions at the molecular level combined with molecular modelling strategies.[32,33] For example, NMR titration,[34,35] UV-Vis studies,[33] and infrared spectroscopies[33] have enabled careful selection of the functional monomer, porogen, and polymerization ratios, thereby resulting in the targeted synthesis of high quality MIPs.

Table 1: Bond energies of non-covalent interactions compared to covalent bond.[36,37]

Bond	**Bond energy (kJ mol^{-1})**
covalent bond	200-800
hydrogen bond	4-60
π-π aromatic stacking	1-50
van der Waals forces	<5
Electrostatic	
Ionic-ionic	250
Ionic-dipole	15
Dipole-dipole	2
hydrophobic interactions	1-3

2.2.3 Intermediate imprinting approach

The intermediate approach also called semi-covalent approach exploits both covalent and non-covalent approaches.[21] Thus the template which is covalently attached to the functional monomer is hydrolytically cleaved in order to remove the template and create room for re-binding *via* non-covalent interactions. Vinyl template derivatives like cholesteryl (4-vinyl) phenyl carbonate and bisphenol A dimethacrylate (BPADM) have been able to produce polymers selective to cholesterol and bisphenol A.[21,38] Just like the covalent imprinting approach, this approach provides polymers with homogeneous binding sites, which translates to high binding capacities and high selectivity.[39] The approach is devoid of template bleeding because any template that is not cleaved during hydrolysis – a more harsh condition compared to the normal MIP application - remains attached to the polymer matrix.

2.3 Relevant aspects for successful imprinting

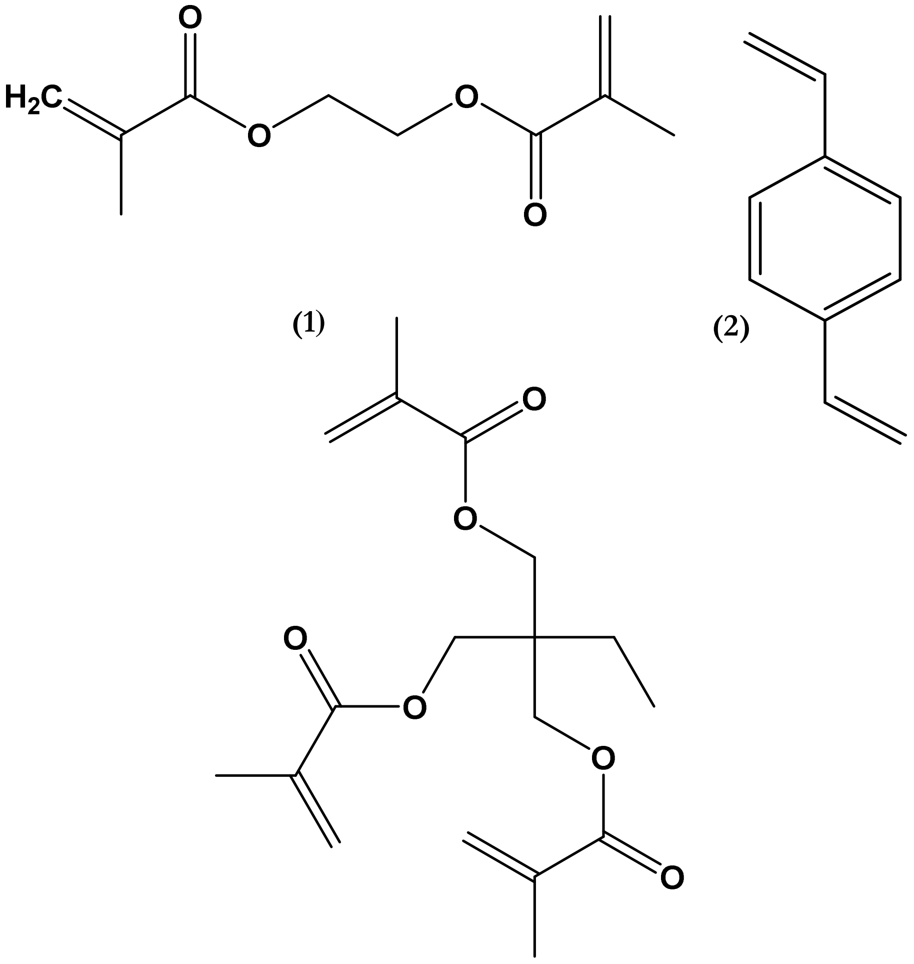
2.3.1 Choice of the porogen

The solvent used in polymerization (usually referred to as porogen) governs the polymer morphology, the strength of non-covalent interactions, and solubilizes the polymerization constituents.[40–42] The porogen of choice should not interfere with the forces of interaction between the template and the functional monomer,[43] since destabilization of the pre-polymerization complex results in polymers with little or no molecular recognition.[40] For instance, when the forces of interaction between the template and the functional monomer are ionic and hydrogen bonding, polar protic solvents may not be recommended, and inert solvents with low dielectric constants such as toluene and dichloromethane are appropriate.[41] The porogen also influences the polarity of the pore walls in the polymer matrix, which plays a decisive role in the rebinding properties of the polymer. Depending on the porogen used the pore wall maybe polar or non-polar. Non-polar porogens give rise to a non-polar wall by exposing the polar groups of the functional monomer inside the bulk of the polymer matrix, while polar porogen exposes more polar groups in the pore wall.[40]

Some studies have used supercritical carbon dioxide (scCO_2) as the porogen owing to the advantages of non-toxicity, availability, diffusivity, and its non-polar nature enabling stabilization of non-covalent interactions.[38,44] scCO2 is also well applicable in template removal due to its rapid diffusion into the polymer matrix at elevated temperatures.[45] Recently, room temperature ionic liquids (RTILs) have been proposed as alternatives to organic solvents, promoting the concept of green chemistry. Attractive attributes of RTILs are high solvating power, stability, negligible vapour pressure, and a wide viscosity range, which enhances the polymerization rates[46] and gives rise to polymers with high binding capacities and selectivity, compared to those synthesized using conventional porogens[46,47] Use of water as a porogen is rarely reported, although in some cases hydrophobic forces have been explored with a mixture of water and methanol (4:1) towards synthesis of water-compatible MIPs. A 2,4-dichlorophenoxyacetic acid (2,4-D) imprinted polymer employing 4-vinylpyridine (4-VP) and ethylene glycol dimethacrylate (EGDMA) as the functional monomer and cross-linker, respectively, resulted in polymers with high selectivity for 2,4-D compared to the analogues.[48,49] Polymerization in aqueous medium resembles the natural environment where the interactions of macromolecules and their ligands are hydrophobic and metal coordination interactions,[50] thus polymers synthesized this way would have a wide scope of application.

2.3.2 Choice of the cross-linking agent

The cross-linker is a component in polymerization which serves to control the morphology and mechanical strength of the resulting polymer, and stability of the binding sites.[42] EGDMA, trimethylolpropane trimethacrylate (TRIM), and divinyl benzene (DVB) (Fig. 2) are some of the cross-linkers used. EGDMA is the commonly used cross-linker, as it is thought to be stable over a wide temperature range. Chromatographic columns packed with polymers synthesized using EGDMA were shown to maintain their selectivity for long periods even at temperature up to 80 °C, while those prepared using DVB showed reduction in selectivity at 70 °C. On the other hand, multifunctional cross-linker like TRIM has been reported to give rise to regular particles with higher imprinting factors.[51,52] Cross-linking amount in tune of 80% is required for the formation of a rigid polymer, and to maintain the integrity of the formed binding cavities. Polymers synthesized with high amount of cross-linker have reported increased ratio of high affinity binding sites to low affinity sites.[53] 17β-estradiol imprinted polymers synthesized using 80% and 0.8% cross-linker resulted in microspheres and sub-microspheres with the later showing about 70 fold reduction in surface area and a median binding affinity constant of 0.0025 mM^{-1}, compared to 0.013 mM^{-1} for the microspheres.[54] Very large amount of cross-linker may, however, lead to rigid polymers with poor accessibility of the binding sites.[43]



(1) (2)

(3)

Figure 2: Commonly used cross-linking agents: (1) ethylene glycol dimethacrylate, (2) divinylbenzene, and (3) trimethylolpropane trimethacrylate.

The amount of cross-linker in relation to the volume of the porogen is reported to determine whether gel-type polymers, macroporous polymers or microgel powders are formed, as illustrated in Fig. 3. Low cross-linking amounts or high cross-linker dissolved in low amount of porogen affects the phase separation of the polymer resulting in gel-type polymers which form glassy gel-type polymers on drying, while higher cross-linking amounts and increased amount of porogen enable the resulting polymer to phase separate forming a macroporous polymer. On the other hand, very high amounts of the porogen (beyond the macroporous polymer region) results in microgel powder.[42] It is also reported that the amount of porogen and cross-linker affects the pore size distribution of the resulting polymers, whereby increase in the amount of porogen or decrease in the amount of cross-linker leads to wide pore size distribution, *i.e.,* mesopores to macropores, while decrease in the amount of porogen or increase in the amount of cross-linker gives rise to polymers with smaller pore size distribution.[33]

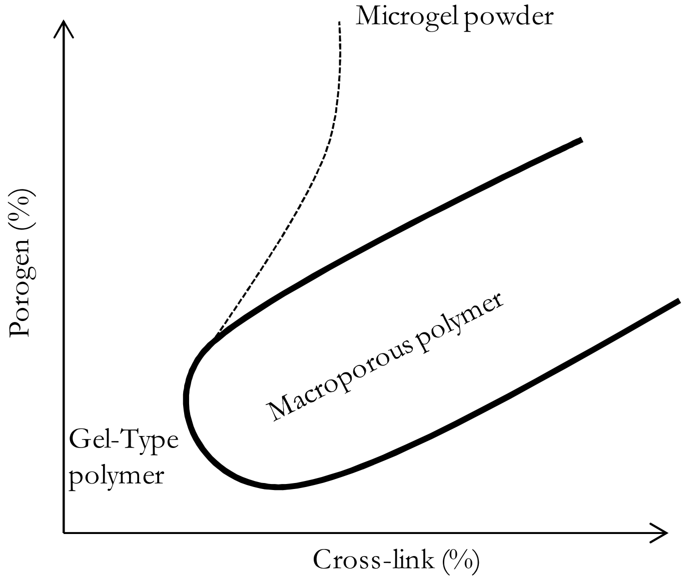
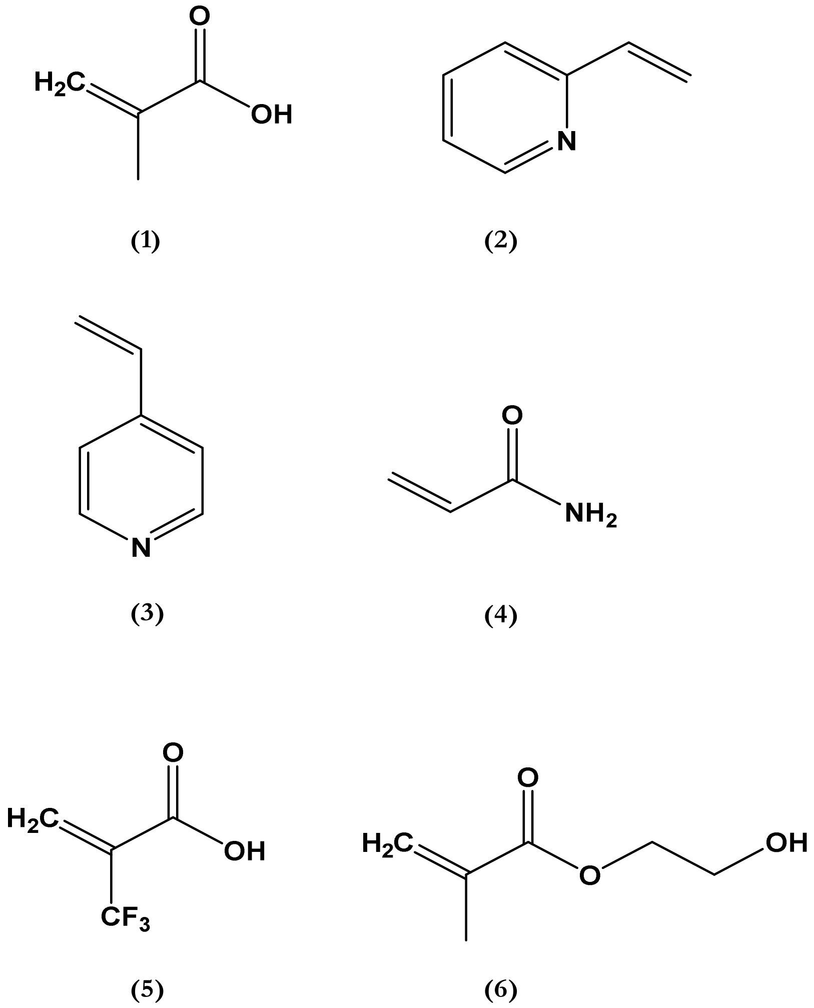


Figure 3: Pseudo phase diagram showing different morphological forms of polymers depending on the amount of cross-linker and porogen. Adapted from Ref. 42. Copyright 2004, with permission from Elsevier.

2.3.3 Choice and amount of functional monomers

Functional monomers are polymerizable constituents containing one or more functional groups for interaction with the template. For this purpose, methacrylic acid (MAA), 2-vinylpyridine (2-VP), 4-VP, acrylamide, trifluoromethylacrylic acid (TFMAA), and 2-hydroxyethyl methacrylate (HEMA) have been employed depending on the perceived interactions with the template (Fig. 4). The functional groups on the functional monomers should complement the template, and MAA has come in handy for templates which are able to form hydrogen bond.[23] Functional monomers with high number of functional groups give rise to polymers with high imprinting effect, probably due to the increased stability of the pre-polymerization complex. For example, a 4-aminopyridine and 2-aminopyridine have given rise to polymers with high capacity factors compared to 4-methyl pyridine.[55] β-cyclodextrins (β-CDs), which bear multiple functionalities are able to interact with different templates *via* hydrogen bonding, van der Waals, and electrostatic forces, and have also acted as functional or co-functional monomers.[56,57] Template to functional monomer (T:FM) ratios up to 1:4 or slightly higher are mostly applied in non-covalent imprinting,[24,25] However, it is worthy to note that this step requires optimization in order to come up with a combination that is appropriate. Surprisingly, exceptionally high T:FM ratios (*i.e.*, 1:500 and 1:1000) have been reported, which resulted in MIPs with significantly higher binding capacities than the controls.[58]



(1) **(2)**

(3) **(4)**

(5) **(6)**

Figure 4: Commonly used functional monomers: (1) methacrylic acid, (2) 2-vinylpyridine, (3) 4-vinylpyridine, (4) acrylamide and (5) trifluoromethylacrylic acid, and (6) 2-hydroxyethyl methacrylate.

2.3.4 Choice of initiators

Initiators are chosen depending on the solubility requirements and the polymerization temperature to be applied. In the list are azo compounds, *i.e.,* 2,2'-azobis-(isobutyronitrile) 2,2'-azobis-(2,4-dimethylvaleronitrile), 2,2'-azobis-(cyclohexylcarbonitrile) and 2,2'-azobis-(2-methylbutyronitrile), organic peroxides and inorganic water-soluble persulfates, which can be decomposed thermally or photochemically to initiate polymerization.[2] The amount of initiator used is usually 1-2 % w/w or 1-2 mol of the polymerizable double bonds.[59,60] Once monomers, their quantity, and the porogen have been settled on, polymerization is initiated thermally or by irradiation with UV-light at 365 nm. Polymers synthesized at low temperatures have exhibited higher recognition capabilities, and high affinity binding sites than those synthesized at high temperatures.[61,62] Non-covalent imprinting is governed by the Le Châtelier's principle and low temperatures push the equation towards pre-polymerization complex formation resulting to polymers with more recognition sites.[53,63] More so non-covalent interactions, especially hydrogen bonding are stabilized at low temperatures.[42]

2.4 Synthetic strategies and MIPs formats

MIPs have been traditionally synthesized by bulk polymerization; however, the demand for polymer formats with enhanced binding efficiency and mass transfer properties has seen synthesis evolving towards strategies that provide microspheres, nanospheres, and membranes. Bulk polymerization usually results in monolith blocks that are then ground and sieved for obtaining the desired particle size; this procedure not only lowers the overall yield,[2,64] but also leads to irregularly shaped particles and potential destruction of binding sites.[65] Consequently, e.g., in chromatographic applications effects such as peak tailing, low column efficiency, and reduced loading capacity have been observed.[4,65] An alternative to bulk polymerization is *in situ* polymerization, which is a direct approach for establishing MIPs within the chromatographic column, thus solving the tedious procedures of grinding, sieving, and column packing. Furthermore, the amount of template and functional monomer is minimized, as no losses have to be anticipated. Polymers synthesized *via* this approach have been successfully applied in column chromatography for the separation of enantiomers and diasteomers,[66] xanthine derivatives (*i.e.,* achieving baseline separation compared to bulk polymers),[67] and for solid phase microextraction (SPME) procedures, e.g., the pre-concentration of 4-nitrophenol.[68] The ultimate alternatives to bulk polymerization are, however, suspension, precipitation, emulsion, mini-emulsion, and multi-step swelling polymerization strategies, which inherently result in – ideally almost monodisperse - polymer beads for direct application. The optimization of parameters such as the amount of cross-linking agent, type of porogen, stabilizing agents, and stirring speed gives rise to particles in the micrometer to nanometer size range,[2,54,64,69–72] which are ideally tailored for application in binding assays,[73] column chromatography,[72] and for capillary electrochromatography.[74] The most attractive approach for membrane synthesis entails phase inversion strategies using cellulose acetate, polyamide, polyacrylonitrile, polysulfone, polystyrene, and polyvinyl chloride as membrane-forming matrices.[75,76] The resulting imprinted polymers (*i.e.,* in membrane or film format) may readily integrate into sensors via *in situ* polymerization, surface grafting, electropolymerization, physical entrapment or chemical coupling to the desired transducer surface.[77]

MIPs in the present thesis were synthesized *via* suspension polymerization, and therefore it is discussed in detail. Here, the polymerization constituents and the initiator dissolved in an appropriate solvent are dispersed in a continuous phase by stirring, and then polymerization left to continue within the monomer droplets. Mineral oil,[78] silicon oil,[79] liquid perfluorocarbon,[80] and water[81] are some of the continuous phases used. Liquid perfluorocarbons have been suggested because they are largely immiscible with most organic solvents and monomers, thus offering an inert environment for polymerization and a wide selection of monomers and porogens. However, the need for droplet stabilizers like fluorinated surfactants and fluorinated polymers,[41] and the high cost of liquid perfluorocarbons has opened the way for mineral and silicon oil which do not require stabilizers owing to their high viscosity. Aqueous suspension polymerization maybe carried out, if the polymerization constituents are insoluble in water and the perceived forces of interaction during the formation of the pre-polymerization complex are not ionic or hydrogen bonding. Fig. 5 is a SEM image of particles synthesized in the present thesis *via* aqueous suspension polymerization.

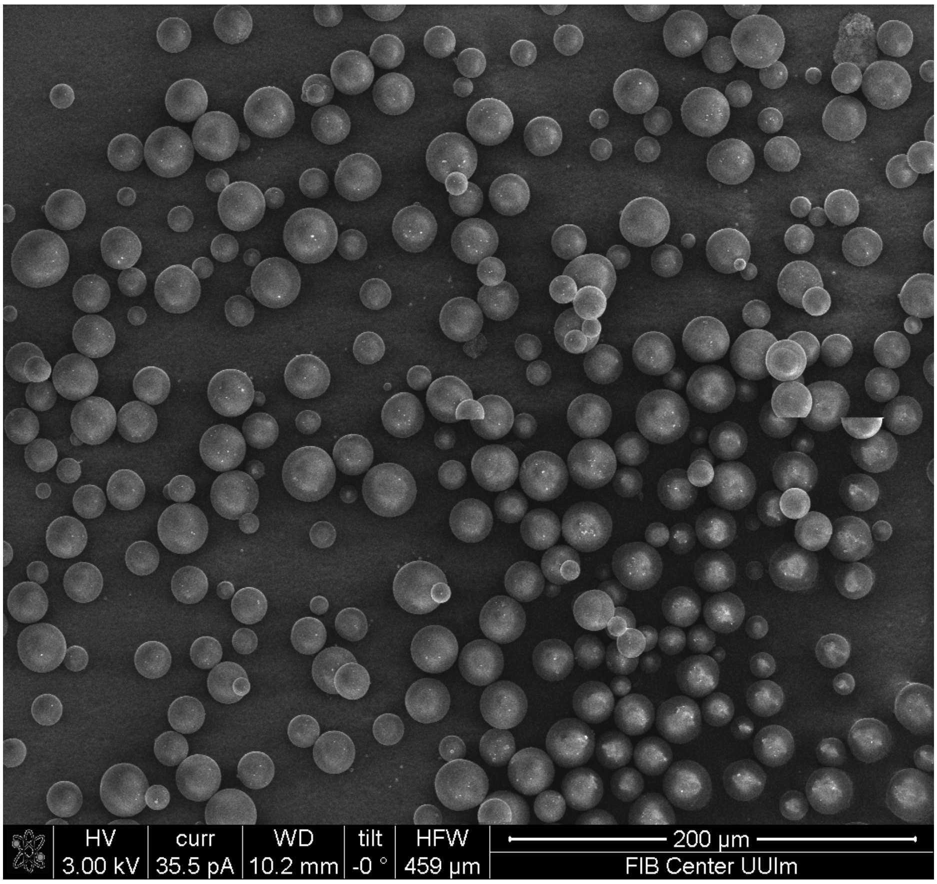


Figure 5: SEM image of particles synthesized in the present thesis *via* aqueous suspension polymerization.

2.5 Evaluation of the imprinting efficiency

After successful synthesis of a MIP, it is important to characterize the novel polymer in order to determine whether it possesses the desired properties, and secondly compare it with the control polymer or non-imprinted polymer (so-called NIP) or other reported polymers. The different methods commonly used to evaluate MIPs are discussed in detail.

2.5.1 Chromatographic retention and batch-rebinding assays

Chromatographic studies and batch-rebinding assays are the two approaches commonly applied to study the extent of recognition and the overall performance of the imprinted polymer. Chromatographic studies are carried out by packing HPLC columns with imprinted or non-imprinted polymers and studying the retention of the target analytes using an appropriate mobile phase. From the retention time of the analytes (t_R), the retention factor or capacity factor (k') (eqn 1), imprinting factor (IF) (eqn 2) and the selectivity (α) (eqn 3) - which is the ability of the MIP to distinguish between the target and structural analogues compounds - are calculated. Imprinted polymers are ideally supposed to retain the template more than the non-imprinted counterpart due to the expected extensive interaction with the polymer matrix. If this happens, IF greater than 1 (*i.e.*, IF >1) is reported and the imprinting process is described to have been successful. The peak symmetry can also serve, at first sight to confirm whether any imprinting has occurred or not. Due to the heterogeneous nature of the binding sites in imprinted polymers, a tailing effect is observed compared to the peak for the non-imprinted polymer.[82]

$$k' = \frac{(t_R - t_0)}{t_0} \qquad (1)$$

$$IF = \frac{k'_{MIP}}{k'_{Blank}} \qquad (2)$$

$$\alpha = \frac{k'_{template}}{k'_{analogue}} \qquad (3)$$

t_R is the retention time of a given species, t_0 is the retention time of the void marker (usually acetone), k'_{MIP} and k'_{Blank} are the retention factors of analytes on the imprinted and blank columns respectively, and $k'_{template}$ and $k'_{analogue}$ are retention factors of the template and its structural analogue.

Batch rebinding experiments facilitate the study of the recognition properties at an equilibrium environment. Here, a given concentration of the template is incubated with a known amount of MIP and NIP, respectively, for a given duration after which the supernatant is filtered and the free analyte quantified using UV-Vis spectrophotometer, HPLC, or GC. The amount of analyte adsorbed at the polymer or the adsorption capacity (Q) is defined by eqn (4), from which the IF and selectivity (α) can be calculated using eqn (5) & (6). Batch rebinding assays of the target analyte in the presence of structural analogues – what is referred to as competitive batch rebinding assays - gives a clear picture on the ability of the imprinted polymer to discriminate between the template and other compounds.

$$Q = \frac{(C_0 - C_f)}{m} V \qquad (4)$$

$$IF = \frac{Q_{MIP}}{Q_{NIP}} \qquad (5)$$

$$\alpha = \frac{IF_{template}}{IF_{ana\log ue}} \qquad (6)$$

C_0 and C_f are the initial and final concentration of the analyte, V is the volume of the sample, *m* is the weight of the polymer, Q_{MIP} and Q_{NIP} represent the adsorption capacity of the imprinted polymer and the non-imprinted polymer, $IF_{template}$ and $IF_{analogue}$ are imprinting factors of the template and its structural analogue.

Single point binding studies may not fully characterize the polymers and are only used to give a rough idea of the imprinting effect. In-depth characterization of the imprinted polymers is therefore carried out by equilibrium binding assays over a wide range of analyte concentration or by frontal chromatographic experiments.[83] The binding data is then fitted to Langmuir, Freundlich, Scatchard plot, Langmuir-Freundlich (LF) adsorption isotherms in order to determine binding parameters such as dissociation constant (K_D), maximum binding capacity (B_{max}), number of binding sites (N_t), and binding affinities.[2]

Langmuir adsorption isotherm

The Langmuir isotherm, eqn (7) was originally used to describe gas-solid-phase adsorption but has been extended to liquid-solid systems. The model assumes that all the binding sites are homogeneous (or equivalent) with uniform adsorption energy, and each site can accommodate only one molecule, thus only a single layer of adsorption (monolayer) with maximum adsorption capacity (q_m) is possible. It is the simplest model commonly used to get information on binding properties such as maximum binding capacity and dissociation constant for imprinted polymers.[71,84]

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \qquad (7)$$

q_e is the bound analyte at equilibrium, C_e is the equilibrium concentration, q_m represents the monolayer coverage or maximum adsorption capacity and K_L is a constant related to the adsorption energy.

Freundlich adsorption isotherm

Freundlich adsorption isotherm, eqn (8) assumes heterogeneous binding site distribution (*i.e.*, binding sites with different adsorption energies),[85] therefore applicable for molecularly imprinted polymers synthesized through non-covalent imprinting approach. A combination of both the Freundlich and Langmuir results in the recently introduced Langmuir-Freundlich isotherm which models both heterogeneous and homogeneous binding sites distribution, thereby covering both sub-saturation and saturation concentrations ranges.[86,87]

$$B = aF^m \tag{8}$$

B and F are the concentrations of bound and free analyte, respectively, *a* is related to the median binding affinity constant K_0, and *m* is the heterogeneity index. *m* ranges from 0 to 1, with 1 indicating homogeneous binding sites.

Scatchard analysis

Scatchard model, eqn (9) has frequently been used to quantify the recognition properties of molecularly imprinting polymers. Thereby, two important parameters, *i.e.*, dissociation constant, K_D (which represents the affinity of the ligand to the receptor), and the maximum density of the receptors (B_{max}) are determined.

$$\frac{B}{F} = \frac{(B_{\max} - B)}{K_D} \tag{9}$$

A plot of B/F versus B is a curve running from sites with high binding strength so sites with low binding strength for heterogeneous materials and a straight line for homogeneous materials.[88] To the curves, two regression lines can be drawn whose slopes and intercept give the dissociation constants (K_D) and subsequent high and low affinity binding sites.

2.5.2 Radioligand binding assays

For more accurate judgement on the selectivity of the polymers, advanced and sensitive binding assays using radiolabeled (^{3}H-labeled) ligands are applied. Radioligand binding assays enable studies of minute differences in binding at very low concentrations of ligands.[88] In these experiments, the analyte (herein referred to as unlabeled ligand) competes with the radiolabeled counterpart for the binding sites. Therefore, the amount of the radiolabeled ligand bound to the polymer is first measured in the absence of the unlabeled ligand, and then unlabeled ligand added at increasing concentrations, whereby it is expected to displace the radiolabeled ligand leading to decrease in the amount bound. The displaced radiolabeled ligand in the supernatant is determined by liquid scintillation counting, and a curve of the ratio between bound radiolabeled ligand in the presence of the unlabeled ligand (B) and bound radiolabeled ligand in the absence of the unlabeled ligand (B_0) against the increasing concentration of the unlabeled ligand is drawn. IC_{50}, which is the concentration of the unlabeled ligand that displaces 50% of the bound radiolabeled ligand is then determined. The template usually has lower $1C_{50}$ compared to the structural analogues, indicating high selectivity for the template. Figure 6 is an exemplary displacement curve for 17β-estradiol imprinted polymer. IC_{50} was lowest for 17β-estradiol, compared to the other structural analogues studied, suggesting high selectivity for the template.[69]

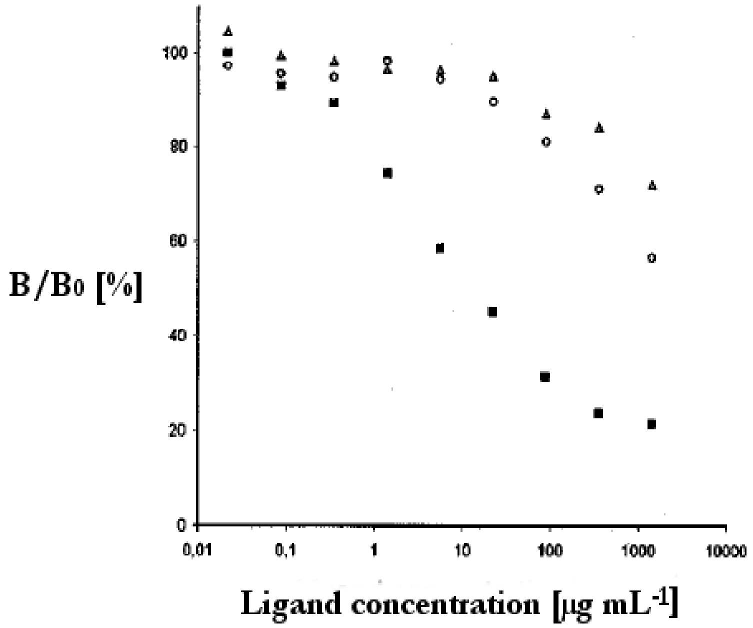


Figure 6: Displacement of [2,4,6,7-^{3}H(N)] estradiol binding to 17β-estradiol imprinted polymer. B/B_0 is the ratio of the amount of radiolabeled ligand bound in the presence of displacing ligand (B) to the amount bound in the absence of displacing ligand (B_0). Displacing ligands are 17β-estradiol (square), 17α-estradiol (circle); 17α-ethynylestradiol (triangle). Adapted from Ref. 69. Copyright 1999, with permission from the Royal Society of Chemistry.

2.6 Class selective molecularly imprinted polymers

By far and large, synthesis of MIPs has predominantly focused on using a single template species to create binding sites specific to a single compound. However, the emergence of 'group-selective MIPs' (gMIPs) or 'class selective MIPs' is increasingly prevalent, which enable molecular recognition for a group of closely related compounds rather than an individual constituent.[89] Two commonly applied approaches for the synthesis of such gMIPs are using template mixtures (a.k.a., multi-template approach), or a single compound as a generic structural analogue. Multi-template approach has successfully been demonstrated by Song *et al.*,[90] and Duan *et al.*,[91] using a mixture of 16 PAHs and 5 acidic pharmaceuticals, respectively, to produce MIPs with recognition properties for these compounds. The polymers were successfully applied as SPE sorbents for the determination of 16 PAHs in sea water, and for the enrichment of pharmaceuticals in environmental samples. For further reference, Krupadam *et al.*[92–95] have executed extensive studies on the use of PAH template-mixtures during the synthesis of selective sorbents for environmental applications.

On the other hand, using a single template serving as surrogate for an entire group of compounds takes advantage of structural analogues among a group of compounds. This approach saves on the amount of template required, and limits the problem of template bleeding. The viability of this approach for environmental studies is evident in the work of Ho *et al.*,[52] who used benzo[a]pyrene to synthesize MIPs for the determination of 16 PAHs in cigarette smoke. The approach owes its success to taking advantage of the usually avoided cross-reactivity resulting from structural analogues. Rather than being a curse, this phenomenon is used as a blessing, especially for PCBs which occur as congeners or homologues, whereby 'group selectivity' would lead to their simultaneous enrichment and detection. Tapping into the advantages of 'group selective MIPs', *i.e.*, being able to work on a number of compounds in one synthesis approach (for multi-template approach), and reduced template requirement (for single compound representing a group of structural analogues), a number of class-selective MISPE cartridges for PAHs, fluoroquinolones, triazines, aminoglycosides - though not yet for PCBs - have been commercialized by Sigma-Aldrich.[96]

Since PCBs occur as homologues, synthesis of gMIPs using one of the PCBs/or a different compound as a dummy template is possible, as evidenced in Paper I and II.

2.7 Application of MIPs: Focus on MISPE

MIPs have found applications in a wide range of analytical scenarios, but it is their use as sorbents in SPE that was the focus of the present study. Among the first reports on MIPs as possible enrichment materials was a study by the group of Sellergen in 1994,[97] whereby they were able to enrich pentamidine in urine up to a factor of 54 compared to 14 using a reference polymer. To date, MISPE has almost established itself in sample pre-treatment and continues to attract extensive research, as evidenced by the annual increase in published articles (Fig. 7). Other notable applications are enrichment of caffeine in Red bull,[33] and zearalenone and α-zearalenol in wheat samples.[98]

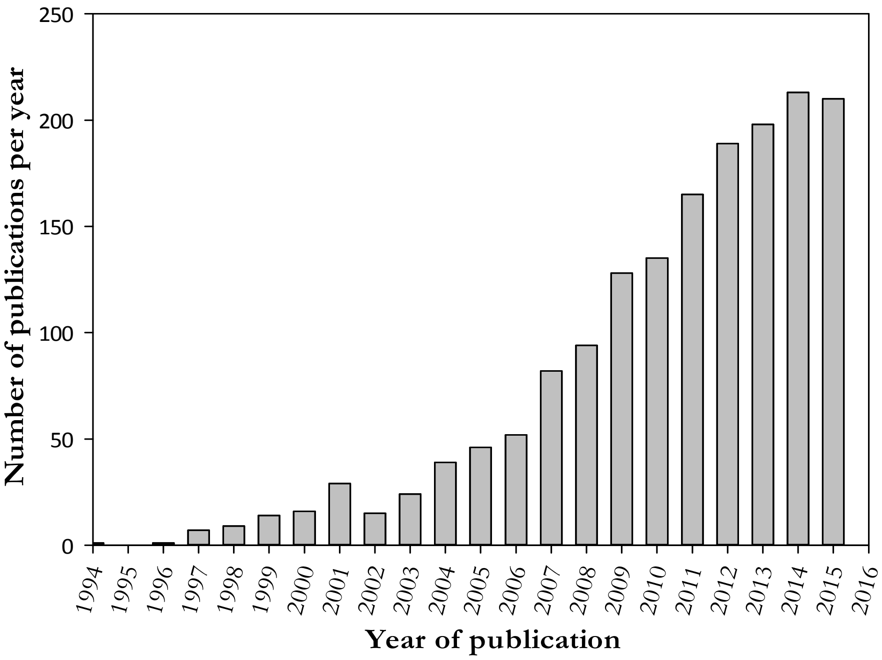


Figure 7: Number of publication on molecularly imprinted solid phase extraction (MISPE) from 1994 to 2015 according to the SciFinder® search engine using the term "molecularly imprinted solid phase extraction".

Like in conventional solid phase extraction, the MISPE procedure follows the traditional conditioning, loading, washing, and elution steps (Fig. 8). Depending on the solvent in which the analytes are dissolved, the MISPE can be normal phase or reversed phase.[99] In normal phase, the analytes are dissolved in a low polarity organic solvent or preferably the porogen used during synthesis. The analytes then selectively interact with the polymer matrix through the earlier established non-covalent forces, whereas most of the interfering molecules are eliminated; a washing step maybe necessary to remove compounds that are non-selectively bound to the polymer. Finally, elution of the analytes is achieved using a solvent that is able to interrupt the specific interactions. Reversed phase applies when the sample is aqueous. Here, both the analytes and the interfering compounds are retained on the cartridge through hydrophobic forces, without any selectivity towards the target analyte. Optimization of the washing solvent is therefore necessary in order to achieve selective desorption. The interfering substances non-specifically bound to the polymer are eliminated during the washing step, at the same time enhancing specific binding of the analyte. There are no guidelines on the best washing solvent, since every SPE procedure is governed by the type of sample and the analytes of interest. However, there has been a suggestion that the best washing effects are realized when the same loading solvent in its pure form is used as a washing solvent.[100]

In the elution step, solvents such as methanol, acetonitrile, THF, chloroform and a mixture of methanol and acetic acid have the ability to disrupt the specific binding leading to elution of the analyte of interest which are non-covalently bound to the polymer.[100]

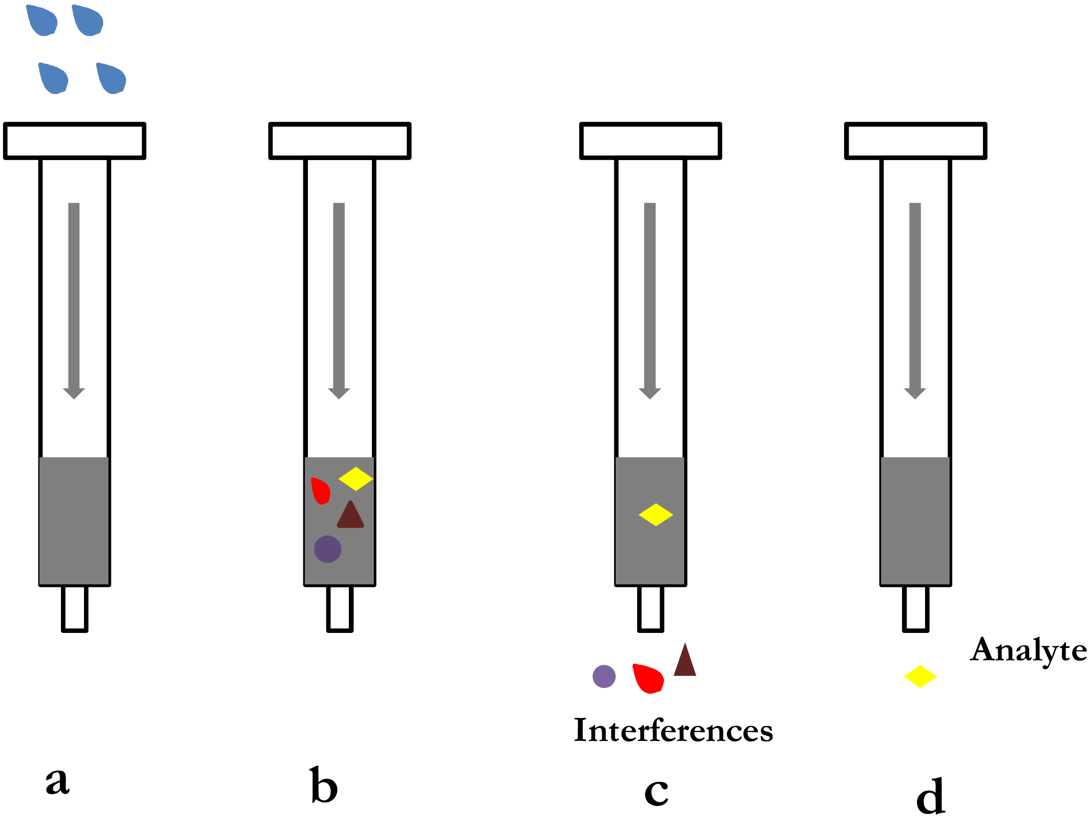


Figure 8: Major steps in solid phase extraction: (a) conditioning, (b) Loading, (c) washing, and d) Elution.

The MISPE can be operated in off-line or on-line mode, whereby in the off-line mode the sample is passed through a polypropylene tube packed with the sorbents materials with the help of SPE manifold connected to a pump. The elution solvent is then reduced or solvent exchanged for quantification of the analytes with high performance liquid chromatography, UV-Vis spectrophotometer, or gas chromatography. In on-line mode, the sorbent is packed in a pre-column which is connected in the loop of the sample injection valve in high performance liquid chromatography. After performing all the 3 steps in SPE, the analytes are eluted by the mobile phase into a separation column and then detected.[13] Despite on-line mode having attractive attributes like reduced sample handling procedures, reduced sample volume, less washing and elution solvents, and minimal analyte losses, its practicality is hampered by the compatibility requirements of the elution solvent with the detection system.

2.8 MIPs selective to PCBs: state-of-the-art

MIPs selective to PCBs would contribute positively to trace level determination of PCBs; however, few reports exist on application of such polymers in environmental studies. The first attempt on imprinted polymers recognizing PCBs applied *p*-xylene, *o*-xylene, and *m*-xylene imprinted polymers as stationary phases in chromatography.[72] Another study combined xylene imprinting effect and sulfoxide modification in synthesis of sorbent materials for separation of PCBs from insulating oil.[101] Recently, Cleland *et al.*[102] rationally designed and characterized imprinted polymers selective to PCBs using 1,2,3,4,5-pentachlorobenzene and 1,2,3-trichlorobenzene as templates. Towards enhancing the performance of imprinted polymers, magnetic particles and MWCNTs have been used as support materials in synthesis of MWCNTs@mMIPs selective to PCBs using 3,4-dichlorobenzene acetic acid as the dummy template.[103]

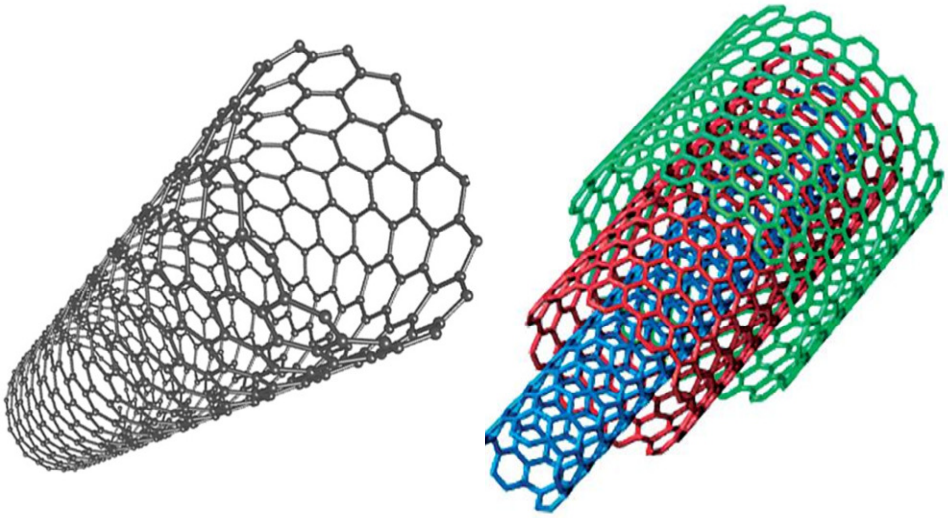
Most of these reports only demonstrated the selectivity of the imprinted polymers to PCBs, without information of their performance in field samples. Therefore further exploitation of these strategies would indeed provide polymers for environmental application. The imprinting effect demonstrated by xylenes is an attractive strategy, as these templates not only surrogate for toxic compounds but also result in polymers with high loading capacity and stable recognition sites since the porogen can be used in high ratios owing to its availability.[104] In addition, fragment chlorinated benzenes as dummy templates also provide alternatives for PCBs but the ratios reported by Cleland *et al.*[102] (template: functional monomer: cross-linker ratios of 1:2:10 and 15:6:29) translate to high amount of template and may not be applicable when large quantity of polymers is required. High amount of either the template or functional monomer in non-covalent imprinting approach is required to push the equilibrium towards the formation of a stable template-monomer complex,[25] however, the latter approach of using large amount of functional monomer maybe recommended, especially when using chlorinated fragments, since these compounds may not be used in large amounts, and needs to be explored in synthesis of polymers selective to PCBs.

MIP synthesis has concentrated on demonstrating the selectivity of the particular MIP towards the template or targeted compounds, without information on performance in real-life scenarios. The present research thesis goes beyond synthesis and characterization of polymers selective to PCBs, to validation of analytical procedures that incorporates these polymers in determination of PCBs from different real-world environmental matrices.

3. Carbon nanotubes in sample pre-treatment

Carbon nanotubes are allotropes of carbon belonging to the fullerene family. Since their discovery by Iijima in 1991,[105] CNTs have attracted enormous scientific attention, thanks to their unique chemical, mechanical, and electrical properties. CNTs, whose basic unit is a graphene sheet exists as either single walled carbon nanotubes (SWCNTs) or multiwalled carbon nanotubes (MWCNTs) (Fig. 9). To arrive at these two forms, the graphene sheet is folded once or several times, resulting in tubes with diameters ranging from 1 to 3 nm for SWCNTs and 5 to 200 nm for MWCNTs.[106] The three main methods used for synthesis of CNTs are laser ablation, arc discharge, and chemical vapour deposition (CVD). These methods are based on vaporisation of a carbon source to produce carbon atoms which recombine to form CNTs. The first two methods depend on solid state carbon precursor as a carbon source, while CVD relies on gaseous hydrocarbons as carbon source (Table 2). CVD is the commonly used synthetic method because it is less costly for large scale production and produces CNTs with few impurities.[107]

Owing to their structure, CNTs are able to interact with organic molecules *via* π- π stacking, hydrogen bonds, electrostatic and van der Waals forces. CNTs have particularly displayed strong interactions with aromatic compounds, thus making them potentially useful materials in analytical science for studies of these compounds. Sample pre-treatment (*i.e.*, clean-up and enrichment), chromatography and sensory are some of the application areas within analytical science for these materials. Ideally, MWCNTs have a higher sorption capacity[108] and are cheaper to produce compared to SWCNTs, thus they have been widely applied as SPE sorbents in determination of polycyclic aromatic compounds,[109] phthalate esters,[110] polyhalogenated compounds,[111] tetracyclines,[112] and as sorbents in removal of dioxins.[113] MWCNTs in their modified form have also been used in removal of PCBs from aqueous solutions,[114,115] pre-concentration of PCBs in water,[116,117] and clean-up of spiked fish samples.[103] These modifications are made to increase the dispersion of CNTs in organic and aqueous media since they are known to agglomerate owing to their strong van der Waals forces. However, excellent performance of CNTs in their raw form (*i.e.,* unmodified) may provide affordable materials for sample pre-treatment. Therefore a ready to use MWCNTs-SPE for pre-concentration of 6 indicator PCBs will be a step forward towards an affordable monitoring protocol for these compounds. In addition, the price of CNTs has decreased over time due to increase in production in order to cope with the current demand,[106] thus placing these materials in a good position for research as alternatives to conventional sorbents.



SWCNTs **MWCNTs**

Figure 9: Structures of SWCNTs and MWCNTs with the graphene sheet rolled once and thrice, respectively.

Table 2: Overview of the main synthetic routes for carbon nanotubes

Synthesis method	Principle	Average diameter of the tubes	Yield	Reference
Chemical vapour deposition (CVD)	Gaseous hydrocarbons (benzene, methane, ethanol, acetylene, ethylene, n-hexane, carbon monoxide, etc) as carbon sources are decomposed at temperatures <1200 °C on catalysts (mainly Fe, Ni, Co, etc) resulting in nanotubes of high purity.	SWCNTS; 0.6-4 nm MWCNTs; 10-240 nm	20-100%	107,118
Arc discharge	electric arc is applied between two opposite graphite electrodes of high purity in a reactor which contain an inert gas. At DC current of ~100 A, the electrodes are vaporised and a carbon rod deposited on the cathode. Doping of the electrodes with a catalysts (Fe, Ni, Co, Mo) results in SWCNTs.	SWCNTS; 0.6-1.4 nm MWCNTs; 1-3 nm (i.d), ~10 nm (o.d)	30-90%	107,118
Laser ablation	A graphite target doped with metal catalysts (CO or Ni), when SWCNTs are desired, is vaporised by a continuous laser in a tube heated at temp approx. 1200 °C in an inert atmosphere. The vapour is transported by He or Argon on a copper collector cooled with water, where the carbon vapours condense forming CNTs.	SWCNTS; 1-2 nm	70-90%	107,118

4. Polychlorinated biphenyls

4.1 Facts about polychlorinated biphenyls

A polychlorinated biphenyl molecule (Fig. 10) consists of two phenyl rings where substitution of hydrogen with chlorine atoms can take place in any of the 10 sites giving rise to 209 different PCBs compounds.

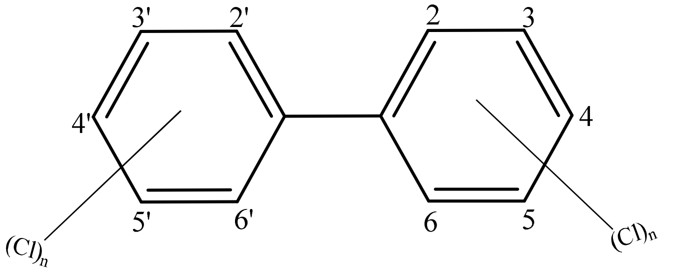


Figure 10: The general structure of PCBs where chlorination at the 10 substitution positions gives rise to 209 compounds, also referred to as congeners

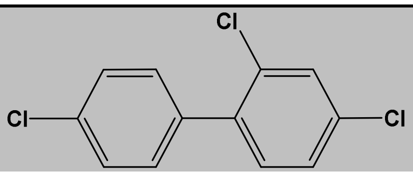
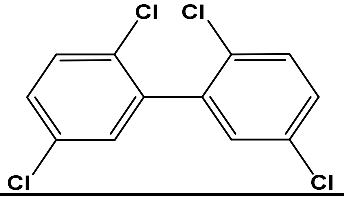
Positions 2,2',6,6'; 3,3',5,5'; and 4,4' are *ortho*, *meta*, and *para* positions, respectively. Depending on the number and the position of the chlorine atoms, the congeners have been assigned IUPAC numbers which range from 1 to 209. For example, 2,2',3,4,4',5,5'-heptachlorobiphenyl is PCB 180. Non-*ortho* and mono-*ortho* substituted PCBs are usually referred to as planar or co-planar PCBs (the benzene rings are in the same plane) while those with two chlorine atoms at the *ortho* position (di-*ortho*) can rotate out of planarity and are called non-planar PCBs. Coplanar PCBs adopt a configuration similar to polychlorinated dibenzodioxins (PCDDs) and are referred to as "dioxin-like PCBs" since they demonstrate the same toxicity patterns as the PCDDs. In addition to being non-*ortho* or mono-*ortho*, they have a minimum of four chlorine atoms at the lateral positions 3,3',4,4',5, 5', thus fully satisfying the qualifications of dioxin-like PCBs. They include: 4 non-*ortho* PCBs (*i.e.,* PCB 77, 81, 126, 169) and 8 mono-*ortho* PCBs (*i.e.,* PCB 105, 114, 118, 123, 156, 157, 167, 189) (Table 3).[119] These dioxin-like PCBs have been assigned toxic equivalent factors (TEFs) that compares their toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which is the most toxic PCDDs with a TEF value of 1.[120]

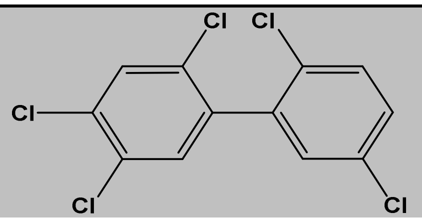
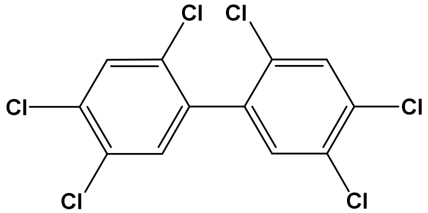
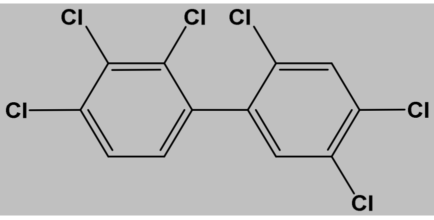
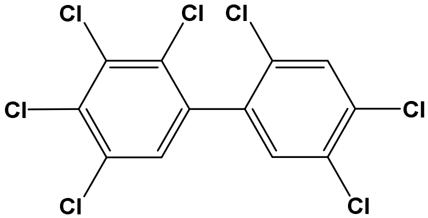
Table 3: Dioxin-like PCBs and their respective TEF values.[120]

IUPAC No.	Homolog	IUPAC Name	TEF
		non-ortho PCBs	
77	tetra-CB	3,3’,4,4’-tetra-CB	0.0001
81	tetra-CB	3,4,4’,5-tetra-CB	0.0003
126	penta-CB	3,3’,4,4’,5-penta-CB	0.1
169	hexa-CB	3,3’,4,4’,5,5’-hexa-CB	0.03
		mono-ortho PCBs	
105	penta-CB	2,3,3’,4,4’-penta-CB	0.00003
114	penta-CB	2,3,4,4’,5-penta-CB	0.00003
118	penta-CB	2,3’,4,4’,5-penta-CB	0.00003
123	penta-CB	2’,3, 4,4’,5-penta-CB	0.00003
156	hexa-CB	2,3,3’,4,4’,5-hexa-CB	0.00003
157	hexa-CB	2,3,3’,4,4’,5’-hexa-CB	0.00003
167	hexa-CB	2,3’,4,4’,5,5’-hexa-CB	0.00003
189	hepta-CB	2,3,3’,4,4’,5,5’-hepta-CB	0.00003

PCBs targeted in the present study were the 6 non-dioxin like PCBs called ‘indicator PCBs’ (Table 4), which have been proposed for continuous monitoring in the environment. Since they were major components in the technical mixtures,[121] they are the most dominant compounds detected in the environment and serve to indicate the extent of contamination with PCBs.

Table 4: Names and structures of the 6 indicator PCBs investigated

IUPAC name (IUPAC number)	Structure
2,4,4'-trichlorobiphenyl (PCB 28)	
2,2',5,5'-tetrachlorobiphenyl (PCB 52)	

2,2',4,5,5'-pentachlorobiphenyl (PCB 101)	
2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138)	
2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153)	
2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180)	

4.2 PCBs production and environmental fate

The chemical and thermal stability that polychlorinated biphenyls (PCBs) portray made them desirable compounds for use as coolants and dielectric fluids in transformers and capacitors (these were the major applications, as shown in Fig. 11), hydraulic fluids, flame retardants, lubricating and cutting oils and as additives in inks, adhesives, carbonless copy paper, paints, pesticides, and plastics[122] Their commercial production spanned between 1930s and 1970s, resulting in total global production of about 1.3 million metric tonnes, out of which a substantial amount is estimated to have been released into the environment.[123,124]

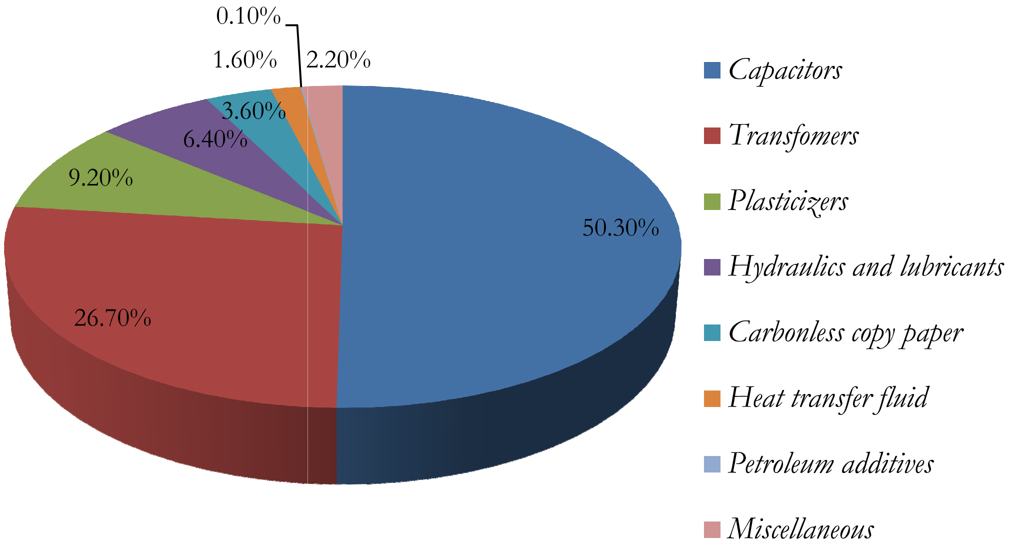


Figure 11: Percent use of PCBs in various systems

Contamination of the environment with PCBs and their possible effect was first brought to the world attention 37 years after the start of their commercial production. PCBs levels as high as 180 μg g^{-1} were detected in samples from a dead white tailed eagle in Sweden.[125] Subsequently, severe contamination was reported in 1960s in Hudson River with concentration as high as 350 μg g^{-1}, which declined to 34 μg g^{-1} in 1991 as a result of a ban in production in USA in 1970s.[126] Due to their persistence in the environment, bioaccumulation in fatty tissues, and the ability to be distributed over long distances, PCBs are ubiquitous environmental contaminants, with traces in soil,[127] sediment,[128] water,[129] air,[130] biota,[131] and remote areas such as the poles.[132] Evidence of toxicity, coupled to their persistence led to ban in production and use through various initiatives, and recently through the globally binding Stockholm convention on persistent organic pollutants (POPs) which came into force in 2004.[14] Despite these measures, PCBs continue to be detected in the environment, albeit at low concentrations. The current sources of contamination are continued use of equipment containing PCBs, landfills and hazardous waste sites where incineration takes place (produced as by-products from chlorine and hydrocarbon containing products) and redistribution of PCBs from past releases.

Once in the environment, depending on the physical-chemical properties, PCBs partition between air, water, sediments, soil, and biota. Their distribution in the environment is mainly through atmospheric transport followed by wet and dry deposition on soil, water, and vegetation. In aquatic environment, PCBs adsorb to sediments and particulate matter due to their low water solubility and high octanol-water partition coefficients (Table 5), persisting with half-lives from 3 years for tetrachlorobiphenyl to 38 years for heptachlorobiphenyl.[133] This makes sediments sinks and potential reservoirs for continuous recycling in the environment. PCBs also tend to bioaccumulate in fatty tissues of animals and humans owing to their high octanol-water partition coefficient and biomagnify through the food chain.[133] Photolysis (photo degradation), biodegradation, and metabolism are the three processes for removal of PCBs from the environment. Due to the large number hydroxyl radicals (OH) generated by sunlight in the atmosphere, reaction of PCBs with OH radicals is a major pathway for their removal from the atmosphere. These reactions results in hydroxylated intermediates which are further degraded to chlorinated benzoic acids,[134] with half-lives of 10 days to 1.5 years for monochlorobiphenyls and heptachlorobiphenyl, respectively.[135] PCBs in water column undergo photodegradation, owing to their sensitivity to ultraviolet (UV) irradiation, thus converting highly chlorinated PCBs to less chlorinated ones through stepwise dechlorination.[136]

In sediments, both aerobic oxidative processes and anaerobic reductive processes are reported to take place, with the degradation process relying on the degree of chlorination and the substitution pattern of the biphenyl molecule. The lower chlorinated PCBs are aerobically degraded by microorganisms, first through conversion of the biphenyl to *cis* 2,3-dihydro-2,3-dihydroxybiphenyl *via* a 2,3- dioxygenase pathway then to chlorobenzoic acids and finally water, carbon dioxide, and chloride ions.[122,137–139] Highly chlorinated PCBs are degraded anaerobically *via* reductive dechlorination, as evidenced by increased concentration of lower chlorinated biphenyls in relation to the higher chlorinated PCBs after incubation with microorganisms. Dechlorination is reported to favour *meta* and *para* chlorines, resulting in reduction of highly chlorinated PCB congeners with corresponding increase in lower chlorinated *ortho* substituted PCB congeners which can be further degraded aerobically.[140] PCBs are metabolized in the bodies of higher organisms via cytochrome P-450 mediated oxidation to mono and dihydroxychlorobiphenyls, which are eliminated in the urine and faeces.[133] The rate of metabolism depends on the level of chlorination and substitution patterns, with hydroxylation favouring para positions of the biphenyl and carbon atoms that are para to the chlorine substituent.[141]

Table 5: Physicochemical properties of 5 indicator PCBs.[142]

Physical properties	28	52	101	138	153
BP, K	610	633	654	673	673
Aqueous solubility (mg L^{-1})^a	0.16	0.03	0.01	no data	0.001
log K_{ow} ^{a}	5.80	6.10	6.40	7.00	6.90
Vapour Pressure (ps,p_L pa) ^{a}	no data	0.0049 0.002	0.0011 0.0035	no data 0.0005	0.00012 0.0007
Henry's law constant (H,pa.m^3/mol) ^{a}	no data	47.59	35.46	48.64	42.9

^{a}determined at 25 °C

4.3 PCBs and human health

Most of the reported health effects of PCBs have been determined from accidental and occupational human exposure and animal studies. For example, mass poisoning of the population in Japan and Taiwan in 1968 and 1979, respectively, through ingestion of rice oil contaminated with PCBs and heat degradation products polychlorinated dibenzofurans, and polychlorinated quarterphenyls. Symptoms included chloroacne, headaches, respiratory problems, jaundice, increased eye discharge, pigmented nails, hepatic damage, etc. Levels of PCBs in the patient's blood was 3-1156 ng g^{-1} in Taiwan, and 1-30 ng g^{-1} in Japan, determined one year and five years after exposure, respectively. PCBs levels in liver and fat tissues of patients who died of cancer related problems ranged from 0.1 to 2.5 mg kg^{-1}.[143] Further animal experiments have led to classification of PCBs as "probable human carcinogens" by U.S. EPA.[141] PCBs are also reported to affect the endocrine system of humans and animals falling under the class of endocrine disrupting compounds. Studies have shown than pre-natal exposure to PCBs affect cognitive development in children. Women in Taiwan with PCBs levels between 2 and 456 ng g^{-1} gave birth to children with low IQ.[144] In another study, children born to women who had consumed Lake Michigan fish contaminated with PCBs reported decreased IQs. Samples taken from the women recorded PCBs levels of 1.25 mg kg^{-1} in breast milk, 4.7 ng mL^{-1} in cord serum, and 9.7 ng mL^{-1} in maternal serum.[145] On the other hand, PCB levels in serum were 2 ng mL^{-1} and 1 ng mL^{-1} for 4 and 11 year old children, respectively. A study targeting the female population around Lake Ontario exposed to PCBs through consumption of contaminated sport fish for a period of 7 years established reduced menstrual cycle length.[146] Earlier studies had reported mean contaminant level for total PCBs as 2.65 mg kg^{-1} in fish samples.[147] Animal studies using male rats exposed to 20 mg kg^{-1} of PCB 77 for approximately 3 months have reported significant increase in weight of testicles, decreased sperm count and motility compared to those treated with 2 mg kg^{-1} and the controls.[148]

For controls, the U.S. EPA has set a maximum contaminant level of 0.5 ng mL^{-1} for ∑PCBs in drinking water. The residue limits set by the food and drug administration is 0.2 mg kg^{-1} in infant foods, 0.3 mg kg^{-1} in eggs, 1.5 mg kg^{-1} in milk and other dairy products (fat basis), 2 mg kg^{-1} in fish and shellfish (edible portions), and 3 mg kg^{-1} in poultry and red meat (fat basis).[149] In addition, to protect the human population against adverse health effects of exposure to environmental contaminants, the U.S. EPA and the agency for toxic substances and disease registry (ATSDR) has set the tolerable daily intake (TDI) - which is the amount of contaminant that humans can ingest daily in a lifetime without any appreciable risk of adverse health effects - at 20 ng kg-bw^{-1} day^{-1} for aroclor 1254 and all 209 PCB congeners based on immunological effects observed in monkeys.[149,150] TDI value for total dioxins (*i.e.*, sum of dioxin and dioxin like PCBs), as given by WHO ranges from 1-4 pg TEQ kg-bw^{-1} day^{-1} based on hormonal, reproductive, and developmental effects in rats and monkeys.[151] Since no TDI value has been established for the frequently monitored 6 indicator PCBs, a guidance value of 10 ng kg-bw^{-1} day^{-1} has been proposed since they represent 50% of the total PCBs.[150] To comply with these limits, the daily dietary intake of PCBs is usually determined, as shown for some countries in Table 6, with some exceeding the set limits.

Table 6: Dietary intake of PCBs (ng kg-bw^{-1} day^{-1}) and dioxins (pg TEQ kg-bw^{-1} day^{-1}) in different countries

Country, year	Age in years	Mean/median dietary intake	PCBs analysed	Ref.
Netherlands, 1998-1999	2 10 40	12.1 *(2.8)* 6.9 *(1.5)* 4.8 *(1.1)*	$\sum_7$ (PCBs)[a] *($\sum$ dioxins)*	152
Denmark, 1998-2003	4-14 15-75	24.9 12.6	$\sum_{10}$ (PCBs)[b]	153
Egypt, 2002-2003		6.04-6.68	$\sum$(dioxins)[c]	154
Korea, 2002-2003		0.68	$\sum$(dioxins)[c]	155
France, 2002-2006	3-14 >15 19-44 (women)	12.9 7.7 7.6	$\sum_6$ (PCBs)[d]	150
Germany, 2005	19-58 (women) 25-56 (men)	11.2	$\sum_6$ (PCBs)[e]	156
Italy, 2008	0.5-6 7-12 13-94	24.6 16.1 10.9	$\sum_6$ (PCBs)[d]	157
China	Infants	151	$\sum$(dioxins)[c]	158

[a] sum of 6 indicator PCBs + PCB 118
[b] sum of 6 indicator PCBs + PCB 105, 118, 156 & 170
[c] dioxin like PCBs + polychlorinated dibenzodioxins and furans
[d] sum of 6 indicator PCBs
[e] sum of 6 indicator PCBs multiplied by 2

4.4 PCBs in Kenyan environment and the existing analytical techniques

Although PCBs were never manufactured in Kenya, PCBs containing transformers and capacitors were imported into the country and have contributed to the present day contamination. In an effort to rid the world of POPs, the Stockholm convention on POPs – to which Kenya is signatory – calls for phase-out of PCB containing equipment by 2025. This noble measure faces challenges, especially in developing countries due to the cost associated with its implementation. Decommissioning process will require technological know-how on safe destruction or storage of PCB containing equipment. A survey carried out in 2005 in Kenya under the international POPs elimination project (IPEP) established that, PCBs containing transformers and capacitors (those manufactured before 1985) are either in use or in storage.[159] There were no clear guidelines on safe disposal of these equipments which are being phased-out. More worrying was the fact that transformer oil maybe in illegal use in informal garages as cooling oils in electrical welding or for medicinal purposes. Besides direct exposure from these illegal uses, these oils eventually end up in the environment, thus raising the need for their continuous monitoring to protect the environment. However, this has not been achieved, probably due to the applied sample pre-treatment techniques, which are demanding in terms of solvent and sorbent requirement (Table 7). Therefore, coupling of the existing and affordable detection techniques such as GC-ECD with affordable sample pre-treatment procedures would ensure there is consistency in monitoring of PCBs.

Table 7: Extraction techniques and clean-up sorbents which have been applied for determination of POPs in Kenya

Matrix	Analyte	Extraction technique	Sorbent	Elution /Extraction solvent	Ref.
Sediments Water	PCBs	Soxhlet LLE	Silica gel (15 g)	*n*-hexane (165 mL) DCM (180 mL)	160
Sediments	PCBs	ASE	Silicagel	*n*-hexane	161
Sediments	OCPs	Soxhlet	Florisil (8 g)	*n*-hexane/acetone (approx. 30 mL)	162
Water		LLE		*n*-hexane (240 mL)	
Soil Water	OCPs	Soxhlet LLE	Florisil (10 g)	*n*-hexane/DE (200 mL) DCM (300 mL)	163

ASE, accelerated solvent extraction; OCPs, Organochlorine pesticides, LLE=liquid-liquid extraction; DCM, dichloromethane; DE, diethyl ether

5. Results and discussion

Sorbents based on molecularly imprinted polymers and multiwalled carbon nanotubes for the determination of 6 PCBs in the environment were investigated, and a brief summary of the results is given. Original articles are included at the end of this section for detailed results. MIPs were synthesized using PCB 15 as the dummy template, 4-VP as the functional monomer and EGDMA as the cross-linker. The resulting polymers gave an imprinting factor of 1.1, whereby the low imprinting factor was attributed to the π-π stacking interactions explored in the formation of the pre-polymerization complex (Paper I). As it is PCB 15 and 4-VP may not have formed a stable pre-polymerization complex, which is important in molecular imprinting. Towards the application of the polymers in SPE for pre-concentration of PCBs in aqueous environment, recoveries >70% were realized in real-world water samples. It was noted that, while in the rebinding studies the difference between the MIP and NIP – though not distinct – was noticeable, there was no observable difference in performance at the SPE step.

Modification of the MISPE cartridge for clean-up enabled the studies of PCBs in complex matrices. The level of PCBs in sediments from the two rivers investigated and soils from different parts of Nairobi city ranged from 0.04 ng g^{-1} to 6.74 ng g^{-1}, which was comparable to what has been reported in different parts of the world, except for extremely polluted sites. Further determination on the extent of contamination with PCBs in selected solid waste disposal dump sites indicated levels between 0.11 and 7.23 ng g^{-1}. Contamination of solid waste disposal sites by PCBs was attributed to open burning of solid waste, especially chlorine containing products. The level of PCBs in sediments (*i.e.*, 0.23-6.43 ng g^{-1} for $\sum_6$PCBs) was below the sediment quality guidelines (SQGs) effect range low (ERL) value of 22.7 ng g^{-1} for $\sum$PCBs (as given by the national oceanic and atmospheric administration (NOAA)).[164] Though there is a rare chance of observing any adverse health effect on benthic organisms at these PCBs levels, these compounds are known to bioaccumulate in fatty tissues, and biomagnify, whereby they may reach toxic levels in animals at the top of the food chain. Furthermore, only 6 PCB congeners were investigated in the present study, and inclusion of more compounds may lead to levels above the ERL. This therefore calls for extended monitoring to cover more PCB congeners and involve extra matrices like humans and animals that depend on the water from the two rivers and scavenge on the waste from the dump sites. The clean-up cartridge developed in the present study resulted in clean extracts, well-defined chromatograms, low detection limits, minimal solvent and sorbent requirement, and additional advantage of re-usability. The extraction technique adopted, *i.e.,* ultrasonic assisted extraction (simply referred to as sonication) complements MISPE in terms of affordability compared to high calibre microwave assisted extraction, supercritical fluid extraction, and pressurized liquid extraction techniques. In addition, the developed procedure can be shortened further by eliminating the sulfur removal step if the detection system is mass spectrometer, since sulfur does not interfere with this type of detector.

Fragment templates comprising of hexachlorobenzene (HCB) and xylenes can serve as alternatives to PCB 15 (Paper II). HCB revealed increased imprinting factor, *i.e.*, 1.2 versus 1.1 for PCB 15, leading to a conclusion that the interaction of HCB with the functional monomer is enhanced resulting in the formation of slightly more stable pre-polymerization complex. The main difference between the two compounds is the level of chlorination, whereby the increased chlorine atoms on HCB makes it more electron poor and therefore able to interact more with electron-rich 4-VP. Evident in this study was the role of increased amount of functional monomer, as polymers prepared using template: functional monomer ratio of 1:16 performed better than 1:8 ratio. These high ratios may compensate for the required multiple interactions that poorly functionalized templates do not achieve. The polymers showed extensive interaction with the investigated compounds when applied as stationary phases in chromatography, compared to C_{18} column. Likewise upon application as sorbents in SPE, recoveries higher than 80% were realized in both organic and aqueous media, demonstrating the utility of these polymers in analysis of PCBs. The other template, *i.e.,* xylene presented an alternative to chlorinated compounds. Due to their substitution pattern, *i.e.,* methyl groups on the benzene rings, they are able to surrogate chlorinated compounds. An imprinting effect for these molecules was evident from the tailing effect and an imprinting factor of 1.56. Recoveries >70% even at SPE adsorbent amount of 60 mg was a clear indication of the promises offered by the porogenic template fragments for imprinting of PCBs. Non-imprinted polymers synthesized using toluene also showed good performance, leading to a conclusion that toluene could also serve as a porogenic template fragment for imprinting of PCBs. This strategy is attractive since it is devoid of template requirement and can be a substitute when the template of choice may not be recommended for use or not available in quantities needed for imprinting.

A method for pre-concentration of PCBs based on commercially available MWCNTs as an alternative to conventional C18, which demonstrates poor interaction with PCBs, was validated in Paper III. It was evident that MWCNTs strongly interact with aromatic compounds from the strength of the elution solvent and the recoveries realized, however, this happens only for the sufficiently chlorinated constituents, as more soluble (*i.e.*, less chlorinated) maybe retained less due to the reduced interaction with MWCNTs. High adsorption capacities (*i.e.,* 555.6 $\mu g\ g^{-1}$) realized clearly suggest that MWCNTs are well applicable for removal of PCBs from the environment, especially in water purification or waste-water treatment. However, this can only be achieved in the absence of oxidizing agents as the introduction of oxygen containing groups reduced the ability of MWCNTs to retain PCBs.

The contribution of molecularly imprinted polymers in studies of polychlorinated aromatic compounds (PACs) is summarized in Paper IV. It was evident that imprinting of poorly functionalized molecules is not a straightforward approach and various innovative strategies have to be employed to imprint these compounds. Strategies such as dummy templating, non-covalent imprinting approach using pre-synthesized complexes, use of electron rich compounds as co-monomers have led to polymers which facilitate the determination of various PACs in the environment. Besides the application of these selective sorbents in SPE, they have been incorporated in sensory as recognition elements (achieving detection at trace levels), and as coatings in solid phase microextraction (SPME).

6. Conclusions and outlook

Current research efforts are being directed at development of techniques that achieve monitoring of PCBs at their environmentally relevant low concentration, and more so at affordable cost. Use of advanced and highly selective instruments such as gas chromatography high resolution mass spectrometry and thorough sample pre-treatment are two ways in which detection at trace levels can be achieved. In the present thesis, use of molecularly imprinted polymers as selective sorbents facilitating removal of substantial matrix interferences in determination of PCBs has been demonstrated. In addition, multiwalled carbon nanotubes are presented as promising materials for studies of PCBs in aqueous environments.

The synthetic protocol followed in this thesis is the non-covalent approach using dummy templates. Characterization of the synthesized polymers by batch rebinding assays gave imprinting factors between 1.1 and 1.2, suggesting that these poorly functionalized templates did not form stable complexes with the functional monomer. It was expected that by applying large amount of functional monomer (template: functional monomer ratio of 1:16), which was higher than the usually applied ratio of 1:4-1:8, the formation of stable complexes would be enhanced, but that was not the case. Since this approach did not yield polymers with high imprinting effect, functionalized dummy templates like hydroxylated PCBs could be applicable, when high imprinting factors are desired. These molecules are able to undergo multiple interactions with 4-VP (*i.e.,* strong hydrogen bonds, hydrophobic and π-π stacking), necessary for formation of a stable pre-polymerization complex. Another strategy, which has been reported to give high imprinting factors even when dealing with poorly functionalized polychlorinated dibenzodioxins (PCDDs) and maybe recommended for imprinting of PCBs is the introduction of methacrylic acid groups onto the polymer matrix *via* the semi-covalent imprinting approach.[165,166] In this case, chlorine atoms form weak hydrogen bonds; however, the approach is limited by the need for a pre-synthesized template-functional monomer complex, which makes it costly. Selectivity can also be enhanced by incorporating electron-rich components as co-monomers during polymerization. This results in electron rich polymer matrix for interaction with electron-deficient chlorinated compounds. [165,167] As an alternative to chlorinate compounds, xylenes evidently surrogate for PCBs[72] and PCDDs,[104] and their extended application in studies of six indicator PCBs is has been shown in this present thesis. This therefore is a promising and affordable route for imprinting of PCBs, and more alkyl benzenes maybe screened for this purpose. Despite the low imprinting factors, the polymers are well applicable in solid phase extraction, serving as possible alternatives to C_{18}, sulfoxide-modified silica and silica gel in pre-concentration and clean-up of soil and sediment extracts during the determination of PCBs. The goal of providing an affordable method that achieves detection of PCBs at trace levels was achieved, as detection limits of 0.008–0.04 ng mL^{-1} for water and 0.05–0.28 ng g^{-1} for solid matrices were reported. In addition, the polymers were re-usable, and reduced amount of solvent was required to achieve clean-extracts. The limits of quantification reported in the present study were within those reported using conventional silica gel and GC-ECD detection, *i.e.,* 0.03-1 ng g^{-1},[128,168,169] clearly indicating a possible adoption of these materials in monitoring of PCBs. The protocol can also be adopted for determination of PCBs in other matrices, especially foodstuffs for compliance with the set residue levels and tolerable daily intake for the protection of human health.

Readily available MWCNTs, can also serve as alternatives to conventional sorbents, as indicated by their enhanced performance in pre-concentration of PCBs from aqueous samples. However, this protocol is only applicable for sufficiently chlorinated compounds since the interaction of PCBs and MWCNTs is governed by π-π stacking. Furthermore oxygen containing groups may interfere with the performance of MWCNTs and should be avoided. Compared to MIPs, a question on whether MWCNTs are indeed selective to PCBs may arise since all kinds of aromatic compounds are bound to be retained by these materials. However, when dealing with simple matrices, these materials can achieve the desired performance. For complex matrices, a two-step SPE, whereby the sample is first cleaned on MWCNTs cartridge, then further on MISPE maybe recommended. That way removal of substantial interferences maybe achieved. Incorporation of MWCNTs as support material during MIPs synthesis is another option of maximizing on the properties of the two materials,[103] and can be explored further. Commercialization of these materials is possible as only small quantities (*i.e.,* >100 mg) are able to achieve the desired results compared to the large amount employed for conventional sorbents.

In conclusion, the present research thesis has presented two practical and affordable procedures that can be adopted for monitoring of PCBs in both aqueous and solid matrices.

7. References

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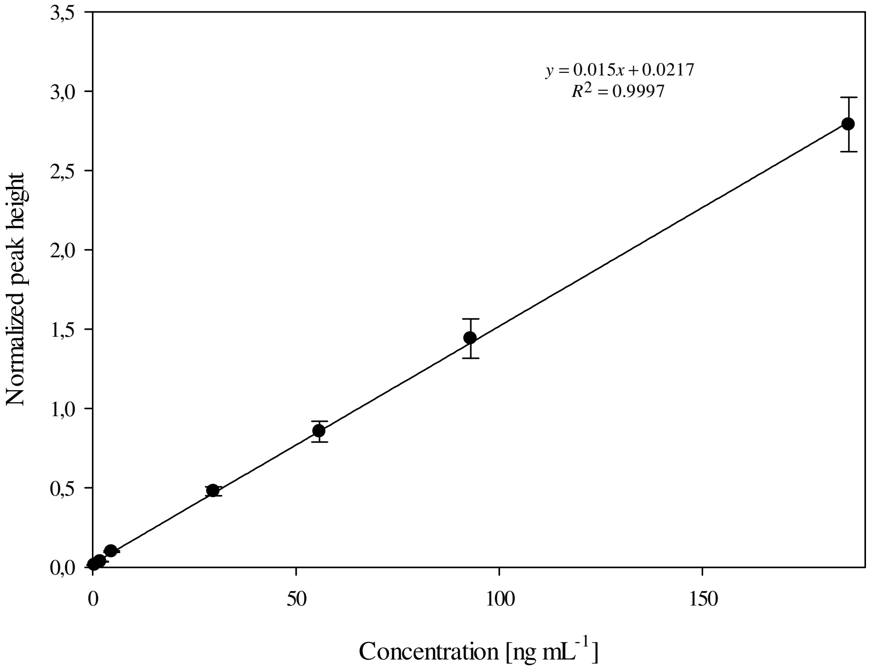
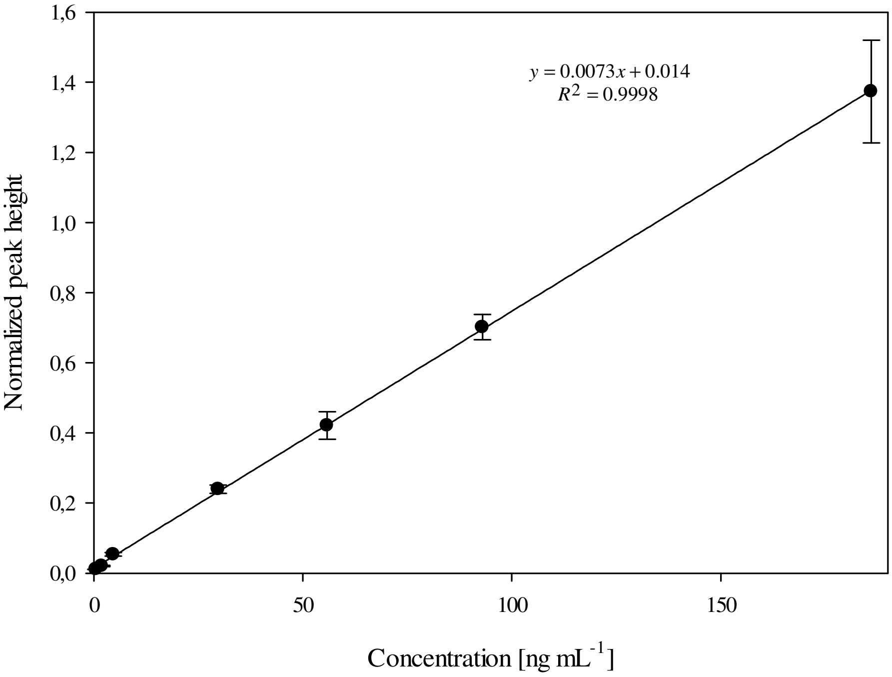
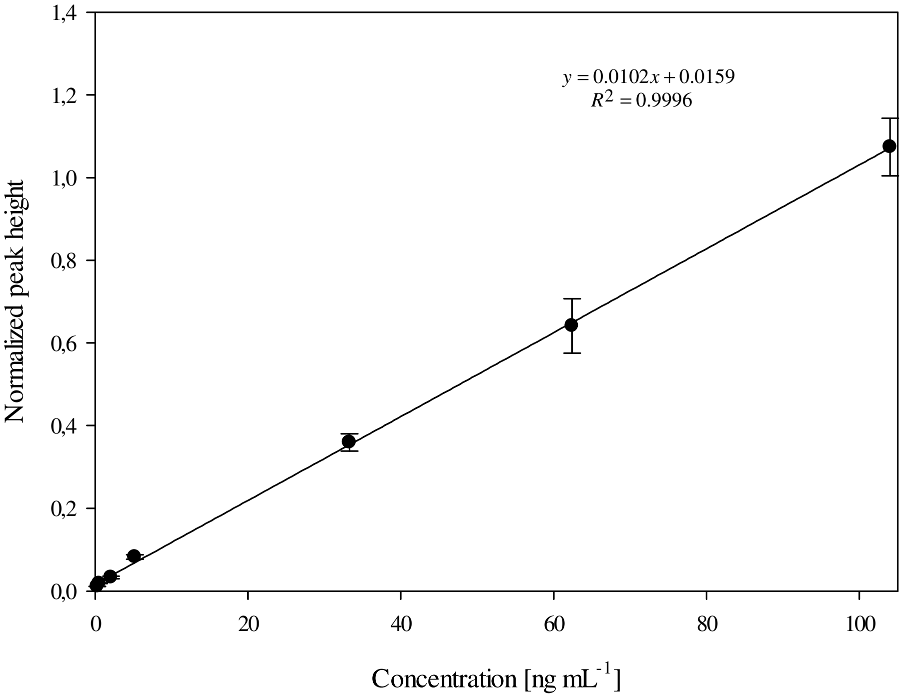


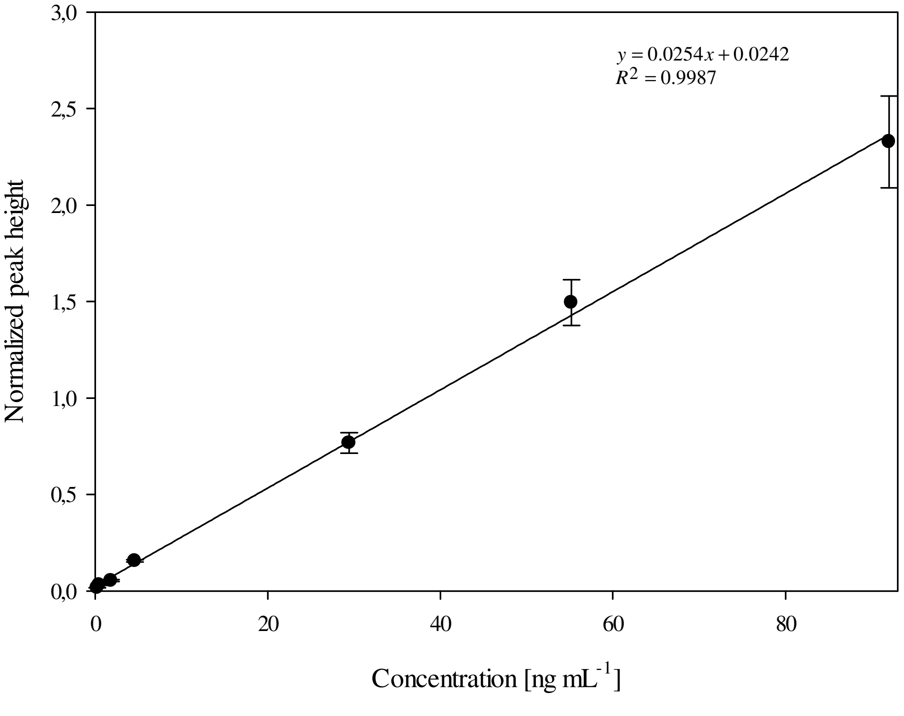
Appendix 8a: Calibration curve for PCB 28 at a concentration range of 0.465-186 ng mL^{-1}.



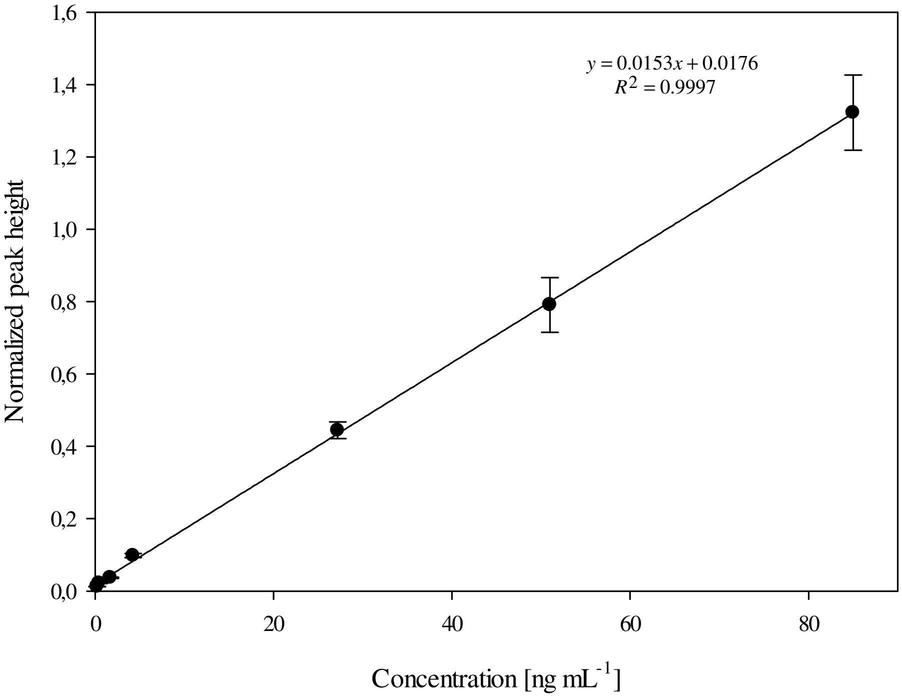
Appendix 8b: Calibration curve for PCB 52 at a concentration range of 0.465-186 ng mL^{-1}.



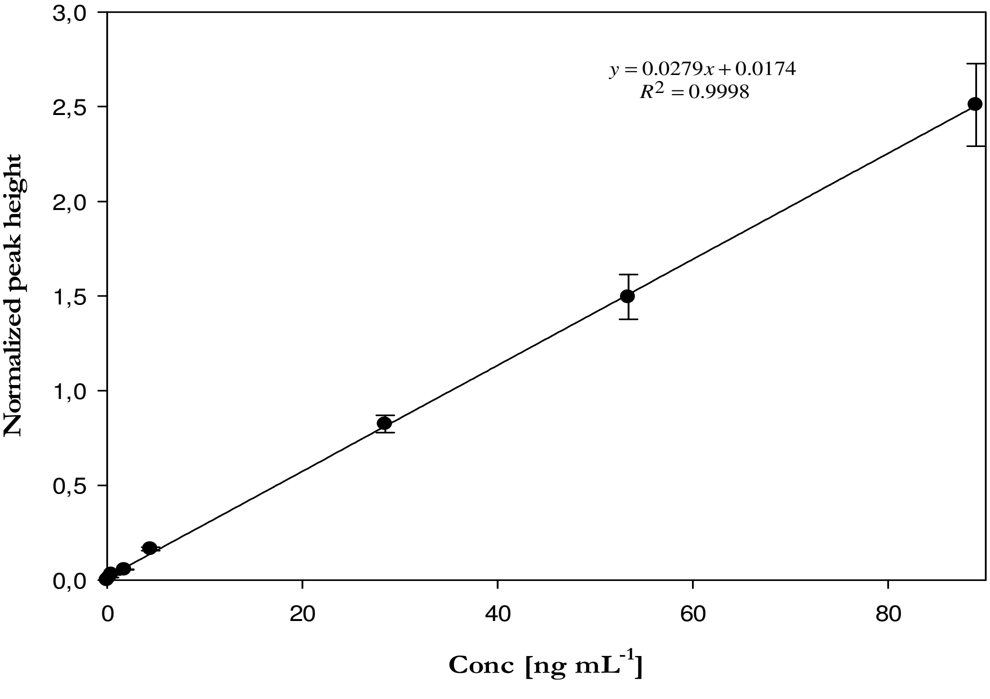
Appendix 8c: Calibration curve for PCB 101 at a concentration range of 0.26-104 ng mL^{-1}.



Appendix 8d: Calibration curve for PCB 138 at a concentration range of 0.23-92 ng mL^{-1}.



Appendix 8e: Calibration curve for PCB 153 at a concentration range of 0.21-85 ng mL^{-1}



Appendix 8f: Calibration curve for PCB 180 at a concentration range of 0.22-89 ng mL^{-1}

Appendix 8g: Some of the sites where samples were collected: (a) Ondiri, (b) Eastern bypass, (c) Ruai, (d) Donholm, (e) Kangundo. (images taken by E.N.Ndunda)

9. Acknowledgements

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10. Declaration of self contribution

Paper I. Elizabeth N. Ndunda, Vincent O. Madadi and Boris Mizaikoff. An alternative clean-up column for the determination of polychlorinated biphenyls in solid matrices. *Environmental science processes & impacts, 2015,* 17: 2101-2109, DOI: 10.1039/C5EM00409H (*Reproduced by permission of the Royal Society of Chemistry*).

- I did all the experimental work, and wrote the manuscript draft myself.

Paper II. Elizabeth N. Ndunda and Boris Mizaikoff. Synthesis of stationary phases that provide group recognition for polychlorinated biphenyls by porogenic fragment template imprinting. *J. Sep. Science,* 2016, 39: 939-946 DOI: 10.1002/jssc.201500960 (*Reproduced by permission of the John Wiley and Sons).*

- I did all the experimental work, and wrote the manuscript draft myself.

Paper III. Elizabeth N. Ndunda and Boris Mizaikoff. Multi-walled Carbon Nanotubes: Innovative Sorbents for Pre-concentration of Polychlorinated Biphenyls in Aqueous Environments. *Analytical methods,* 2015, 7: 8034-8040, DOI: 10.1039/C5AY01496D) *(Reproduced by permission of the Royal Society of Chemistry).*

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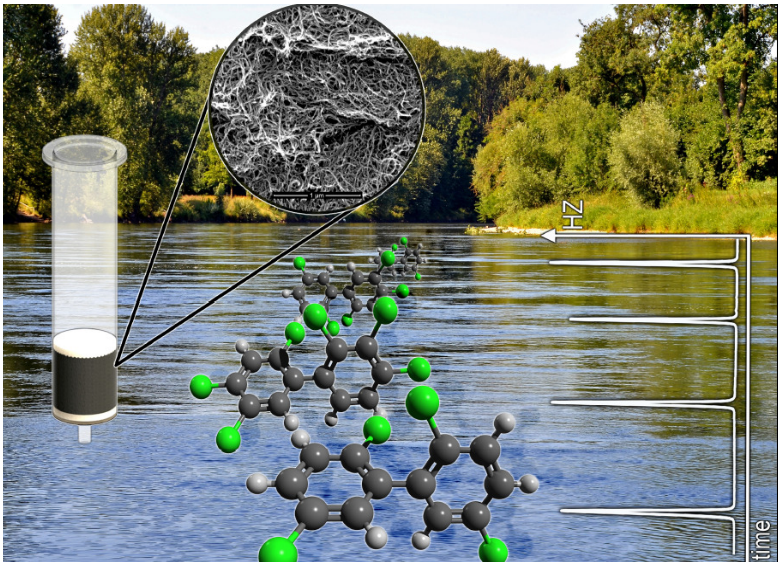

- I did all the experimental work, and wrote the manuscript draft myself. A. Wilk and M. Dinc helped design the journal front cover.

Paper IV. Elizabeth N. Ndunda and Boris Mizaikoff. Molecularly imprinted polymers forthe analysis and removal of polychlorinated aromatic compounds in the environment: a review. *Analyst, 2016, 141*: 3141-3156, DOI: 10.1039/C6AN00293E *(Reproduced by permission of the Royal Society of Chemistry).*

- I wrote the manuscript draft myself.

11. Curriculum vitae

Personal details

Name: Elizabeth Nthambi Ndunda
Place of Birth: Machakos
Nationality: Kenya

Education

4/2012 – 3/2016	ULM UNIVERSITY, ULM, GERMANY Doctoral candidate at the Institute of Analytical and Bioanalytical chemistry under the advisory of Prof. Dr. Boris Mizaikoff - Dissertation title: Development of Selective Sorbent Materials for the Determination of Polychlorinated Biphenyls (PCBs) in the Environment. Overall grade for the Dissertation: magna cum laude
10/2007-10/2010	UNIVERSITY OF NAIROBI, NAIROBI, KENYA Master of Science (M.Sc.) Degree in Chemistry
9/2001-10/2005	UNIVERSITY OF NAIROBI, NAIROBI, KENYA Bachelor of Science (B.Sc.) Degree in Chemistry (First class honours)
2/1996 - 11/1999	Limuru Girls' School, Limuru, Kenya Kenya Certificate of Secondary Education (B+)
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Work Experience

8/2011 - 11/2011: Assistant Lecturer at the Department of pure and applied sciences, Kenya Methodist University (KeMU), Meru, Kenya.

3/2011 - 6/2011: Part-time Lecturer at the Department of Chemistry, University of Nairobi, Nairobi, Kenya.

11/2008 - 11/2009: Research student at the Nairobi River Basin Project (NRBP) aimed at development of water quality monitoring protocol for Nairobi River Basin

11/2006 - 6/2007: Graduate student assistant at the Department of Chemistry, University of Nairobi, Kenya, responsible for introducing practical lessons at undergraduate level

9/2005 - 11/2005: Analytical chemistry trainee at the Government Chemists' Department, Mombasa, Kenya

Peer-reviewed Publications

E. N. Ndunda, B. Mizaikoff. Multi-walled Carbon Nanotubes: Innovative Sorbents for Pre-concentration of Polychlorinated Biphenyls in Aqueous Environments. *Analytical methods*, 2015, 7: 8034-8040

E. N. Ndunda, V.O. Madadi, B. Mizaikoff. An alternative clean-up column for determination of polychlorinated biphenyls in solid matrices. *Environmental science processes and impacts*, 2015, 17: 2101-2109

E. N. Ndunda, B. Mizaikoff. Synthesis of stationary phases that provide group recognition for polychlorinated biphenyls by porogenic fragment template imprinting. *J. Sep. Science*, 2016, 39: 939-946.

E. N. Ndunda, B. Mizaikoff. Molecularly imprinted polymers for the analysis and removal of polychlorinated aromatic compounds in the environment: a review. *Analyst,* 2016, 141: 3141-3156.

Books and Reports

E. Ndunda, S. Wandiga, P. Shiundu. Assessment of Polychlorinated Biphenyls (PCBs) in Nairobi River: Polychlorinated Biphenyls (PCBs) as environmental contaminants. MSc. thesis. Saarbrücken, LAP LAMPERT academic publishing GmbH & Co.KG, 2012. ISBN 9783848444823.

S. O. Wandiga, K. M. Mavuti, F. D. Oduor, D. K. Kariuki, C. W. Mirikau, G. Wafula, V. O. Madadi, N. Mwenda, F. Masese, F. K. Gitari, E. Masenge, **E. N. Ndunda**, D. Situma, V. C. Wang'ondu, and G. Ooko. *Mini-monitoring report for the Nairobi River Basin.* Final report submitted to UNEP regional office for Africa (ROA) in November 2009.

S. O. Wandiga, K.M. Mavuti, F.D. Oduor, D. K. Kariuki, C.W. Mirikau, G. Wafula, V.O. Madadi, N. Mwenda, F. Masese, F.K. Gitari, E. Masenge, **E. N. Ndunda**, D. Situma, V.C. Wang'ondu, and G. Ooko. *Water Quality Monitoring Protocol for Nairobi River Basin.* Final report submitted to UNEP regional office for Africa (ROA) in November 2009.

Oral presentations

E. Ndunda, V. Madadi, B. Mizaikoff
Composite-MISPE for determination of PCBs in solid matrices. 6th Graduate students' symposium on molecular imprinting. Kent, UK. 27-28th August 2015

E. Ndunda, B. Mizaikoff
Molecularly imprinted solid phase extraction (MISPE) for endocrine disrupting compounds
5th Graduate students' symposium on molecular imprinting. Belfast, Northern Ireland, UK. 15-17th August 2013

E. Ndunda, S.O. Wandiga, P. Shiundu
Assessment of Polychlorinated Biphenyls (PCBs) in Sediments and Water from Nairobi River
4th Society of Environmental Toxicology and Chemistry (SETAC) Africa meeting and International Conference on Environmental Pollution and Toxicology. Kampala, Uganda. 2-5. *November 2009*

E. Ndunda, S.O. Wandiga, P. Shiundu
Assessment of Polychlorinated Biphenyls (PCBs) in Sediments and Water from Nairobi River
6th Kenya Chemical Society and East and Southern Africa Environmental Chemistry/8th Theoretical Chemistry in Africa conference. Mombasa, Kenya. 5-10. October 2009.

Poster presentations

E. Ndunda, B. Mizaikoff
Development of a fast extraction and clean-up method for rapid analysis of six indicator PCBs in soil, sediment, and water. 9. Deutsches Biosensor Symposium. München, Germany. 11-13th March 2015

E. Ndunda, B. Mizaikoff
Multiwalled carbon nanotubes solid-phase extraction for determination of six indicator PCBs in aqueous samples. ANAKON Conference. Graz. Austria. 23-26th March 2015

Trainings

- Scientific writing workshop organized by Ulm University International office.
- Powerful presentation skills workshop organized by Ulm University International office
- Participated in an instrumental workshop on GC-MS at the Jomo Kenyatta University of Agriculture and Technology, Kenya. Topic: Contemporary mixture analysis using Gas Chromatography Mass spectrometer.
- Trained on analysis of Persistent Organic Pollutants (POPs) in Air and Milk in the UNEP-GEF workshop held at the University of Nairobi, Kenya
- Trained on publishing and communication of scientific information in scientific communication and publishing course held at the University of Nairobi, Chiromo Campus
- Trained on Risk Assessment at the 7th African Network for Chemical Analysis of Pesticides (ANCAP) Summer School held at Makerere University, Kampala, Uganda.

Skills

- Experience in chromatographic techniques, especially high performance liquid chromatography (HPLC) and gas chromatography (GC)
- Basic knowledge of gas chromatography mass spectrometer (GC-MS)
- Good knowledge in working and operation of UV/Vis spectroscopy
- Languages: English, Swahili, Kikamba, and basic understanding of German language

Professional Membership

- Gesellschaft Deutscher Chemiker (Germany chemical society)
- Kenya Chemical Society (KCS)

12. Eidesstattliche Erklärung

Hiermit erkläre ich, das ich die vorliegende Dissertation selbstständig angefertigt und keine anderen als die in der Arbeit aufgefürten Hilfsmittel verwendet habe.

Ulm, den 31.03.2016 ....……………………….............

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An alternative clean-up column for the determination of polychlorinated biphenyls in solid matrices†

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The need for continuous monitoring of polychlorinated biphenyls (PCBs) has necessitated the development of analytical techniques that are sensitive and selective with minimal reagent requirement. In light of this, we developed a column for clean-up of soil and sediment extracts, which is less demanding in terms of the amount of solvent and sorbent. The dual-layer column consists of acidified silica gel and molecularly imprinted polymers (MIPs). MIPs were synthesized *via* aqueous suspension polymerization using PCB 15 as the dummy template, 4-vinylpyridine as the functional monomer and ethylene glycol dimethacrylate as the cross-linker and the obtained particles characterized *via* SEM, BET, and batch rebinding assays. Pre-concentration of the spiked real-world water sample using MISPE gave recoveries between 85.2 and 104.4% (RSD < 8.69). On the other hand, the specific dual-layer column designed for clean-up of extracts from complex matrices provided recoveries of 91.6–102.5% (RSD < 4%) for spiked soil, which was comparable to clean-up using acidified silica (70.4–90.5%; RSD < 3.72%) and sulfoxide modified silica (89.7–103.0%; RSD < 13.0%). However, the polymers were reusable maintaining recoveries of 79.8–111.8% after 30 cycles of regeneration and re-use, thereby availing a cost-effective clean-up procedure for continuous monitoring of PCBs. Method detection limits were 0.01–0.08 ng g^{-1} and 0.002–0.01 ng mL^{-1} for solid matrices and water, respectively.

Environmental impact

Monitoring of polychlorinated biphenyls (PCBs) is of substantial interest due to the threat these chemicals pose to human and environmental health. Consequently, the development of advanced yet affordable analytical techniques will ensure that these compounds can be monitored at minimal cost. The present study demonstrates the advantages of utilizing molecularly imprinted polymers (MIPs) for the determination of polychlorinated biphenyls in complex matrices, thereby revealing the potential of tuneable sorbent materials for environmental analysis of such relevant pollutants.

1. Introduction

The chemical and physical stability exhibited by polychlorinated biphenyls (PCBs) made them major components in electrical equipment as coolants and lubricants, and additives in various open systems, thus rendering them ubiquitous environmental contaminants. It is the evidence that they cause adverse health effects in humans and animals that led to ban in production and use of these compounds in many countries in the 1970s. Though measures like adoption of the Stockholm convention on persistence organic pollutants (POPs) have been put in place to curb any further releases into the environment, monitoring of these compounds in the environment is of substantial interest, in order to protect humans and animals, and detect any illegal releases.

To this end, efforts have been geared towards developments of techniques that are rapid with minimal reagent requirement so as to facilitate monitoring of these compounds at an affordable cost. For analysis of PCBs in aqueous media, the conventional liquid–liquid extraction (LLE) has been largely replaced by solid phase extraction (SPE),[1] solid phase microextraction (SPME),[2] and star bar sorptive extraction (SBSE).[3] Although the last two techniques have almost eliminated the need for a solvent, SPE still remains a technique of choice attracting continuous development of new sorbent materials to replace the conventional C18 sorbent which is characterized by poor performance.[4] On the other hand, analysis of organic compounds in solid matrices has seen the introduction of ultrasonic assisted extraction (UAE),[5,6] microwave assisted extraction (MAE),[7,8] pressurized liquid extraction (PLE),[9–12] and super-critical fluid extraction (SFE),[13] as substitutes for the

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conventional Soxhlet extraction which is associated with long extraction hours and large amounts of solvent. Apart from PLE which has been designed to achieve both extraction and clean-up by incorporating clean-up sorbents in the extraction cell, all other techniques employ an additional purification step using silica gel, acidified silica gel, Florisil, alumina (or a combination of these materials), and Bio-beads S-X3.[14–16] In addition to these, alternative sorbents that impact selectivity and stability during sample purification have aroused scientific interest in the last 2 decades. The sorbents, *i.e.*, molecularly imprinted polymers (MIPs) are tailored to show selectivity to a specific compound or a group of similarly structured compounds, which then facilitates elimination of matrix interferences during sample purification.

Selectivity in these polymers is achieved by carrying out polymerization of functional monomers in the presence of a target analyte (a.k.a., template), followed by removal of the template leaving behind nanocavities that re-bind the target analyte with high specificity. In addition, MIPs have readily demonstrated their robustness as molecular recognition matrix in a variety of environments, and can be regenerated for multiple uses maintaining their performance even after 10 cycles of usage or more.[17]

MIPs in solid phase extraction, *i.e.*, MISPE, as either pre-concentration or clean-up sorbents is the most advanced application area, and some of the successful applications are in the study of citalopram in human serum and urine, PAHs in water, methamidophos in water and soil, and quercetin in red wine,[17–20] among many others. To the best of our knowledge, only three studies have reported imprinted polymers with recognition properties for PCBs using 1,2,3,4,5-pentachlorobenzene (1) and 1,2,3-trichlorobenzene (2),[21] 3,4-dichlorobenzene acetic acid,[22] and xylenes,[23] as dummy templates and porogenic templates, respectively. Although recognition of the PCBs was demonstrated, applications in environmental studies have only been reported for fish samples. Therefore, the focus of our study was to incorporate imprinted polymers in determination of 6 indicator PCBs in soil and sediments, which are important matrices for monitoring of these compounds. Our extraction technique was ultrasonic assisted extraction, followed by purification of the extracts on specific columns prepared using acidified silica gel and MIPs, and finally quantification using gas chromatography micro-cell electron capture detector (GC-μECD). The optimized method enabled determination of PCBs in real-world samples, provided well-defined chromatograms, and demonstrated minimal solvent requirement, thereby unveiling a reliable and affordable protocol.

2. Materials and methods

2.1 Chemicals and reagents

Chemicals and reagents were purchased from the following sources: PCB standards mixture (28, 52, 101, 138, 153, 180), PCB 14, 15 and 209, hexachlorobenzene (HCB), poly(vinyl alcohol) (PVA, M_w 13 000–23 000, 87–89% hydrolyzed), ethylene glycol dimethacrylate (EGDMA, >98%), 2,2′-azobis isobutyronitrile (AIBN), 4-vinylpyridine (4-VP, 95%), silver nitrate ($AgNO_3$), copper powder (<45 μm), Supelclean C18 SPE cartridges (6 mL, 500 mg, 51.7 μm, 490 $m^2\ g^{-1}$), and Supelclean sulfoxide SPE from Sigma-Aldrich (Steinheim, Germany); toluene, pesticide grade *n*-hexane (≥99%), methanol, dichloromethane (DCM), acetone, chloroform, empty SPE cartridges (6 mL) and frits (20 μm porosity) from Carl Roth Chemicals (Karlsruhe, Germany); silica gel 60 0.063–0.200 mm (70–230 mesh), sulphuric acid (95–97%) from Merck (Darmstadt, Germany); and nitrogen (99.999%) for gas chromatography from MTI IndustrieGase AG (Neu-Ulm, Germany). EGDMA and 4-VP were distilled under reduced pressure before use to remove the inhibitors, while water used was purified using a Milli-Q academic filter system (Millipore, Billerica, USA).

2.2 Sample collection

Sampling was done in Kenya in January 2014 where soil was collected from Dandora and Mt. Kenya, and sediment at five sites along Nairobi River which included: Ondiri, Kijabe, Outering, Eastern bypass, and Ruai (Fig. 1). Tap water was collected from a laboratory at the Institute of Analytical and Bioanalytical Chemistry, University of Ulm, whereas river and lake water were collected from the Danube River (Ulm) and Ludwigfelder See (Neu-Ulm). Water samples were contained in glass bottles and transported to the laboratory where they were filtered through 0.45 μm pore filters and stored at 4 °C until analysis.

2.3 Preparation of molecularly imprinted polymers by suspension polymerization

PCB 15 imprinted microspheres were prepared following a previously reported protocol by Lai *et al.*,[24] with slight modifications. Thus, the continuous phase was prepared by dissolving 2.0 g of PVA in 50 mL Milli-Q water at 95 °C while stirring, which was then allowed to cool to room temperature. Then, the organic phase consisting of PCB 15 (dummy template, 0.3 mmol), 4-VP (functional monomer, 4.8 mmol), EGDMA (cross-linker, 24 mmol), and AIBN (radical initiator, 2% mol of the polymerizable double bond) as the radical initiator dissolved in a mixture of toluene (5 mL) and chloroform (2 mL) was added to the aqueous phase while stirring at 1000 revolutions per min (rpm). The mixture was stirred for 5 min, and the suspension left under UV irradiation (50 W, 365 nm) at room temperature for 4 hours to allow polymerization to take place. A control polymer (a.k.a., non-imprinted polymers; NIPs) and a blank were synthesized using the same procedure, however, in the absence of the template for the NIPs and without the functional monomer and the template for the blank. The resulting particles were then wet filtered under vacuum using borosilicate filters with a pore size of 10–16 μm.

Removal of the template and unreacted monomers was achieved on ULEX[25] (*i.e.*, an extraction device developed at the Institute of Analytical and Bioanalytical Chemistry, University of Ulm) under sonication using methanol : acetic acid (90 : 10, v/v) until no traces of PCB 15 were detected within the extraction solution *via* GC-μECD. To ensure complete removal and avoid template bleeding, the polymers were packed into SPE cartridges and further treated with 12 mL of methanol followed

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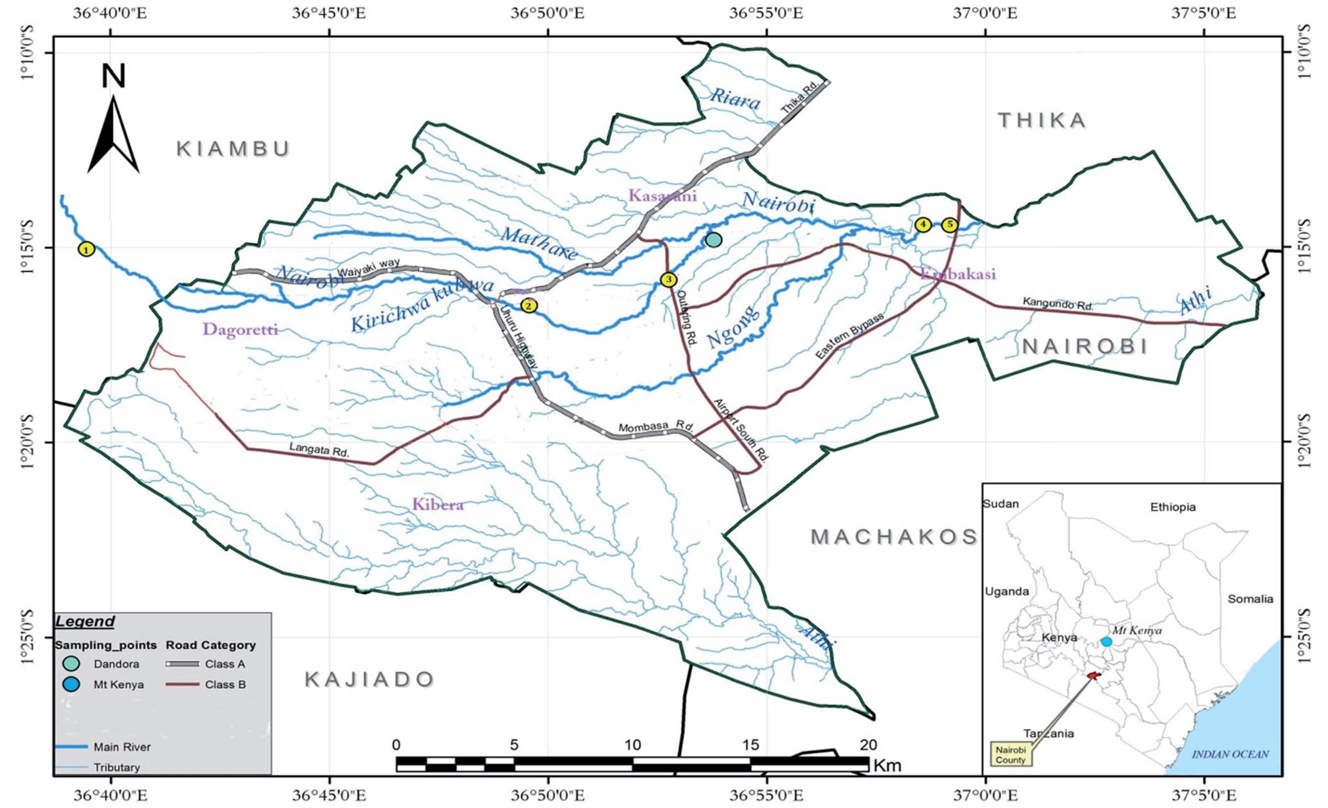

Fig. 1 Map of the study area showing 5 sampling sites along Nairobi River, *i.e.*, Ondiri (1), Kijabe (2), Outering (3), Eastern bypass (4), and Ruai (5); and two other sites, *i.e.*, Mt. Kenya and Dandora where soil was sampled.

by 12 mL of *n*-hexane : acetone (3 : 1, v/v). Twenty mg of the washed particles were then shaken with hexane for 4 h, and the supernatant was analyzed for the presence of the template, whereby absence of the peak of interest confirmed complete removal of the template. The MIP particles were then sieved under acetone to the desired size fraction using sieves of different mesh sizes, and dried in an oven under vacuum at 45 °C overnight.

2.4 Characterization of the polymer particles

The particle shape, size, and surface morphology were determined using a DualBeam Helios Nanolab 600 (Hillsboro, OR, USA) scanning electron microscope (SEM), while specific surface area, pore size, and pore volume were determined *via* the nitrogen adsorption–desorption BET and BJH method on particles that had been degassed for 4 h at 100 °C under vacuum prior to analysis.

2.5 Kinetics and equilibrium rebinding experiments

Thirty mg of the polymer particles was weighed into 2 mL tubes and 1.0 mL of 0.44 μg mL^{-1} PCB 15 standard in *n*-hexane was added and then vortexed for 3 h. To determine the extent of adsorption with time, the supernatant was analyzed after every 30 min. The tubes were centrifuged at 4000 rpm for 10 minutes and the supernatant was filtered through a 0.45 μm PTFE filter (VWR International GmbH, Darmstadt, Germany) into 1.5 mL GC autosampler vials, and the concentration of PCB 15 determined using GC-μECD. The amount of the bound analyte was determined by subtracting the final concentration from the initial concentration and dividing by the mass of the polymer used. After establishing the time required to reach equilibrium, the procedure was repeated with PCB 15 over the concentration range 0.088–0.968 μg mL^{-1}. Kinetic data were applied on pseudo-first-order and pseudo-second order kinetics models, while the binding parameters were calculated using Langmuir and Freundlich adsorption isotherms (see ESI†).

2.6 Optimization of molecularly imprinted solid phase extraction (MISPE)

Five hundred mg of the polymer particles (size fraction: 32–60 μm) were suspended in methanol, and then slurry packed into a 6 mL empty polypropylene cartridge with a frit at the top and bottom (20 μm porosity). Prior to use, the column was conditioned with 12 mL of methanol followed by 12 mL of *n*-hexane : acetone (3 : 1, v/v), and finally equilibrated with 6 mL of *n*-hexane. During these steps, the column was not allowed to dry. One mL (20 ng mL^{-1}) of PCB standard mixture in *n*-hexane was then introduced to the column at a flow rate of 0.5 mL min^{-1}, and the column was dried for 10 min under full vacuum. Elution of the adsorbed analytes was performed using 5 mL *n*-hexane : DCM (9 : 1, v/v), and reduced to near dryness under a gentle flow of argon then reconstituted in *n*-hexane to

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a volume of 1 mL for GC-μECD analysis. For comparison, conventional C18 and sulfoxide modified silica columns were processed similar to the MISPE cartridges, as well as the control non-imprinted solid phase extraction (NISPE) columns.

Validation of the MISPE cartridges for analytes in aqueous media was done by first conditioning the columns with 6 mL of methanol, followed by equilibration with 6 mL of Milli-Q water. Five mL of Milli-Q water (containing 125 μL of methanol, organic modifier) spiked at a concentration of 0.2 ng mL^{-1} was loaded onto the column and washed with 2 mL of methanol then dried under full vacuum for 15 min. The elution step and eluate treatment before analysis were as described above. C18 and sulfoxide cartridges were treated *via* the same procedure, however, washed with 2 mL of 10% methanol in water. The optimized MISPE protocol was then applied for real-world water samples spiked at 0.2 ng mL^{-1}; these samples were analyzed before spiking using the validated protocol to determine the level of contamination.

2.7 Preparation of specific clean-up columns

After establishing the performance of the MISPE cartridges using PCB standards, the specific MISPE column (composite-MISPE) was prepared by adding 750 mg of sulphuric acid impregnated silica gel (SiO_2–H_2SO_4) on top of the MIP beads column and then subjected further to a validation process using PCB standards.

2.8 Study of matrix effect and sample analysis

In order to determine the performance of the specific column in the presence of interfering matrices, real-world soil and sediment samples from background sites, *i.e.*, Mt. Kenya & Ondiri were spiked with the constituents of interest, and taken through extraction and clean-up steps following the validated protocol. The samples had been analyzed prior to spiking following the validated protocol to ascertain the initial level of contamination. Therefore, 5 g of soil spiked at 4 μg kg^{-1} was sonicated for 5 min with 20 mL of *n*-hexane : acetone (8 : 2, v/v), and the extract separated from the soil by centrifugation. Then it was transferred into a round bottomed flask and extraction repeated twice using 10 mL of the solvent mixture. The final volume was reduced to 1 mL using a rotary vacuum evaporator with the water bath set at 30 °C. The extract was then subjected to a clean-up procedure using the validated specific columns with an additional sulphur removal step as described in the ESI.† The sediment sample was subjected to similar steps, yet, were dried using K_2SO_4 before extraction. Once the recoveries were determined, the collected field samples were analyzed accordingly for the levels of PCBs.

2.9 Quantification of PCBs by GC-μECD

Gas chromatography was performed using an Agilent 6890 GC (Agilent Technologies) coupled to a micro-cell ECD detector (GC-μECD). Separation of PCBs was achieved on a ZB5-MS capillary column of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness with a 1 m deactivated fused silica guard column, which was connected to the analytical column through a glass capillary connector. The temperature program applied was an initial temperature of 60 °C (hold time 2 min), ramped at 15 °C min^{-1} to 210 °C (hold time 2 min), and finally ramped at 10 °C min^{-1} to 275 °C (hold time 5 min) resulting in a total GC run time of 25.5 min. The detector temperature was set at 280 °C. One μL of standards and samples was manually injected using the on-column injection mode. Nitrogen (>99.999% purity) was used as both carrier gas at a flow rate of 2 mL min^{-1} and detector make-up at 30 mL min^{-1}. Data were processed *via* Chemstation software version A.01.08 supplied by Agilent Technologies.

2.10 Quality control

Quality control measures involved matrix spike, cleaning of silica gel before activation, analysis of blank samples, replicate analysis, rinsing of glassware with acetone before use, and use of high purity standards and solvents. The method detection limit (MDL) was determined following the EPA guidelines[26,27] with K_2SO_4 and Milli-Q water as blank matrices for solids and water, respectively. The method is based on collecting and analysing a series of blanks spiked at a concentration corresponding up to 5 times the expected MDL *via* the same protocol. The MDL is then calculated as

$$\mathrm{MDL} = T_{(n-1,1-\alpha=0.99)} \times \mathrm{SD} \tag{1}$$

where $T_{(n-1,1-\alpha=0.99)}$ is the student's t value at $n-1$ degrees of freedom and at a 99% confidence level, n is the number of replicates, and SD is the standard deviation of replicate analyses.

Quantification was based on an internal standard calibration method using PCB 209 as the injection standard. Seven point calibration curves covering low, middle, and high concentrations of 0.25, 2, 5, 50, 100, and 200 ng mL^{-1} for all the PCB congeners were prepared. A chromatogram of 100 ng mL^{-1} PCB mixture standard is shown in the ESI, Fig. S1.†

3. Results and discussion

3.1 Microsphere synthesis

Suspension polymerization was the synthetic protocol of choice, as in contrast to conventional bulk polymerization, which requires extended procedures of polymer grinding and sieving leading to losses in the process.[28,29] The choice of the polymerization constituents, *i.e.*, the solvent or porogen, the functional monomer, and the cross-linker was also taken into consideration since they play a role in determining the performance of the resulting polymer. The porogen is particularly important because it governs the polymer morphology, the strength of non-covalent interactions, in addition to solubilizing the functional monomers.[30–33] In this study, we optimized a mixture of toluene (less polar) and chloroform (more polar) as porogen. Since imprinting of constituents such as PCBs which are poorly functionalized is a challenge, we chose the electron-rich 4-VP as our functional monomer in order to facilitate the only possible interactions, which are π-stacking of the aromatic rings.

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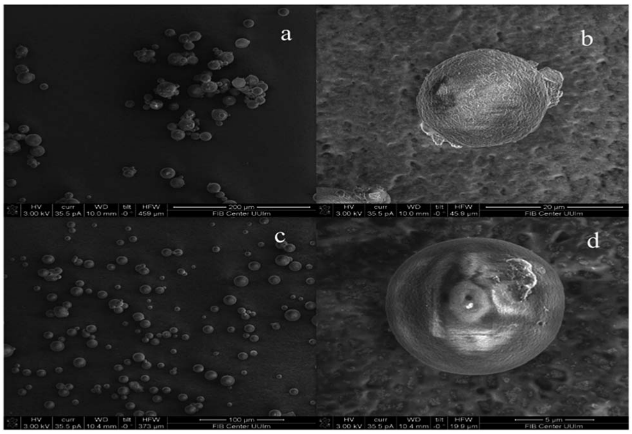

Fig. 2 Scanning electron micrographs of (a and b) MIP, and (c and d) NIP.

The obtained particles were microspheres (Fig. 2) with dimensions ranging from 6 to 60 μm, and a repeat synthesis gave particles within this size range (RSD < 10%). The SEM images of the MIP revealed a comparatively rough surface as compared to the NIP, which was confirmed by the obtained BET surface area of 285.56 ± 20.36 and 134.45 ± 7.55 $m^2\ g^{-1}$ for the MIP and NIP respectively, and MIP pore volume of 0.69 ± 0.12 $cm^3\ g^{-1}$ *vs.* 0.33 ± 0.027 $cm^3\ g^{-1}$ for the NIP.

3.2 Template extraction

Template removal is a general challenge in molecular imprinting, and frequently complete removal is not achieved leading to template bleeding. A previous study established that up to 1.38% of the template remains incorporated within the polymer matrix even after extensive extraction,[34] which is detrimental for trace-level analytical applications of these materials. Incomplete removal of the template also limits the fraction of available binding sites for rebinding of the template. In the present study, template removal by the ULEX and monitoring by GC-μECD achieved almost complete removal (see ESI Fig. S2†). Owing to the low detection limit exhibited by the used GC-μECD (0.01–0.08 ng g^{-1}), the extraction process was considered sufficient for trace analytical applications of the generated MIPs. In addition, as a precautionary measure, we used PCB 15 as a dummy template to represent the 6 indicator PCBs targeted in our present study, meaning that any PCB 15 which bleeds out during the polymer applications does not affect the final quantification due to the different retention times exhibited by the molecules.

3.3 Binding characteristics of the polymer sorbents

To study the binding characteristics, batch rebinding assays were carried out at a concentration range of 0.088–0.976 μg mL^{-1}, where the adsorption capacities of all the polymers increased with increasing initial concentration; though slightly for the blank (Fig. 3). The MIP curve progressing slightly above the NIP, where the rather low imprinting effect realized was attributed to weak π–π interactions explored in the synthesis as PCBs are poorly functionalized molecules. On the other hand, the blank polymer prepared in the absence of both the template and the functional monomer revealed significantly lower binding capacities compared to both the MIP and NIP ($p < 0.05$). It is therefore concluded that 4-VP significantly increases the binding capacity by providing an electron-rich polymer matrix, which extensively interacts with PCB molecules. Introduction of the template during the imprinting process further enhances the binding capacity *via* the creation of binding sites specific to PCBs. The binding isotherms were well fitted on the Freundlich isotherm as was given by high correlation coefficients (Table 1). The MIP was slightly more heterogeneous than the NIP which is characteristic of imprinted polymers due to the formed binding cavities. In contrast, the blank gave a heterogeneity index almost equal to 1, indicating the homogeneous nature of the matrix due to lack of imprinting effect.

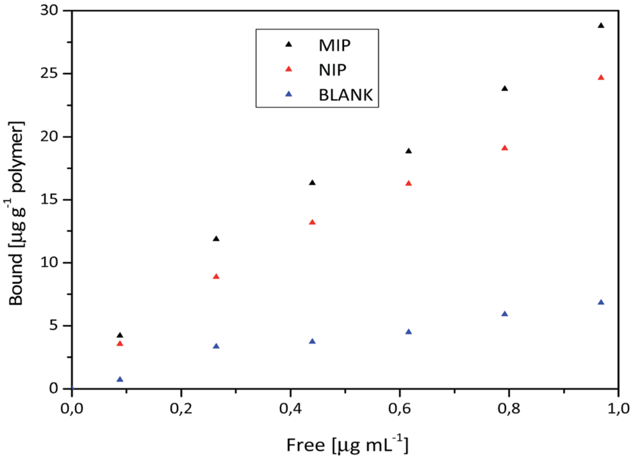

Fig. 3 Binding isotherms of MIP (black), NIP (red), and blank (blue).

Selectivity studies by competitive batch rebinding assays showed that the MIP was indeed selective for PCB 15 among other related compounds as was given by the high binding capacities (see ESI Fig. S3†). PCB 14 – which has the same number of chlorine atoms as PCB 15 though a different substitution pattern – could also be recognized by the MIP in contrast to HCB, which has only one benzene ring. The different behaviour for the PCBs and HCB can be attributed to the dimensions of the binding sites entailed *via* the imprinting process binding well-fitting PCBs more tightly compared to HCB, thus yielding reduced binding capacities for the latter.

3.4 Optimization of the MISPE

This step focused on six "indicator PCBs" which have been globally proposed for monitoring in the environment.

Table 1 Langmuir and Freundlich adsorption isotherm constants

		MIP	NIP	Blank
Langmuir	Q_m (μg g^{-1})	60.2	54.9	23.5
	K_L (mL $μg^{-1}$)	0.8539	0.7344	0.4233
	R^2	0.9037	0.8924	0.3695
Freundlich	K_F (μg g^{-1})	29.5	24.3	7.60
	n	0.7742	0.7845	0.9035
	R^2	0.9882	0.9966	0.9412

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Optimization was done in both organic and aqueous media using *n*-hexane and Milli-Q water, respectively. In the organic media, *n*-hexane was used as the loading solvent due to its non-polar nature, and the fact that it is commonly applied as the extraction solvent for the analysis of chlorinated compounds in solid matrices. The elution solvents investigated herein were 3 mL of *n*-hexane : acetone (3 : 1, v/v) followed by 2 mL of DCM, and 5 mL of *n*-hexane : DCM (9 : 1, v/v), which yielded almost similar recoveries, *i.e.*, 89.4–99.15% and 90.4–99.0% respectively; the latter solvent was selected because of its reduced polarity, which should limit the elution of polar interferences. MISPE and NISPE cartridges provided recoveries of 94.9–99.0% and 90.4–98.9%, respectively, which were higher than 69.2–78.9% for C18 (see ESI Table S1†). The inability of C18 to retain non-polar compounds is also confirmed by recoveries of 25.5%, 58.8%, 1–97% for caffeine in green tea, benzo[*a*]pyrene (BAP) in instant coffee, and 16 PAHs in acetonitrile.[24,35,36] C18 adsorbent retains molecules through hydrophobic forces while the MIPs employed additional π–π interaction forces, and molecular recognition thus increasing chances of high recoveries. On the other hand, commercially available sulfoxide-modified silica gave recoveries between 83.8 and 90.2% in organic media. These high recoveries were attributed to interactions between the π-electron cloud of the PCBs aromatic moieties and the rather electrophilic sulfur.

For potential applications of the polymers in enrichment of PCB in contaminated aqueous samples, optimization was done using Milli-Q water at a pH of 7 giving recoveries >70%. C18 and sulfoxide modified silica gave recoveries of 68.1–73.0% and 35.6–83.1% respectively, which decreased to 33.9–67.7% and 17.9–56.5% with increase in flow rate from 1 mL min^{-1} to 5 mL min^{-1}. The performance of the two sorbents in aqueous phase was contrary to what was realized in organic phase, thus ruling out their application in aqueous media. The MISPE cartridge was further applied for pre-concentration of PCBs in real-world samples which consisted of tap (pH = 7.69), river (pH = 8.42), and lake water (pH = 8.12). The samples had been analyzed before spiking providing no measurable signals. Recoveries >80% were recorded for all the water samples (Table 2). The reproducibility of the method expressed as relative standard deviation (RSD) was in the range of 0.3–8.7%. The water samples had been spiked at a concentration of 0.2 ng mL^{-1} which is less than 0.5 ng mL^{-1} maximum allowable contaminant levels for PCBs in drinking water (as given by US environmental protection agency, EPA),[27] meaning that the polymers can be applicable in monitoring of PCBs. Molecular recognition by the polymers is further demonstrated by the high recoveries given by PCB 138, 153, and 180 which have two chlorine atoms at the *para* positions just like the template used, *i.e.*, PCB 15. In addition, the high chlorinated compounds are much electron poor and therefore have got enhanced π–π interaction with the electron-rich polymer matrix.

Table 2 Recovery (% ± SD) of 6 PCBs in real-world water samples spiked at 0.2 ng mL^{-1a}

	Tap water	River water	Lake water
PCB 28	83.6 ± 5.20	96.0 ± 8.34	83.7 ± 5.87
PCB 52	85.2 ± 2.68	88.2 ± 1.06	83.5 ± 4.03
PCB 101	95.6 ± 0.28	94.1 ± 1.70	92.4 ± 5.80
PCB 153	98.9 ± 1.59	103.1 ± 3.11	95.9 ± 2.67
PCB 138	100.9 ± 2.04	97.1 ± 2.97	95.8 ± 6.01
PCB 180	97.8 ± 5.48	104.4 ± 4.31	98.5 ± 1.06

[a] SD is the standard deviation of replicate analysis.

3.5 Optimization of the specific clean-up columns and study of the matrix effect

Due to the complexity of certain matrices, a simple MISPE may not be sufficient for removal of all interfering components, and some studies have proposed a two-step process whereby the sample extract is first passed through a pre-column packed with either the non-imprinted polymer, C18, or even restricted access materials (RAMs), and then further cleaned-up using MISPE.[37–39] In our case, we modified the MISPE cartridge for clean-up of soil and sediment extracts by incorporating acidified silica gel (SiO_2–H_2SO_4) which helped in the removal of lipids that may be present in soil and sediment extracts.[40,41] Validation of the composite-MISPE column using PCB standards gave recoveries ranging from 89.6–96.0%, and an optimized elution volume of 5 mL *n*-hexane : DCM (9 : 1, v/v). Acidified silica, which is among the conventionally applied sorbents in clean-up was also optimized resulting in recoveries of 92.1–94.9%, and elution volumes of 10 mL *n*-hexane : DCM (9 : 1, v/v). To optimize the whole procedure from extraction to clean-up, we used spiked real-world samples. We tested two solvent systems in the extraction step, *i.e.*, 9 : 1 and 8 : 2 of *n*-hexane : acetone which were less polar compared to the commonly reported *n*-hexane : acetone (3 : 1 or 1 : 1)[1,42,43] or *n*-hexane : DCM (1 : 1).[44] PCB recoveries were determined by subtracting the peak values of non-spiked soil and sediments from the spiked samples which resulted in recoveries in the range of 62.9–79.5% and 91.6–102.5% for the two solvent systems studied herein. The second solvent system (*n*-hexane : acetone; 8 : 2) gave the best recoveries and was adopted for sediment resulting in recoveries of 70.2–94.6%. In addition to increasing the polarity of the extraction solvent, the extraction time was reduced to 15 min with three extraction cycles of 5 min each, compared to one continuous extraction cycle of 30 min using the first solvent system (*n*-hexane : acetone; 9 : 1). Acidified silica resulted in recoveries between 70.4 and 90.5% for spiked soil samples. In addition, commercially available sulfoxide-modified silica was tested for clean-up, which resulted in recoveries between 89.7 and 103.0% (Table 3). This sorbent was initially developed for the extraction of PCBs from oil transformers, waste, and mineral oils. The results obtained in the present study confirm that it is also applicable as a clean-up sorbent for the determination of PCBs in solid matrices.

The developed clean-up method produced highly refined extracts with substantially reduced matrix interferences, as demonstrated by the baseline of the associated chromatograms (see ESI, Fig. S4–S6†) and recoveries within the recommended range for analysis of PCBs (70–120%).[45] The protocol was

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Table 3 Recoveries (% ± SD) of PCBs in background soil and sediments spiked at 4 ng g^{-1}[a]

	Soil			Sediment
	MISPE	Acidified silica	Sulfoxide-modified silica	MISPE
PCB 28	92.6 ± 1.13	83.0 ± 0.28	97.0 ± 2.19	84.6 ± 0.86
PCB 52	102.5 ± 2.12	90.5 ± 0.49	103.0 ± 13.4	73.0 ± 1.47
PCB 101	91.6 ± 0.47	70.4 ± 2.62	89.7 ± 1.06	70.2 ± 9.49
PCB 153	97.0 ± 4.27	73.6 ± 0.57	91.9 ± 1.34	72.5 ± 6.98
PCB 138	94.5 ± 1.05	75.3 ± 1.34	94.7 ± 1.56	73.3 ± 8.27
PCB 180	92.0 ± 1.36	73.7 ± 2.33	91.5 ± 0.71	94.6 ± 3.19

[a] SD is the standard deviation of replicate analysis.

comparable to the acidified silica sorbent and sulfoxide-modified silica in terms of recoveries and removal of matrix interferences (see ESI, Fig. S7 & S8†), suggesting that it could be used as an alternative for the determination of PCBs. The polymers, however, offer an additional advantage of re-usability (see ESI, Fig. S10†), which therefore makes monitoring of PCBs affordable. In summary, to process one sample, a total of 40 mL solvent was required for extraction, and 9 mL for clean-up.

3.6 Levels of PCBs in real-world soil and sediments

Mt. Kenya is considered an ideal background site for the analysis of POPs in Kenya due to its remote location. Background PCB levels were below the detection limits except for PCB 153 and 138, which were determined at 0.07 and 1.43 ng g^{-1}, respectively (Table 4). The presence of these higher-chlorinated PCBs can be related to the fact that they are less volatile, and therefore bind more strongly to solid matrices. On the other hand, PCB 153, 138, and 180 have been reported to be more dominant in the environment.[46,47] Levels of PCBs in Dandora ranged from 0.22–6.74 ng g^{-1}, with PCB 28 giving the lowest concentration and PCB 153 the highest. Compared to the earlier survey in 2008 by UNEP on POP levels in Africa, which reported PCB levels below the limit of quantification (LOQ) for Mt. Kenya soils, and levels ranging from 0.7–1.9 ng g^{-1} in Dandora,[48] the levels of PCBs in the present study were high in Dandora which could be attributed to its proximity to a municipal waste disposal, where continuous human activities like burning of waste may be contributing to PCB burden in the environment close by.

The other matrix studied was sediment, which is important for monitoring of organics in aquatic systems as it is considered a sink for these compounds which bind to sediments owing to their high octanol–water partition coefficient (K_{ow}).[49,50] Sediment samples had contamination levels ranging from 0.04–1.94 ng g^{-1} (Table 4). The level of PCBs increased along the river profile except at the Eastern bypass, which recorded lower levels probably due to its location away from human influence. The sampling site downstream of the river, *i.e.*, Ruai had the highest ∑PCBs of all the samples collected along the Nairobi River profile, where the rather high levels were attributed to additional contributions of the PCBs by the industrial and municipal effluents discharged into the river. Outering recorded the second highest levels of PCB contamination. The site is subjected to numerous human activities in addition to burning of plastic waste – especially tyres – in the area, and also due to its close proximity to light industries. As was expected, Ondiri which was our background site and the source of Nairobi River was less contaminated mainly due to its location in the upstream where there is limited human influence.

Compared to past studies in East Africa, levels of PCBs in sediments in the present study were slightly higher than what has been reported along the Kenyan coast (0.15–1.16 ng g^{-1} dry weight),[51] Winam Gulf, Lake Victoria, Kenya (6.9×10^{-5} to 4×10^{-4} ng g^{-1}),[52] Napoleon Gulf, Lake Victoria, Uganda (3.6×10^{-4} to 8.4×10^{-4} ng g^{-1}),[16] and less than levels reported at the Kavirondo Gulf, Lake Victoria, Kenya (bdl-60 ng g^{-1}).[53] In relation to other parts of the world, PCB levels in the present study were comparable except in extremely polluted areas. The reported PCB levels are 0.33–8.08 ng g^{-1} in Lake Bosumtwi, Ghana,[42] 0.019–1.206 ng g^{-1} dry weight in Ghal El Melh lagoon in Tunisia,[54] and 0.03–1.00 ng g^{-1} dry weight in Xiamen offshore area of China.[10] Exceptionally, high levels of PCB contamination have been reported in Kentucky Lake, USA (11–660 ng g^{-1} for PCB 180), North west Persian Gulf, Iran (100–18 400 ng g^{-1}), Scheldt River, Belgium (14.8–46.4 ng g^{-1} for PCB 153), where the high levels were linked to a transformer manufacturing company and industrial waste water discharge.[43,46,55]

3.7 Method performance

K_2SO_4 and Milli-Q water were used as the blank matrices and subjected to the validated protocol for soil and aqueous samples respectively, revealing no peaks for the six PCBs, thus

Table 4 Levels of PCBs (ng g^{-1} dry weight) (mean ± SD) in surface soil and sediments (0–5 cm top layer); $n = 3$

	Mt. Kenya[a]	Dandora[a]	Ondiri[b]	Kijabe[b]	Outering[b]	Eastern bypass[b]	Ruai[b]
PCB 28	Nd	0.22 ± 0.05	0.23 ± 0.07	0.68 ± 0.10	0.73 ± 0.43	1.03 ± 0.35	1.21 ± 0.43
PCB 52	Nd	0.88 ± 0.10	Nd	nd	0.49 ± 0.16	0.39 ± 0.10	1.94 ± 0.36
PCB 101	Nd	1.78 ± 0.005	Nd	nd	0.37 ± 0.0004	nd	0.66 ± 0.06
PCB 153	0.07 ± 0.03	3.51 ± 0.15	Nd	0.05 ± 0.01	0.13 ± 0.05	nd	0.54 ± 0.04
PCB 138	1.43 ± 0.20	6.74 ± 0.26	Nd	1.68 ± 0.14	1.54 ± 0.27	0.09 ± 0.02	1.15 ± 0.34
PCB 180	Nd	0.78 ± 0.02	Nd	0.44 ± 0.36	0.11 ± 0.0006	0.04 ± 0.007	0.20 ± 0.02
∑PCBs	1.50	13.91	0.23	2.85	3.37	1.41	5.70

[a] Soil. [b] Sediment, nd = not detected, SD = standard deviation.

Table 5 Analytical figures of merit for the proposed method

Congener	Linear range (ng mL^{-1})	Equation	Linearity (R^2)	Method detection limit (MDL) Solids (ng g^{-1})	Method detection limit (MDL) Water (ng mL^{-1})
PCB 28	0.465–186	$y = 0.015x + 0.0217$	0.9997	0.08	0.01
PCB 52	0.465–186	$y = 0.0073x + 0.014$	0.9998	0.05	0.007
PCB 101	0.26–104	$y = 0.0102x + 0.0159$	0.9996	0.02	0.003
PCB 153	0.21–85	$y = 0.0153x + 0.0176$	0.9997	0.02	0.003
PCB 138	0.23–92	$y = 0.0254x + 0.0242$	0.9987	0.02	0.003
PCB 180	0.22–89	$y = 0.0279x + 0.0174$	0.9998	0.01	0.002

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indicating no cross-contamination originating from the solvents and glassware. The calibration functions were established by plotting normalized peak heights (*i.e.*, ratio of the analyte peak and the PCB 209 response) *vs.* the concentration of the analyte. An exemplary sample calibration function for PCB 180 is shown in the ESI, Fig. S9.† Excellent linearity was obtained for all the compounds in the relevant concentration range of 0.21–186 ng mL^{-1} with correlation coefficients (R^2) ranging from 0.9987–0.9998. Method detection limits were 0.01–0.08 ng g^{-1} and 0.002–0.01 ng mL^{-1} for solid matrices and water respectively, while limit of quantification ranged between 0.05–0.28 ng g^{-1} and 0.008–0.04 ng mL^{-1} (Table 5). The detection limits realized were within those reported using other clean-up methods,[1,12,56] meaning that our developed protocol could as well be applied for the determination of PCBs in the environment. Since the cartridge was reusable, after the validation steps and sample analysis, it was subjected to recovery tests using the background samples which resulted in recoveries of 79.8–111.8% (see ESI, Fig. S10†), thus confirming the stability of the cartridge for environmental applications.

4. Conclusions

The present study reports a first time clean-up cartridge which incorporates imprinted polymers in the determination of PCBs in complex matrices. The developed protocol from extraction to instrumental quantification reported minimal solvent requirement, *i.e.*, approximately 50 mL for both the extraction and clean-up. In addition, the adopted clean-up method resulted in neat extracts with minimal interference levels, as indicated by well-defined chromatograms. The method was comparable to other conventional clean-up strategies, meaning that the cartridge can serve as alternative to conventional techniques, offering additional advantages of reusability. For the determination of PCBs in aqueous samples, the MISPE outperformed both the C18 and sulfoxide modified silica, thereby availing a reliable cartridge for monitoring of these compounds at their environmentally relevant low concentrations.

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Supporting information

An alternative clean-up column for the determination of polychlorinated biphenyls in solid matrices

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1. Experimental

1.1 Preparation of sulphuric acid and silver nitrate impregnated silica gel

Silica gel 60 for column chromatography 0.063-0.200 mm (70-230 mesh) was activated by baking at 130 °C for 12 hrs. It was cooled in a desiccator and 44 g of concentrated sulphuric acid (96%) added to 56 g of the activated silica to make (44% H_2SO_4 on silica) then shaken until there were no more lumps and left for 2 hrs to equilibrate. Soil and sediment samples normally contain sulfur which is extracted with the analytes and presents challenges in the GC-ECD analysis. Therefore both activated copper and silver nitrate impregnated silica gel (SiO_2-$AgNO_3$) were applied to ensure total removal of sulfur. Copper was activated using dilute hydrochloric acid (1:3) and rinsed 3 times with methanol then stored in cyclohexane. $AgNO_3$ impregnated silica gel was prepared by adding 0.2 g silver nitrate in 1 mL water dropwise to 2 g silica gel to make a 10% $AgNO_3$ on silica gel. It was then shaken and dried in an oven for 1 hr at 80 °C.

1.2 Sulfur removal

Soil and sediment samples contain sulfur which is sensitive to ECD detection method, and interferes with PCBs quantification,[1] so after the clean-up step using the specific columns, the extracts were subjected to a sulfur removal step. Approximately 250 mg of activated copper powder was added to the 1 mL eluate and allowed to equilibrate for a few minutes. To ensure that no traces of sulfur were left and to remove the copper sulfide (CuS) formed, the eluate was then passed through a column packed with 500 mg of silver nitrate impregnated silica gel (SiO_2-$AgNO_3$), and eluted with 4 mL hexane: DCM (9:1,v/v) followed by blow down and GC-μECD determination.

1.3 Sampling protocol for soil and sediment

Soil and sediment were collected using a shovel from at least three points within a radius of 50 m around the sampling site, homogenised on aluminium foil, and placed in zip-lock bags for transportation to the laboratory where they were kept in a freezer at -20 °C.

1.4 Kinetics and adsorption isotherms

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t \qquad \text{Pseudo-first-order (1)}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \qquad \text{Pseudo-second-order (2)}$$

q_e is amount adsorbed at equilibrium, q_t is the amount adsorbed at any given time (t, min), k_1 is rate constant for the pseudo-first-order (min^{-1}), and k_2 is rate constant for the pseudo-second-order (min^{-1} g μg^{-1}).

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \qquad \text{Langmuir isotherm (3)}$$

$$q_e = K_f C_e^n \qquad \text{Freundlich isotherm (4)}$$

q_e is the bound analyte at equilibrium (μg g^{-1}), C_e is the equilibrium concentration in μg mL^{-1}, q_m represents the monolayer coverage or maximum adsorption capacity (μg g_{-1}), and K_L is a constant related to the adsorption energy (mL μg^{-1}), K_f and n are Freundlich constants representing the adsorption capacity and heterogeneity index.

1.5 Selectivity studies

Selectivity of the imprinted polymer was determined by competitive batch rebinding assays using PCB 14 and hexachlorobenzene (HCB) as the structural analogues of PCB 15. The three analytes each at a concentration of 0.88 μg mL^{-1} were treated as described in the section 2.5.

1.6 Results on the kinetic data

To understand the mechanism of adsorption, the kinetic data was applied on the pseudo-first-order (1) and pseudo-second-order kinetic models (2). Adsorption of PCB 15 followed the pseudo-second-order kinetic model as was indicated by high correlation coefficient (R^2) of 0.9918 for the MIP and 0.9897 for the NIP. The values for the equilibrium concentration (q_e) 7.23 and 6.41 μg g^{-1} for the MIP and NIP in the pseudo-second-order were also near the experimental 8.54 and 7.63 μg g^{-1}, thus confirming that the adsorption involved sharing or exchange of electrons,[2,3] which is consistent with the expected π-π interaction between the template and the polymer.

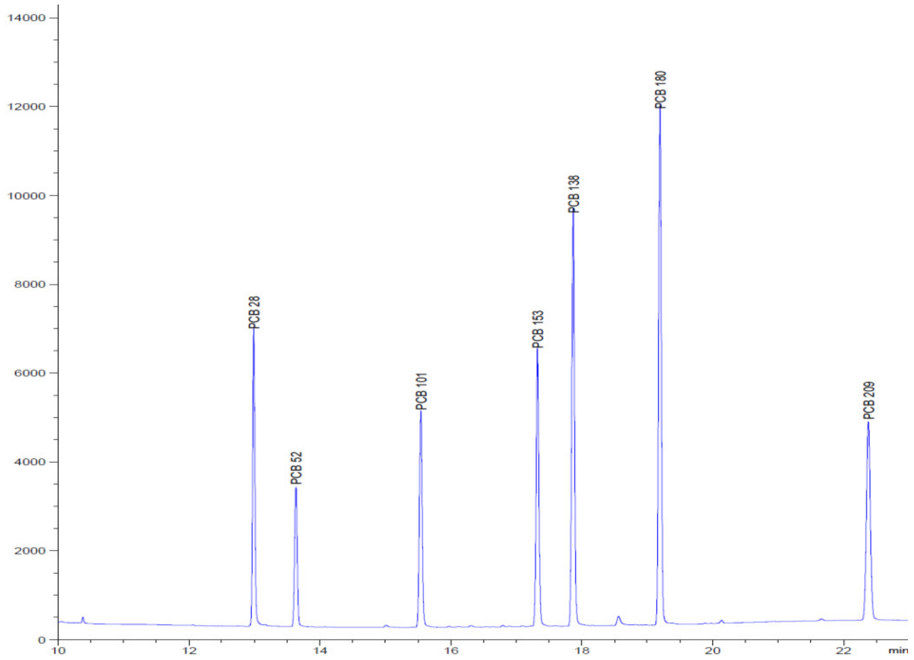

Fig. S1 Chromatogram of 100 ng mL^{-1} PCBs standard mixture with PCB 209 as the internal standard

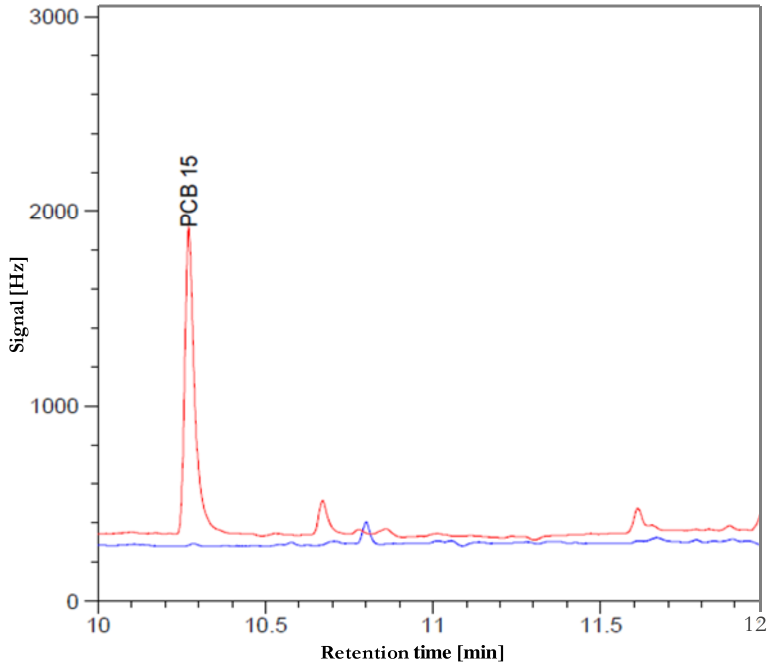

Fig. S2 Chromatogram of cleaned MIP (blue) and PCB 15 standard (red) using GC-μECD and Nitrogen as both the carrier and make-up gas at 2 mL min^{-1} and 30 mL min^{-1}, respectively.

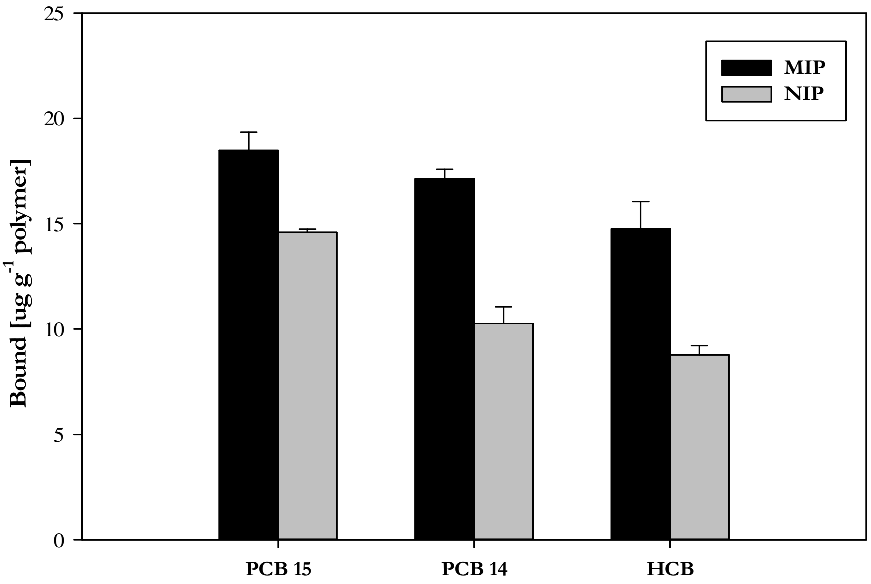

Fig. S3 Selectivity studies using PCB 15 and structurally similar PCB 14 and HCB.

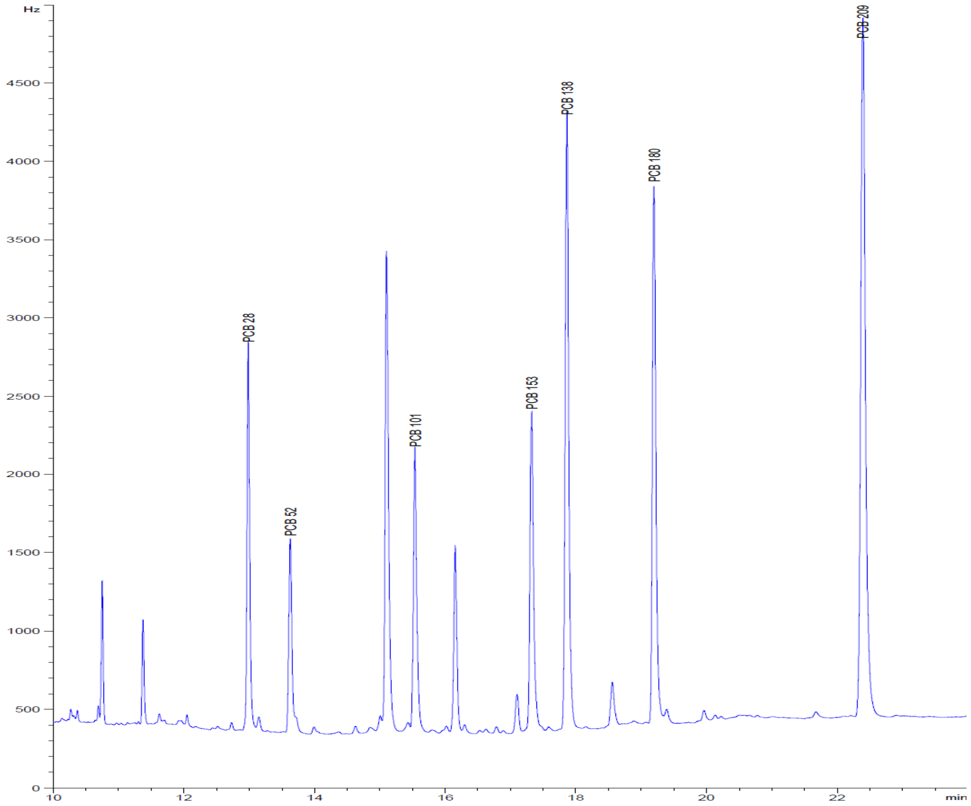

Fig. S4 Chromatogram of Mt. Kenya soil sample spiked at 4 ng g^{-1} with PCB standard mixture and cleaned using MISPE.

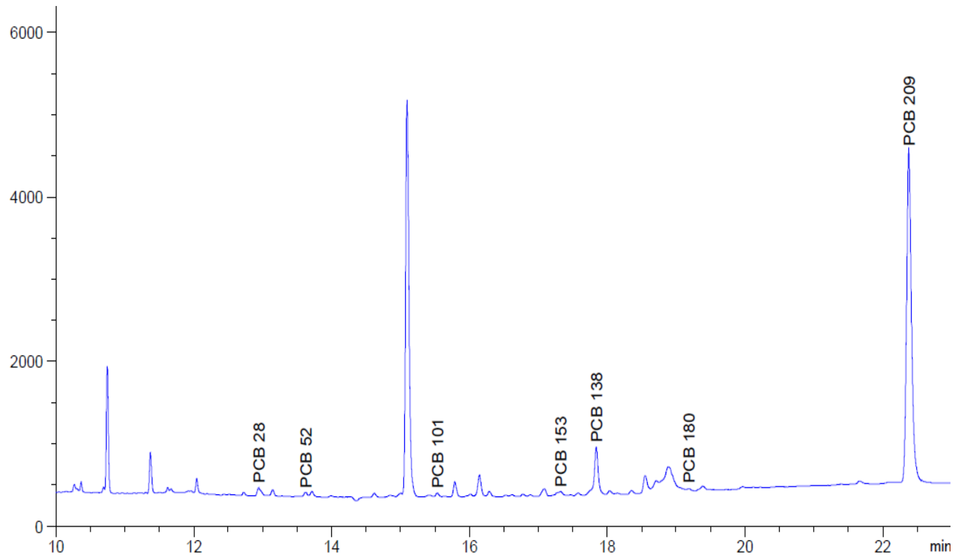

Fig. S5 Chromatogram of a soil sample from Dandora cleaned using MISPE.

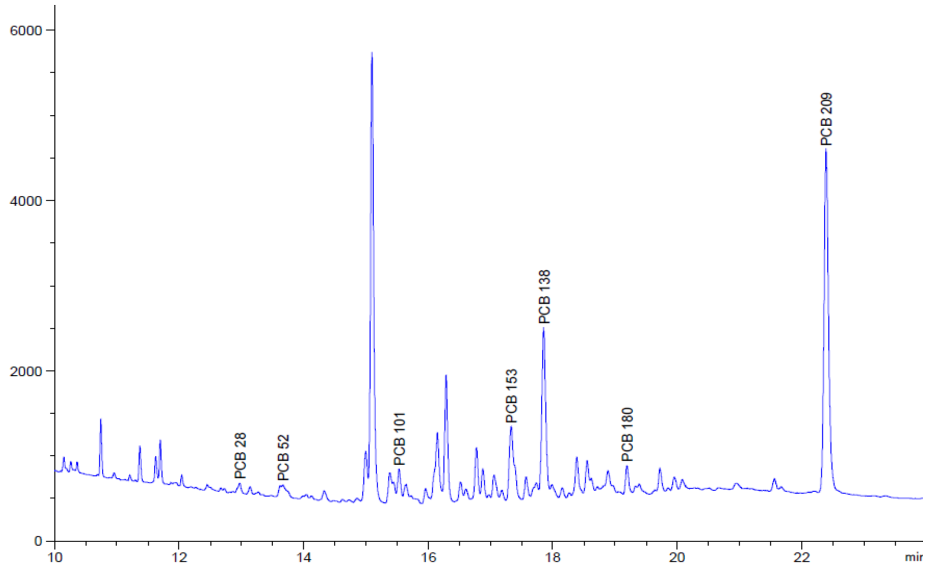

Fig. S6 Chromatogram of a sediment sample from Kijabe cleaned using MISPE.

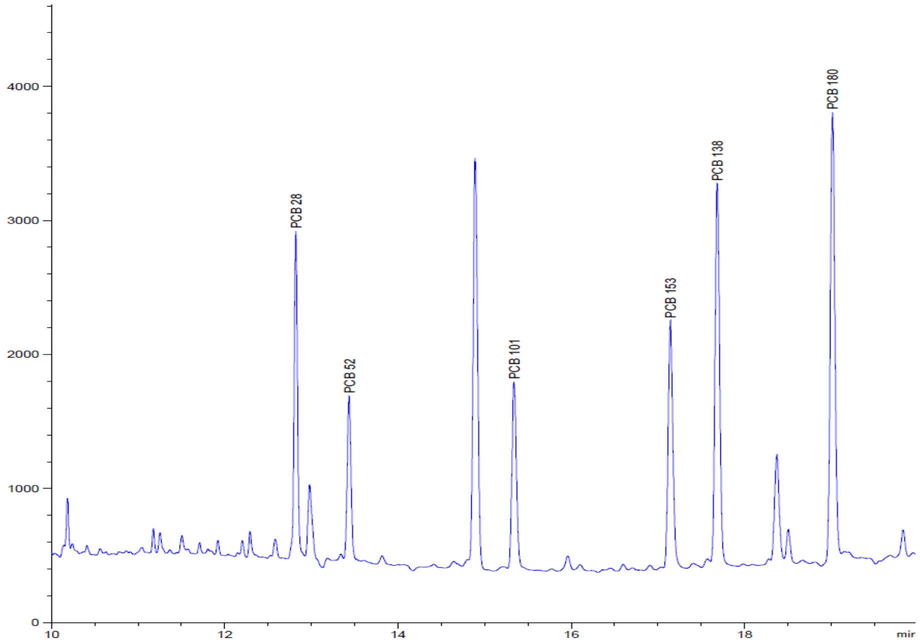

Fig. S7 Chromatogram of Mt. Kenya soil sample spiked at 4 ng g^{-1} with PCB standard mixture and cleaned using acidified silica gel.

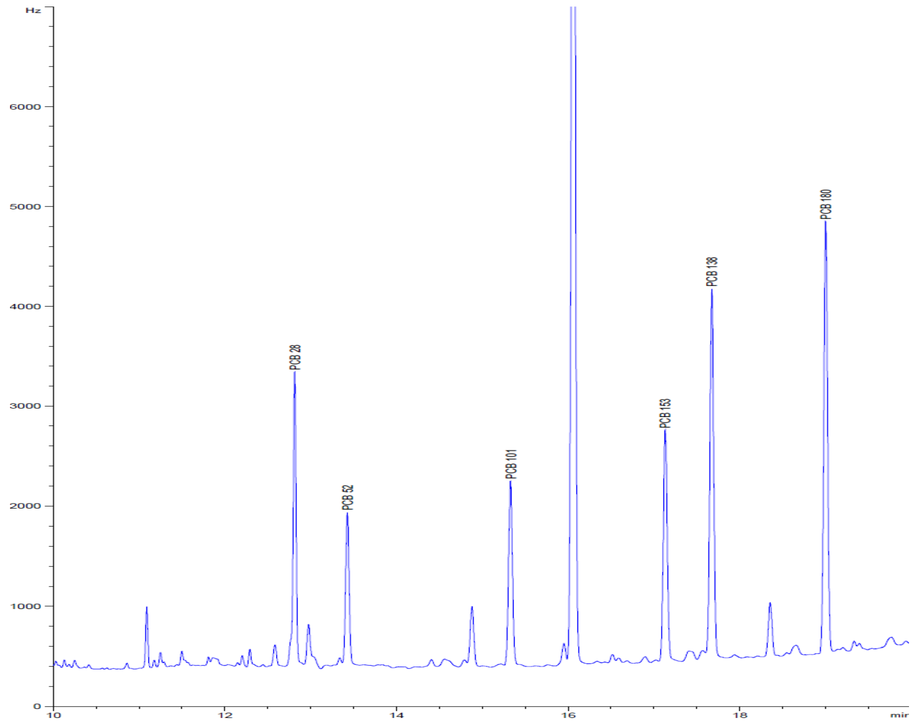

Fig. S8 Chromatogram of Mt. Kenya soil sample spiked at 4 ng g^{-1} with PCB standard mixture and cleaned using sulfoxide modified silica.

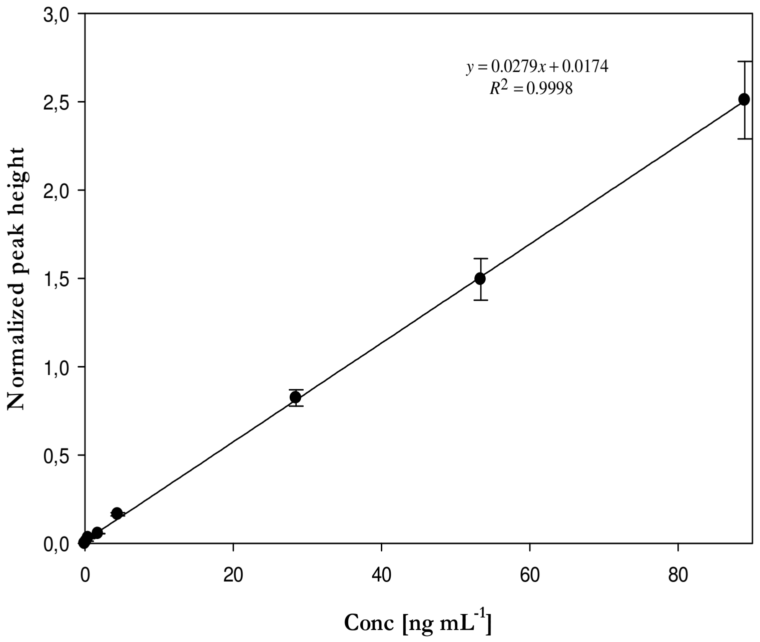

Fig. S9 Exemplary calibration curve for PCB 180 at the concentration range between 0.22 and 89 ng mL^{-1}, with PCB 209 as the internal standard. The error bars represent the standard deviation of 3 measurements.

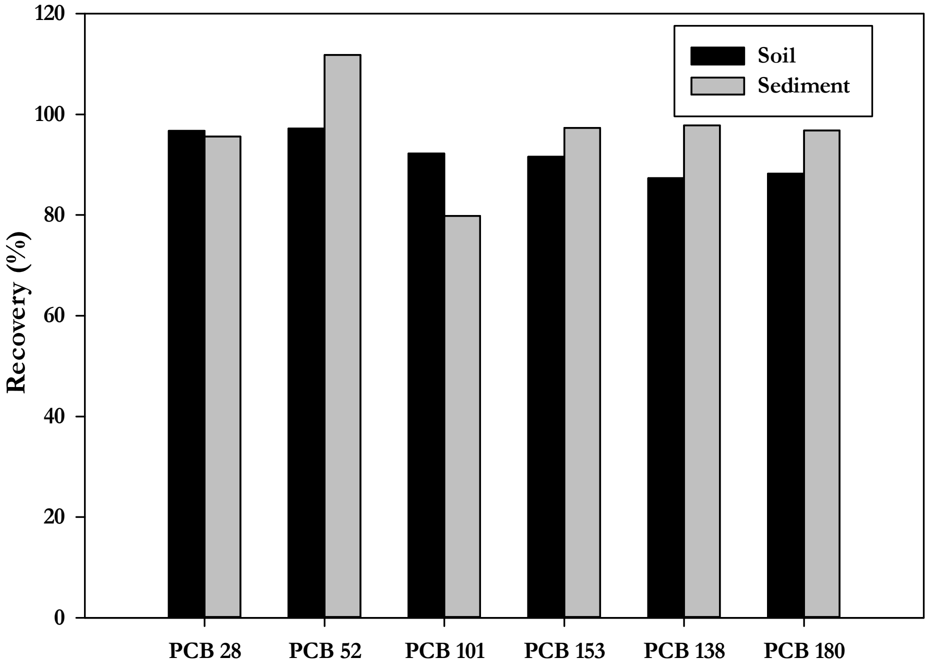

Fig. S10 Recoveries of PCBs on spiked soil and sediment on specific clean-up columns after 30 cycles of usage.

Table S1 Recoveries and losses (% ± SD) of PCBs on MISPE, NISPE, C18, Sulfoxide silica columns using PCBs standards in hexane at a concentration of 20 ng mL^{-1} and elution with *n*-hexane: DCM (9:1; v:v)

	Recovery (%)				Losses (%)			
	MISPE	**NISPE**	**C18**	**SMS**	**MISPE**	**NISPE**	**C18**	**SMS**
PCB 28	99.0 ± 0.64	91.7 ± 3.96	72.5 ± 2.83	83.8 ± 2.62	1.20 ± 0.14	0.80 ± 0.0	26.1 ± 5.30	7.10 ± 0.37
PCB 52	96.6 ± 0.78	90.4 ± 2.19	78.9 ± 7.64	88.0 ± 0.35	4.05 ± 1.20	7.90 ± 0.84	32.4 ± 1.98	5.03 ± 1.71
PCB 101	95.0 ± 8.41	93.6 ± 0.49	72.5 ± 1.98	90.2 ± 0.78	1.70 ± 0.14	2.60 ± 0.57	31.0 ± 3.18	4.97 ± 0.85
PCB 153	94.9 ± 2.16	98.9 ± 0.42	69.2 ± 8.49	84.3 ± 1.27	0.70 ± 0.0	0.70 ± 0.0	32.8 ± 4.10	5.00 ± 0.32
PCB 138	96.1 ± 5.16	95.3 ± 6.79	71.4 ± 0.85	85.8 ± 0.21	0.75 ± 0.07	0.55 ±0.07	30.1 ± 0.92	3.44 ± 0.33
PCB 180	96.3 ± 5.15	94.0 ± 4.88	71.5 ± 2.33	87.6 ± 0.85	1.25 ± 0.07	0.0	31.1 ± 2.40	0.0

SD = standard deviation of replicate analysis; SMS= sulfoxide-modified silica

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Additional information relevant to Paper I but not published

1. Sampling and sample analysis

Ngong' River and various solid waste disposal sites in Nairobi were selected for further studies on the level of contamination with PCBs. Soil samples were collected in August 2015 from Dandora –the main dump site – and 4 other sites located at Kariobangi, Outering, Donholm, and Enterprise. Sediments were collected at 5 sites along the Ngong' River in January 2015, with the sites being distributed to cover the upstream, midstream and downstream of river profile (Fig. 1). Sampling was done as described in paper 1, and analysis done following the method developed in the same paper.

2. Results and discussion

2.1 Level of PCBs in dump sites

Despite the evidence that open dump sites in developing countries are "hot spot" for toxic chemicals such as, *eg.*, polychlorinated dibenzodioxins and furans (PCDD/Fs) and dioxin-like PCBs,[1] often than not, these sites are ignored as major sites for investigation and not much data exists on the level of contamination. Besides incomplete combustion of chlorine containing products during incineration and uncontrolled open burning of waste in dump sites, dismantling and waste recycling of electronic equipments, which is rampant in waste disposal sites, is another source of PCBs. Concentrations as high as 12.9 mg kg^{-1} for penta-chlorinated PCBs analyzed in soil from an abandoned electronic waste recycling area in China have been reported, which was higher than the canadian soil quality guidelines for environmental health of 1.3 mg kg^{-1} for total PCBs in residential areas.[2]

In the present study, PCBs levels in the waste disposal sites ranged from 0.11 to 7.23 ng g^{-1} (Table 1). Samples from Dandora showed the highest level of contamination. Dandora dump site is the biggest in Nairobi receiving about 2000 tonnes of waste which is generated per day. Dandora1 was located at the entrance of the dump site and recorded the highest level of PCBs. Inside the dump site, soil samples were taken from two sites, *i.e.,* Dandora2 and Dandora3. A complete survey of the dump site, which would have included more samples from deep inside was not possible due to security reasons as the dump site is manned by street boys who are a threat to strangers. Nevertheless, we were able to determine $\sum_6$PCBs in these three sites at 12.35 ng g^{-1}, 10.88 ng $g,^{-1}$and 11.30 ng g^{-1} for Dandora1, Dandora2, and Dandora3, respectively. Though one expects the level of contamination to increase as one gets deep into the dump site, this was not the case, as Dandora1 located at the entrance of the dump site showed the highest level of contamination. It may therefore be concluded that, the entrance may as well serve as a monitoring point for preliminary studies since getting inside the dump site requires elaborate logistics. Compared to an earlier study in 2008, which indicated PCBs levels ranging from 0.7 to 1.9 ng g^{-1},[3] the level of PCBs in the present study were significantly higher, however, not different from the $\sum_6$PCBs 13.9 ng g^{-1} from samples collected at the entrance of the dump site in 2014.[4] An earlier study on the levels of dioxin like PCBs (dl-PCBs) in free-range chicken eggs around Dandora indicated levels of dl-PCBs at 8.1 pg TEQ g^{-1} of fat, which exceeded the WHO limit of 2 pg TEQ g^{-1} of fat in foodstuffs by four fold.[5] The sum of 7 indicator PCBs ($\sum_7$PCB) was 31.1 ng g^{-1} fat (or 3.58 ng g^{-1} fresh weight). The presence of dl-PCBs was attributed to burning of chlorine containing waste products such as poly (vinyl chloride) (PVC) plastics, which could be true because out of the 2000 tonnes of waste generated in Nairobi, 225 tonnes consists of polythene bags, plastics and PVC items.[6] This conclusion is also supported by a study which identified combustion of PVC as the main contributor to dioxins and coplanar PCBs, ruling out other plastics like polyethylene and polystyrene.[1] Contamination of the dump site with PCB leads to a conclusion that humans and animals that scavenge there are at risk of exposure, and further monitoring should be done to determine the extent of exposure in humans.

Donholm and Outering sampling sites recorded levels of 9.06 and 7.68 ng g^{-1} for $\sum_6$PCBs. The sites were located at places with high population density, meaning that the large quantity of waste generated and subsequent open burning maybe contributing to PCBs in the environment. In addition, these sites are notorious for combustion of car tyres. Kariobangi and enterprise recorded the lowest level of contamination, probably indicating minimal human activities at these sites. The levels of PCBs in the studied waste disposal sites were comparable to lightly contaminated sites, *i.e.*, 0.20-15 ng g^{-1} in soil from a e-waste recycling area in China,[7] 0.43-1.50 ng g^{-1} in soil from a burnt plastic dump site in Guiyu, southern China.[8] However, levels as high as 12 900 ng g^{-1} have been reported in extremely polluted e-waste recycling sites in China.[9]

2.2 Levels of PCBs in Ngong' River

Ngong' River together with Nairobi and Mathare River form part of the larger Nairobi River basin (Fig. 1). It is an important river for monitoring any form of contamination because it drains the industrial area and may serve to indicate any illegal activities taking place. It starts at Ngong' forest, then flows through Kibera slums, industrial area, before finally draining into the Athi River. PCBs levels ranged from 0.19 to 2.39 ng g^{-1} , with PCB 153 giving the lowest concentration and PCB 138 the highest (Table 2), which is expected because it is among the main contributors of PCBs contamination. Donholm recorded the highest $\sum_6$PCBs. The site is located below the dense industrial area and also suffers from massive solid waste disposal which could be contributing to increased levels of pollution. Kibera gave the second highest $\sum_6$PCBs. While the level of contamination is expected to increase as the river flows downstream, an unlikely observation was the lower levels recorded at Mbagathi which is located after Kibera. The possible explanation for this could be the presence of Nairobi Dam between the two sites, where the water maybe undergoing some natural cleansing through sedimentation of particulate matter resulting in reduced levels of PCBs as water flows down the river. The dam also suffers from massive eutrophication from water hyacinth and the vegetation could be accumulating some of the pollutants, thus removing them from the water column. There is evidence that plants and vegetation can bioconcentrate PCBs, as given by 95% recovery of tetra and hexa-CB at a point 5 cm from the point of injection of a tomato plant without any evidence of metabolism.[10] There is also a possibility of aquatic plants adsorbing PCBs on the stems and leaves followed by subsequent movement to the apoplast or symplast.[11]

Motoine which is the source of Ngong' River gave unexpectedly high levels of PCBs. While the site is quite a distance from human settlements, there were numerous activities taking place around the site. Kangundo site is located downstream of the river before it joins the Nairobi River and finally the Athi River. On contrary, this site recorded the lowest levels of PCBs which was attributed to the napier grass slightly above the sampling site and might be involved in the rivers cleansing process by accumulating some of the pollutants.

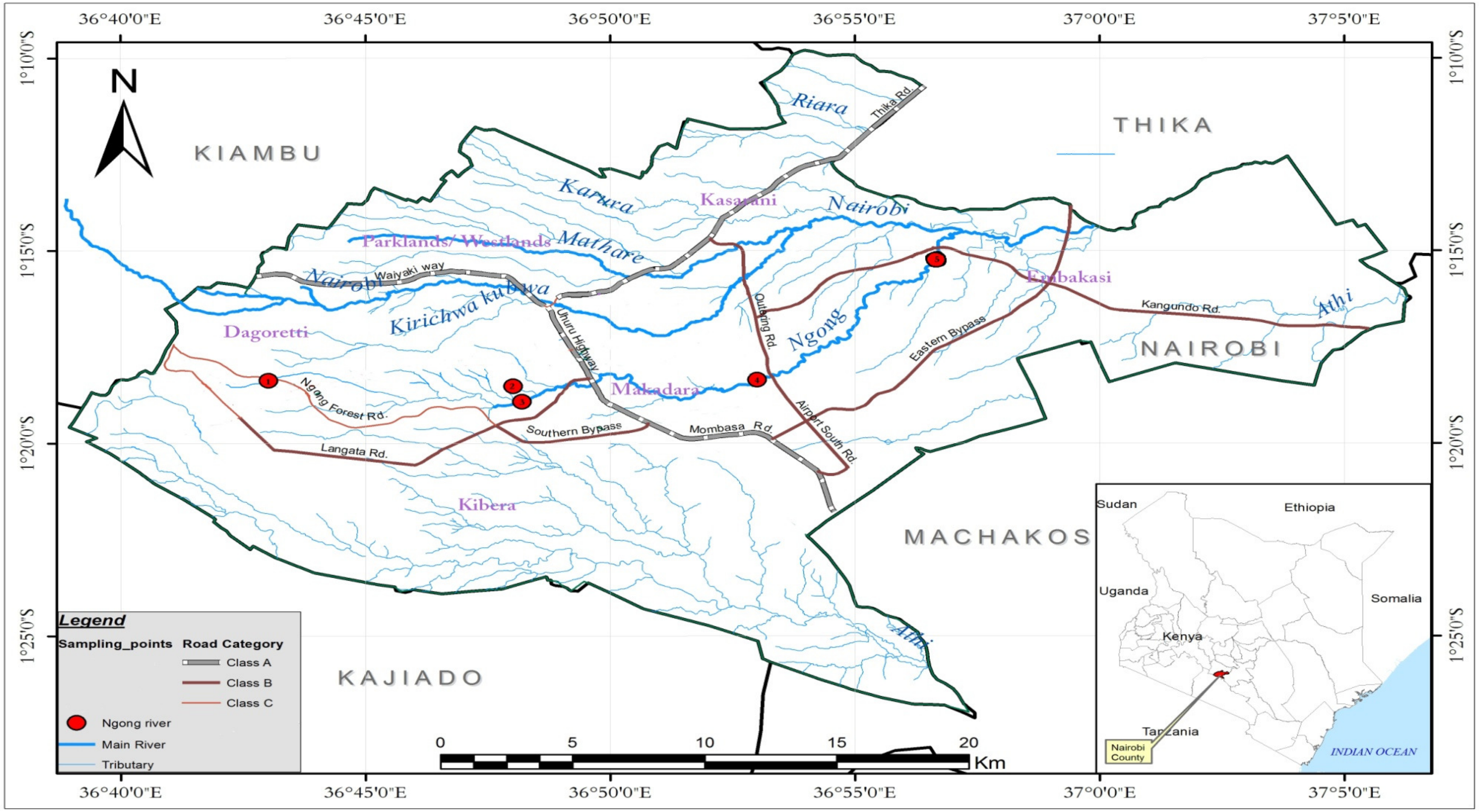

Fig. 1 Map of the study area showing 5 sampling sites along Ngong' River, *i.e.*, Mutoine (1), Kibera (2), Mbagathi (3), Donholm (4), and Kangundo.

Table 1: Mean concentration ± SD of six polychlorinated biphenyls (ng g^{-1} dry weight) in soil in selected solid waste dump sites

	Dandora1	Dandora2	Dandora3	Kariobangi	Enterprise	Outering	Donholm
PCB 28	0.43 ± 0.02	0.56 ± 0.21	0.63 ± 0.01	0.81 ± 0.07	0.36 ± 0.01	0.13 ± 0.002	0.52 ± 0.05
PCB 52	3.78 ± 0.45	6.94 ± 0.01	4.56 ± 0.18	1.48 ± 0.13	0.20 ± 0.005	1.75 ± 0.02	2.05 ± 0.44
PCB 101	0.17 ± 0.11	0.72 ± 0.03	0.37 ± 0.02	nd	nd	3.94 ± 0.55	1.11 ± 0.02
PCB 153	0.34 ± 0.09	0.96 ± 0.19	0.95 ± 0.008	0.11 ± 0.08	nd	nd	1.61 ± 0.03
PCB 138	7.23 ± 0.59	0.99 ± 0.31	3.95 ± 0.98	0.51 ± 0.31	nd	0.83 ± 0.53	2.70 ± 0.18
PCB 180	0.50 ± 0.004	0.71 ± 0.06	0.84 ± 0.006	1.71 ± 0.27	1.46 ± 0.22	1.03 ± 0.10	1.07 ± 0.0009
$\sum_6$PCBs	12.45	10.88	11.30	4.62	2.02	7.68	9.06

Dandora1, the site was located outside the dump site
Dandora2, the site was located 200 m from the entrance of the dump site
Dandora3, the site was located 400 m from the entrance of the dump site

Table 2: Mean concentration ± SD of six polychlorinated biphenyls (ng g^{-1} dry weight) in surface sediments (0-5 cm) from Ngong' River

	Mutoine	Kibera	Mbagathi	Donholm	Kangundo
PCB 28	1.12 ± 0.22	0.54 ± 0.13	0.42 ± 0.08	0.93 ± 0.23	0.24 ± 0.05
PCB 52	1.76 ± 0.66	0.61 ± 0.22	0.79 ± 0.22	1.50 ± 0.25	0.29 ± 0.02
PCB 101	nd	0.53 ± 0.14	nd	nd	nd
PCB 153	0.58 ± 0.17	0.29 ± 0.02	0.44 ± 0.08	1.11 ± 0.24	0.19 ± 0.01
PCB 138	0.76±0.44	2.39 ± 0.18	0.41 ± 0.02	2.28 ± 0.57	0.25 ± 0.05
PCB 180	0.50 ± 0.00	0.21 ± 0.01	0.22 ± 0.04	0.61 ± 0.03	0.14 ± 0.01
$\sum_6$PCBs	4.72	4.57	2.28	6.43	0.65

3. Conclusions

This is the first report on levels of PCBs in different solid waste dump sites in Nairobi, and as evidenced in the study, these sites are sources of PCBs contamination and cannot be ignored anymore, thus the need for their adoption as monitoring sites to protect human and animals from exposure to toxic compounds. The presence of non-dioxin like PCBs is further indication that dioxin like PCBs are present in these soils and should therefore be determined to ascertain the extent of contamination. $\sum_6$PCBs in Ngong' River ranged from 0.65 to 6.43 ng g^{-1}, and were therefore below the sediment quality guideline effect range low (ERL) value of 22.7 ng g^{-1} for $\sum$PCBs, as given by the national oceanic and atmospheric administration (NOAA). This means that there is a rare chance of observing any adverse health effect in benthic invertebrates, however, this cannot be ignored as even low concentrations of PCBs constitute an important route of exposure, and may entail diseases if the exposure is prolonged. PCBs levels in soil were lower than the canadian soil quality guideline for environmental health value of 500 ng g^{-1} and 1300 ng g^{-1} for agricultural and residential areas, respectively.[2]

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Research Article

Synthesis of stationary phases that provide group recognition for polychlorinated biphenyls by porogenic fragment template imprinting

Molecular recognition based on imprinted polymers results from the polymerization of functional monomers and cross-linkers in the presence of a target analyte (i.e. template), with subsequent removal of the template to create synthetic binding sites. However, complete removal of the template is difficult to achieve, thereby leading to template leaching, which adversely affects real-world analytical applications. To overcome this challenge, the present study utilizes porogenic fragment template imprinting techniques to provide an alternative synthetic strategy to generate molecularly imprinted polymers with molecular recognition toward polychlorinated biphenyls. Thereafter, thus-generated imprinted polymers have been applied as stationary phases in molecularly imprinted solid-phase extraction for preconcentrating six “indicator polychlorinated biphenyls” in both organic and aqueous media. Recoveries of up to 98.9% (imprinted polymers) versus 73.0% (conventional C_{18}) in an organic phase, and up to 97.4% (imprinted polymers) versus 89.4% (C_{18}) in an aqueous phase have been achieved corroborating the utility of this advanced sorbent material. Finally, porogenic fragment template imprinting strategies have yielded molecularly imprinted polymers that are useful for the quantitative determination of polychlorinated biphenyls in environmental matrices, which provides a low-cost strategy for tailoring stationary phases that avoid template leaching in applications in solid-phase extraction as well as liquid chromatography.

Keywords: Gas chromatography / Molecularly imprinted polymers / Polychlorinated biphenyls / Solid-phase extraction
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1 Introduction

The molecular imprinting technique provides materials that ideally reveal molecular recognition properties similar to bioreceptors, such as antibodies. Following the pioneering work of Arshady and Mosbach [1] in 1981 on molecular imprinting by noncovalent interactions (i.e. electrostatic forces, charge transfer, van der Waals, and hydrophobic forces), significant scientific attention in this area of research has been witnessed [2]. Molecular imprinting by noncovalent interactions mimics biological processes such as antibody–antigen or enzyme–substrate interactions, thus providing) analogous yet biomimetic molecular recognition schemes [3,4].

Because of their inherent properties including robustness, multiple usage, lifetime, and selectivity, molecularly imprinted polymers (MIPs) have been applied as stationery phases in column chromatography [5], recognition elements in sensors [6], bioassays [7], and adsorbents in SPE, so called molecularly imprinted solid-phase extraction (MISPE) [8–12]. However, despite such successful reports, several drawbacks such as heterogeneity of the binding sites, low average binding affinities, incompatibility with aqueous media, and template leaching have limited a more widespread application as antibody mimics. Template leaching, which results from incomplete removal of the template, is a substantial problem during the application of MIPs. A previous study, for example, showed that between 0.47 and 1.38% of the template remains within the polymer matrix even after extensive

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Abbreviations: **CL**, cross-linker; **EGDMA**, ethylene glycol dimethacrylate; **FM**, functional monomer; **HCB**, hexachlorobenzene; **MIP**, molecularly imprinted polymer; **MISPE**, molecularly imprinted solid-phase extraction; **NIP**, nonimprinted polymer; **PCB**, polychlorinated biphenyl; **PVA**, polyvinyl alcohol; **XIP**, xylene-imprinted polymer; **4-VP**, 4-vinylpyridine; **μECD**, microcell electron capture detector

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extraction [13]. Therefore, particularly for trace analytical applications and quantitative analysis template leaching has to be minimized.

One of the strategies for limiting template leaching is the dummy template approach, which involves the use of structural analogues as the template or, even more advanced, the presynthesis of a dummy template [14–16]. Within the family of dummy templating approaches, fragment imprinting specifically aims at using only a fragment of the target molecule as a pseudotemplate. Using this approach, the synthesis MIPs with recognition properties for homologues of chlorinated bisphenol A has been demonstrated applying 2,6-dimethylphenol and *p-tert*-butylphenol as fragment templates [17]. Chlorinated bisphenol A homologues with chlorine substitutions at the 3rd and 5th positions resulted in the highest capacity factors for MIPs synthesized using 2,6-dimethylphenol. Substitutions at the 2nd and 6th positions at the phenol moiety correspond to the 3rd and 5th positions at a biphenyl ring, whereby the recognized molecule followed the substitution patterns of the template.

Recently, so-called effective fragment potentials have been used aiming at rational design of MIPs with recognition properties for polychlorinated biphenyls (PCBs) [18]. Here, the fragment templates were 1,2,3-trichlorobenzene and 1,2,3,4,5-pentachlorobenzene yielding imprinting factors of 6.57; 3.46 and 5.80; and 1.38, 1.38, and 1.41 for PCB 44, PCB 105, and PCB 174, respectively [18]. Though high imprinting factors were evident in this approach, the amount of template in the optimized imprinting ratios 1:2:10 (template/functional monomer [FM]/cross-linker [CL]), and 15:6:29 were exceptionally high preventing the useful synthesis of large quantities of such MIPs, as required for practical applications and potential commercial scaling of the synthesis.

Another promising strategy, which has to date been less explored is porogenic template imprinting, whereby a porogenic solvent able to form a macroporous structure within a cross-linked polymer network is used as both, the porogen and the template [19]. This technique was first reported by Hosoya et al. [19] who used *p*-xylene, *o*-xylene, and *m*-xylene as porogenic templates to synthesize MIPs with recognition properties for PCBs. Upon application as stationary phases in HPLC, *p*-xylene MIPs were highly selective toward PCB 15, which is chlorinated at the *p*-position while *o*-xylene MIPs were selective toward PCB 14, which is an *o*-chlorinated PCB.

In the present study, we have combined fragment imprinting and porogenic template imprinting into porogenic fragment template imprinting for synthesizing polymers enabling group recognition of PCBs based on hexachlorobenzene (HCB) as the fragment template, and a mixture of xylenes as porogenic template. The choice of xylenes was governed by the fact that they are "aromatic solvents" in addition to being substituted at two positions of the benzene moiety. Therefore, they may act as "dummy templates" for aromatic constituents such as PCBs. The obtained MIPs have successfully been applied as preconcentration matrix in MISPE providing superior results in both aqueous and organic environments compared to conventional C_{18} phases, thus offering advanced preconcentration strategies for analyzing PCBs.

2 Materials and methods

2.1 Chemicals and reagents

PCB 14, 15, and PCB standard mixtures (28, 52, 101, 138, 153, and 180), HCB, polyvinyl alcohol (PVA; Mw 13 000–23 000, 87–89% hydrolyzed), ethylene glycol dimethacrylate (EGDMA, >98%), 2,2′-azobisisobutyronitrile, 4-vinylpyridine (4-VP, 95%), *o*-, *m*-, *p*-xylene, toluene, cyclohexane, and Supelclean C_{18} SPE cartridges (6 mL, 500 mg, 51.7 μm, 490 m^2/g) were purchased from Sigma–Aldrich (Steinheim, Germany). HPLC grade methanol (≥99.9%), pesticide grade *n*-hexane (99%), dichloromethane (99.8%), acetone (99.8%), empty SPE cartridges (6 mL), and frits (20 μm porosity) were purchased from Carl Roth Chemicals (Karlsruhe, Germany). Nitrogen (99.999%) for GC was from MTI IndustrieGase AG (Neu-Ulm, Germany). EGDMA and 4-VP were distilled under reduced pressure before use to remove the inhibitors. Water used in the study was purified using a Milli-Q filter system from Millipore (Billerica, MA, USA).

2.2 Preparation of imprinted polymers by suspension polymerization

Polymers were synthesized using HCB as the fragment template (T), 4-VP as the FM, and EGDMA as the CL. Two ratios 1:16:80 (T/FM/CL) and 1:8:40 with toluene (5 mL) as the porogen were applied. Likewise, porogen-imprinted polymers were synthesized with a mixture of *o*-, *m*-, *p*-xylene (5 mL) as porogenic template, and 4-VP and EGDMA as comonomers. The controls (i.e. nonimprinted polymers [NIPs]) were synthesized using the same procedure, however, for the xylene-imprinted polymers (XIPs) controls cyclohexane was applied as the porogen. Therefore, the organic phase consisting of the polymerization constituents and 2,2′-azobisisobutyronitrile (2% mol of the polymerizable double bond) was dispersed in 50 mL water (containing PVA as the stabilizer) by stirring at 1000 rpm. The mixture was further stirred for 5 min, and the polymerization was initiated by UV irradiation (UV lamp: 50 W, 365 nm) and allowed to proceed at room temperature for 4 h. The resulting microspheres were filtered under vacuum using a borosilicate P4 filter. To remove unreacted monomers and the template, the particles were washed with methanol/acetic acid (90:10, v/v) using an Ulm Extractor (ULEX) [20] under sonication until no significant peaks were observed during GC–microcell electron capture detector (GC–μECD) analysis of a hexane supernatant obtained by incubating the particles with hexane. Thus, obtained neat particles were then sieved under acetone for selecting the desired particle diameters using sieves of different mesh size, and dried in an oven under vacuum at 45°C overnight.

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2.3 Morphology and surface area

Particle shape, size, and surface morphology were investigated using a dual-beam FEI Helios Nanolab 600 focused ion beam SEM system, while specific surface area, pore size, and pore volume were determined by nitrogen adsorption–desorption Brunauer–Emmett–Teller and Barrett–Joyner–Halenda methods, respectively, using a QuadraSorb SI system (Quantachrome Instruments).

2.4 Rebinding experiments

Kinetic experiments were executed by equilibrium batch rebinding assays using XIPs and their nonimprinted counterparts (XIP-NIPs). Thirty milligrams of XIP was weighed into Eppendorf vials, and 1.0 mL of 0.66 μg/mL PCB 15 standard in *n*-hexane was added and vortexed for 3 h. To determine the extent of adsorption with time, the supernatant was filtered and analyzed every 30 min by GC–μECD, as described in Section 2.5, and the adsorbed amount was determined using Eq. (1). After establishing the equilibrium time, rebinding experiments were performed in the concentration range of 1.2–4.4 μg/mL.

$$Q = \frac{(C_0 - C_f)\ V}{m} \tag{1}$$

where Q is the binding capacity in μg/g, C_0 is the initial concentration of the analyte in μg/mL, C_f is the concentration of the analyte in the supernatant in μg/mL, V is volume of the solution in mL, and m is mass of the polymer in grams.

2.5 Quantification of PCBs

A gas chromatograph (Agilent 6890) coupled to a μECD (GC–μECD) and equipped with the Chemstation software package (Version A.08.03, Agilent Technologies, Waldbronn, Germany) for instrument control and data processing was used for the analysis achieving separation on a ZB5-MS capillary column with dimensions 30 m × 0.25 mm id × 0.25 μm film thickness. The column oven temperature was programmed as follows: initial temperature 60°C (hold time 2 min), ramp at 15°C/min to 210°C (hold time 5 min), and final ramp at 10°C/min to 275°C; finally, this temperature was held for 5 min with the detector temperature set at 280°C. Nitrogen (purity ≥ 99.999%) was used as both carrier and make-up gas at a flow rate of 2.0 and 30 mL/min, respectively. Volumes of 1 μL for both the samples and standards were manually injected using the on-column injection mode. Congener peaks were identified by comparing their retention times with those of standards, while quantification was based on external standard calibration.

2.6 Packing of HPLC column and chromatographic studies

A Dionex HPLC system comprising a UVD340S diode array detector, an ASI-100 automated sample injector, a P580 pump, and the Chromeleon 6.80 software package (Dionex, Idstein, Germany) for instrument control and data acquisition was used throughout the studies reported herein. An amount of 1.5 g of the synthesized neat microspheres (size fraction: 6–32 μm) was suspended in methanol, sonicated for 5 min, and then packed into stainless-steel HPLC columns of dimensions 150 × 4.6 mm id using a slurry packer (Alltech 1666, Deerfield, IL, USA) at 4000 psi with acetone as the packing solvent. The column was then connected to the HPLC and equilibrated with methanol until a stable baseline was obtained. Analysis was performed by injecting 20 μL of analyte solution with methanol (100%) as the mobile phase at a flow rate of 1.5 mL/min; detection was performed at 254 nm for PCBs, and 235 nm for HCB. Comparison studies were performed applying conventional RP Phenomenex Luna column C_{18} (5 μm, 150 × 4.6 mm id, Aschaffenburg, Germany).

2.7 Optimization of molecularly imprinted SPE

Five hundred milligrams of microspheres (size fraction: 32–60 μm) was suspended in methanol, sonicated, and slurry-packed into 6 mL polypropylene cartridge with a frit at the top and the bottom (20 μm porosity). The column was then mounted onto an SPE manifold, and then conditioned with 12 mL of methanol followed by 12 mL of *n*-hexane/acetone (3:1, v/v), and finally equilibrated with 6 mL of *n*-hexane. One milliliter of 20 ng/mL PCB standard mixture in *n*-hexane was then introduced to the column at a flow rate of 0.5 mL/min, and the column was dried for 10 min under full vacuum. Elution was performed using 5 mL *n*-hexane/DCM (9:1, v/v), and the eluate blown down to near dryness under a gentle flow of argon. The residue was reconstituted in *n*-hexane into autosampler vials to a volume of 1 mL for GC–μECD analysis.

2.8 Molecularly imprinted SPE of PCBs in deionized water

The columns prepared in Section 2.7 were conditioned with 6 mL of methanol and equilibrated with 6 mL of deionized water (Milli-Q system). Five milliliter of deionized water (containing methanol as the organic modifier) spiked with the PCB mixture at a concentration of 4 ng/mL was then loaded onto the column at a flow rate of 1 mL/min followed by washing with 2 mL of methanol and 100 μL of dichloromethane. To ensure that all the water was removed from the polymers, a vigorous drying procedure was applied for the polymers. The columns were dried under full vacuum for 10 min, and then centrifuged at 2957 × *g* for 5 min followed by another 5 min of drying under full vacuum. Elution of the adsorbed analytes

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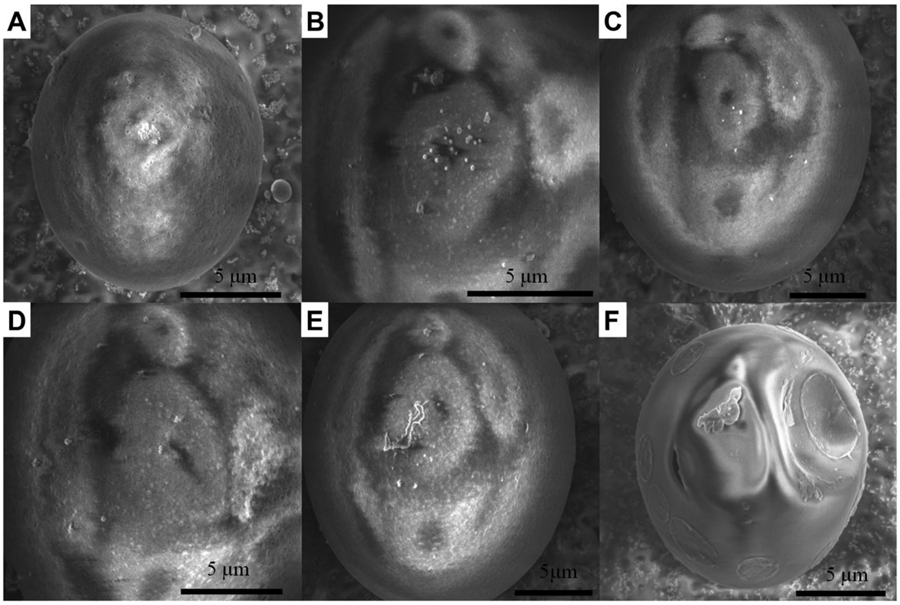

Figure 1. SEM images of (A) MIP1; (B) NIP1; (C) MIP2; (D) NIP2; (E) XIP; and (F) XIP-NIP).

was performed as described in Section 2.7. For comparison, conventional C_{18} cartridges were subjected to the same procedures using 10% methanol in water as the washing solvent, however, without the vigorous drying process.

3 Results and discussion

3.1 Polymer synthesis and characterization

A suspension polymerization protocol was followed synthesizing particles for direct application, thereby avoiding loss from grinding and sieving procedures associated with bulk polymerization strategies. Water mixed with PVA as stabilizer served as the continuous phase, as noncovalent π–π interactions and hydrophobic forces dominate the formation of the prepolymerization complex. HCB was applied as the fragment template of choice due to its similarity with PCBs. For porogenic template imprinting, the selection of xylenes was governed by the fact that they are considered “aromatic solvents,” which are additionally substituted at two positions of the benzene ring, thus surrogating the chlorine substitution at the PCBs phenyl rings. Two different T/FM/CL ratios, 1:16:80 (MIP1) and 1:8:40 (MIP2) for HCB fragment templates, and 16:80 (FM/CL) for XIPs were applied. The obtained particles were porous microspheres (Fig. 1), as confirmed by SEM images and by the rather high specific surface areas (Table 1). XIP gave the highest specific surface area of 329.7 m^2/g with an average pore diameter and pore volume of 1.39 nm and 0.85 cm^3/g, respectively. On the other hand, the XIP-NIPs gave the lowest surface area of 4.03 m^2/g, which was in agreement with the nonporous nature of the materials, as confirmed by SEM images. In all cases, the amount of CL in relation to the total monomer ratio was approx. 80%, which is in accordance with literature suggesting that the ideal amount of CL is in the range of 50–80% [21]. Besides controlling the morphology of the resulting polymers, the CL also governs the mechanical strength and integrity of the obtained binding cavities [22].

3.2 Adsorption kinetics

To understand the mechanisms of adsorption, XIP and the corresponding nonimprinted particles were investigated and the obtained results were fitted with two kinetic models: a pseudo first order Eq. (2), and a pseudo second order kinetic model Eq. (3). Equation (2) assumes that the rate of adsorption is controlled by the number of unoccupied sites, while Eq. (3) considers the adsorption controlled by a chemical process where exchanging or sharing of electrons is involved. A plot of $\log(q_e–q_t)$ versus t gives the values for q_e and k_1 from the intercept and slope, respectively. Likewise, a plot of t/q_t versus t results in a straight line from which k_2 and q_e can be derived.

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t \tag{2}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \tag{3}$$

Here, q_e is the amount adsorbed at equilibrium, q_t is the amount adsorbed at any given time (t, min), k_1 is the rate constant for the pseudo first order (min), and k_2 is the rate constant for the pseudo second order (min^{-1} g μg^{-1}) model.

The obtained kinetic data readily followed the pseudo second order kinetic model with a correlation coefficient (R^2) of 0.9962 and 0.9711 for the XIP and XIP-NIP, respectively, and values of 0.7058 and 0.8411 for the pseudo first order kinetic model. The value of q_e at 10.8 and 2.89 μg/g for both the XIP

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Table 1. Specific surface area of the synthesized polymers, $n = 2$

Polymer	Specific surface area (m^2/g)[a]	Pore surface area (m^2/g)[b]	Pore diameter (nm)[b]	Cumulative pore volume (cc/g)[b]
MIP1	200.8 ± 6.90	275.5 ± 3.67	$1.28 \pm 3.18 \times 10^{-3}$	0.62 ± 0.04
NIP1	200.5 ± 5.46	280.0 ± 8.99	$1.29 \pm 3.54 \times 10^{-4}$	0.64 ± 0.12
MIP2	287.6 ± 30.1	379.6 ± 84.0	$1.39 \pm 2.12 \times 10^{-3}$	0.81 ± 0.15
NIP2	287.3 ± 23.1	374.8 ± 15.4	1.39 ± 0.0	0.75 ± 0.22
XIP	329.7 ± 99.0	423.4 ± 147.1	$1.39 \pm 7.07 \times 10^{-3}$	0.85 ± 0.25
XIP-NIP	4.03 ± 0.13	5.33 ± 0.79	3.07 ± 0.03	0.009 ± 0.001

a) The specific surface area was determined by nitrogen adsorption using QuadraSorb SI from Quantachrome (Odelzhausen, Germany). Samples were degassed at 100°C under vacuum before data collection and the surface area was determined by the Brunauer–Emmett–Teller method.

b) The Barrett–Joyner–Halenda method was used to determine pore surface area, pore diameter, and cumulative pore volume.

Table 2. PCB 15 binding by polymers in hexane[a]

	PCB 15 bound on imprinted polymers (μg/g)	PCB 15 bound on NIPs (μg/g)	IF
MIP1	9.53 ± 0.23	8.14 ± 0.12	1.17
MIP2	8.04 ± 0.45	8.37 ± 0.04	0.96
XIP	8.23 ± 0.06	5.29 ± 1.40	1.56

a) Experiments were executed at 0.32 μg/mL PCB 15 in *n*-hexane with 20 mg of polymer particles. Results are expressed as mean ± SD.

and XIP-NIP, respectively, in the pseudo second order model was close to the experimentally obtained values, thus confirming that the adsorption of PCB 15 is governed by chemisorption involving sharing or exchanging electrons [23]. This is consistent with the expected π–π interaction between analyte and polymer matrix. Previous studies on the adsorption of 4-nitrophenol and perfluorooctane sulfonate on their respective MIPs also indicated that the adsorption followed a pseudo second order kinetic model [24, 25].

3.3 Adsorption isotherms

After establishing the equilibrium time at approx. 90 min, the polymers were incubated for this period with PCB 15 in *n*-hexane. While MIP1 gave an imprinting factor of 1.2, MIP2 did not reveal any pronounced imprinting effect (Table 2). In fact, the only difference between the two polymers was the amount of FM applied during the synthesis, which confirms that an increase in FM results in an additional stabilization of the prepolymerization complex, and consequently, enhanced molecular recognition properties of the resulting MIP. XIP particles bound 8.23 μg/g compared to 5.29 μg/g for the nonimprinted material resulting in an imprinting factor of 1.56, thereby indicating a distinct imprinting effect for the XIP.

To further quantify the associated binding parameters, equilibrium rebinding experiments were executed with PCB 15 at a concentration range of 1.2–4.4 μg/mL. The obtained data were fitted using a Freundlich adsorption isotherm (Eq. (4)), which characterizes the heterogeneous binding site distribution of imprinted polymers [26, 27]. While the XIP particles clearly revealed the expected heterogeneous binding site distribution, as reflected by an *n*-value of 0.86, the XIP-NIP was more homogeneous with an *n*-value of 0.98. The parameter *a*, which is related to the median binding affinity was 13.9 and 8.6 for the XIP and the XIP-NIP, respectively.

$$B = aF^{\mathrm{m}} \tag{4}$$

Here, B is the amount of analyte bound to the polymer, F is the amount of free analyte in solution after equilibrium, a is related to the median binding affinity K_{o} by $K_{\mathrm{o}} = a^{1/\mathrm{m}}$, and m is the heterogeneity index, which varies from 0 to 1 and equals 1 for perfectly homogeneous materials.

3.4 Chromatographic studies

MIP, NIP, XIP, and XIP-NIPs columns were packed and connected to an HPLC system to study the retention factors of PCB 15 and similarly structured constituents (i.e. PCB 14 and HCB). Similarly, the retention of these compounds using a conventional C_{18} column was evaluated, and the retention factor (k') was calculated using Eq. (5).

$$k' = \frac{(t_{\mathrm{r}} - t_0)}{t_0} \tag{5}$$

Here, t_{r} is the retention time of the analyte, and t_0 (a.k.a. dead time) is the time it takes for the nonretained compound to migrate through the column.

Due to so-called "porogen effects," it is usually recommended that the mobile phase in chromatographic experiments be the same as the porogen used during MIP synthesis so as to replicate the microenvironment during the polymerization [28]. A different solvent may lead to difference in swelling behavior, thus affecting the recognition properties

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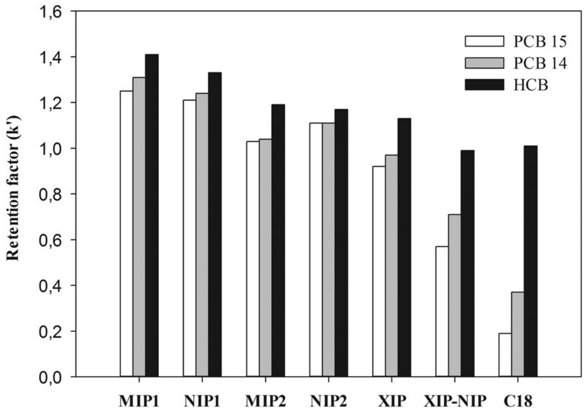

Figure 2. Retention factors of PCB 15, PCB 14, and HCB at the synthesized polymers and at a C_{18} column using methanol (100%) as the mobile phase at a flow rate of 1.5 mL/min; detection was performed at a wavelength of 254 nm for PCBs and 235 nm for the HCB.

of the polymer [29, 30]. However, in the present study reverse phase chromatography using methanol (100%) as our choice mobile phase was performed. MIP1 gave higher retention factors as compared to MIP2 (Fig. 2) with the enhanced performance attributed to the increased FM concentration presumably leading to the formation of additionally stabilized prepolymerization complexes. Due to the rather weak noncovalent interaction forces, an excess of the FM shifts the equilibrium toward the formations of more stable prepolymerization complexes during self-assembly in solution [31, 32].

The obtained results were consistent with past chromatographic studies where an increase in FM resulted in polymers providing improved capacity factors. For example, *t*-BOC-D-phenylalanine-imprinted polymers synthesized using a ratio of 1:2 (T/FM) gave capacity factors of 1.3 compared to 0.8 for 1.1 ratio [33]. Likewise, sulfadimethoxine-imprinted polymers gave capacity factors of 0.26 versus 0.43 for 1:4 and 1:6 imprinting ratios [34].

As the synthesis of XIP particles does not entail distinct template molecules, conventional NIPs prepared in absence of the template may not be synthesized; however, in the present study NIPs were defined as materials synthesized using cyclohexane, which is a nonaromatic analogue. The imprinting factor was given by the ratio of k'_{XIP} and $k'_{\mathrm{XIP\text{-}NIP}}$, where PCB 14 and 15 revealed the highest imprinting factor compared to HCB; meaning that they were retained more than HCB (see Supporting Information Table S1). PCB 14 and 15 gave almost equal value for the imprinting factor, which was attributed to the equal number of chlorine atoms. In addition, the XIP column revealed clearly observable peak tailing effects, which is characteristic of imprinted polymers due to the generation of binding sites with a heterogeneous binding affinity distribution [30]. Hence, from these observations it was evident that xylenes indeed induced a memory effect for recognizing PCBs, and may therefore act as templates for such constituents (see Supporting Information Fig. S1).

Even though C_{18} had the highest specific surface area (490 m^2/g), in comparison the lowest retention factors were obtained. It was also observed that the retention factor did not increase with an increase in specific surface area; thus, it evidenced that the retention of the analytes was predominantly based on molecular recognition mechanisms rather than the specific surface area. The low retention factors by C_{18} suggest the least interaction of the analytes with the adsorbent. While the other stationary phases provide additional interaction forces such as π–π stacking, the only interaction at C_{18} is provided by hydrophobic forces.

3.5 Optimization of molecularly imprinted SPE in organic and aqueous media

To investigate the suitability of the developed materials in real-world application scenarios, MIP1, NIP1, and XIP were used as preconcentration sorbents for group recognition of six nondioxin-like PCBs frequently referred to as "indicator PCBs" (i.e. IUPAC Nos. 28, 52, 101, 138, 153, and 180; Fig. 3). These PCBs have been globally proposed for environmental monitoring, whereby their presence in the environment indicates potential PCB contamination. These particular PCBs were selected for representing major components in the technical mixtures manufactured between 1930 and 1970. Because of their substantial chlorination (3–7 chlorine atoms), these indicator PCBs are considered representative for an entire suite of 209 PCBs congeners [35, 36].

n-Hexane was the solvent of choice for studying PCBs in organic media due to its nonpolar nature, and the fact that it is the most commonly applied extraction solvent for the analysis of chlorinated compounds in the solid matrices. MIP1 gave slightly higher recoveries (93.0–97.1%) than the corresponding NIP1 (90.6–95.6%; see Supporting Information Table S2). Comparable to the chromatography results with PCB 14 and 15 providing the highest imprinting factor on XIP, PCB 138, 153, and 180, which have two chlorine atoms at the *para* positions and at least two chlorine atoms at the *meta* positions gave the highest recoveries on the XIP cartridge, thereby confirming the dominance of *para and meta*-xylenes during the imprinting process. C_{18} resulted in reduced recoveries as approx. 30% of the analytes were already lost during the loading step. In general, all developed polymer cartridges outperformed C_{18}, which could be attributed to the enhanced molecular recognition properties associated with π–π stacking interactions, as compared to only hydrophobic forces for C_{18}.

Deionized water was used to model studies in aqueous media with recoveries between 64.7–97.3% for MIP1, 64.2–97.4% for XIP, and 74.0–89.4% for C_{18}. The lower chlorinated congeners (PCB 28 and 52) showed the lowest recoveries in almost all the cases. Since the lower chlorinated compounds are more volatile, losses were attributed to the vigorous drying step that was applied. The drying step ensured the absence of water residues within the polymer matrix, as traces of water

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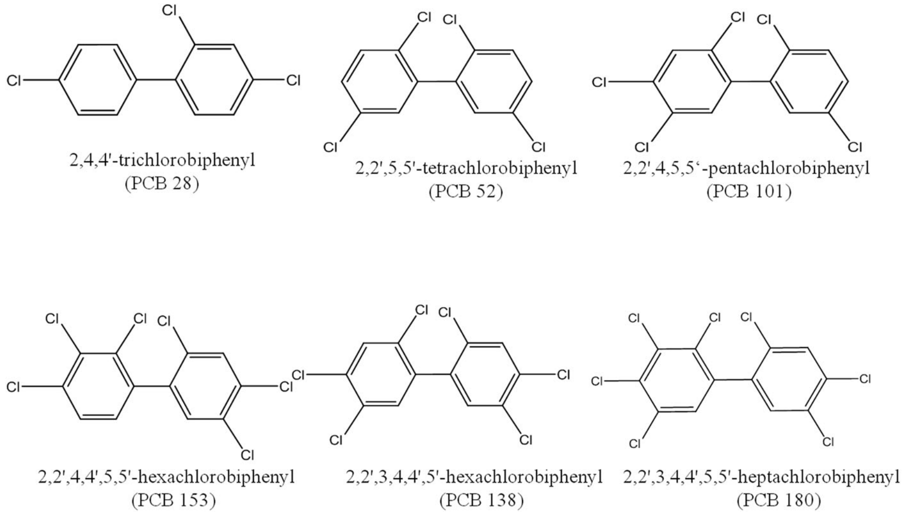

Figure 3. Structures of the six indicator PCBs studied herein.

reduce the total recovery due to the limited number of pores accessible to the eluting solvent. In addition, the presence of water in the eluate demands an additional drying step using Na_2SO_4 leading to further losses. The porogen-imprinted polymer performed exemplarily well, thereby offering a suitable alternative sorbent to C_{18}.

To determine that a reduced amount of the adsorbent would be applicable for the preconcentration of PCBs, a cartridge was packed with only 60 mg of particles using the same protocol. The cartridge gave almost similar recoveries in organic phase, while revealing a tiny decrease in recoveries in aqueous phase, which therefore confirms that indeed porogen-imprinted polymers are superior to conventional C_{18} phases. In addition, the cartridge was reusable, as demonstrated by the recoveries >80% after the fourth usage cycle, while C_{18} indicated a significant decrease ($P < 0.05$) in recovery upon reuse (see Supporting Information Fig. S2).

4 Concluding remarks

As environmental contaminants, PCBs occur at trace to ultratrace concentration levels, which renders template bleeding from MIPs used as sorbent material in SPE or chromatographic stationary phase materials a serious problem in real-life analytical applications. Therefore, porogenic fragment template imprinting was applied as an alternative synthesis strategy for MIPs selective for PCBs, which avoids using the actual target molecule(s) as template. It was shown that xylenes and toluene are promising solvents/fragment templates for the synthesis of polymers selective to PCBs. HPLC studies using thus prepared MIPs as stationary phase retained these chlorinated compounds superior to conventional C_{18} sorbents; likewise, in MISPE applications recoveries > 60% in both organic and aqueous media were achieved, thereby confirming the potential of such materials for selectively preconcentrating PCBs in environmental trace analysis.

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The authors have declared no conflict of interest.

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Supporting Information

Synthesis of stationary phases that provide group recognition for polychlorinated biphenyls by porogenic fragment template imprinting

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Table S1. Retention factors and imprinting factors for PCB 14, 15, HCB on the imprinted columns

	Retention factors (k')		
	PCB 14	PCB 15	HCB
XIP	0.97	0.92	1.13
XIP-NIP	0.71	0.57	0.99
		Imprinting factors	
XIP	1.37	1.61	1.14

Table S2. Recoveries of six indicator PCBs (% ± SD) on MIP, XIP, and C_{18} cartridges in organic and aqueous media.

	Recovery (%)					
	PCB 28	**PCB 52**	**PCB 101**	**PCB 153**	**PCB 138**	**PCB 180**
			organic media			
MIP1	95.8 ± 2.40	95.0 ± 0.71	93.0 ± 8.98	94.9 ± 6.58	97.1 ± 3.54	96.8 ± 1.91
NIP1	90.7 ± 1.98	92.1 ± 4.24	95.6 ± 7.50	95.4 ± 4.95	94.3 ± 6.65	90.6 ± 12.6
XIP	93.6 ± 7.35	95.0 ± 4.03	94.3 ± 5.52	95.4 ± 5.0	98.9 ± 1.70	95.9 ± 7.57
XIP (60 mg)	95.6 ± 0.78	93.4 ± 4.81	97.8 ± 4.17	93.8 ± 1.98	91.1 ± 5.09	91.9 ± 2.05
C_{18}	68.1 ± 6.22	73.0 ± 8.41	71.1 ± 1.80	68.7 ± 0.71	69.2 ± 3.18	69.1 ± 3.39
			aqueous media			
MIP1	64.7 ± 3.18	71.9 ± 2.19	94.9 ± 5.44	90.1 ± 0.85	97.3 ± 0.57	91.5 ± 0.14
XIP	64.2 ± 2.69	72.6 ± 2.12	93.6 ± 1.41	97.4 ± 1.62	96.5 ± 0.78	93.2 ± 3.04
XIP (60 mg)	70.3 ± 1.13	81.8 ± 1.95	78.6 ± 7.96	72.7 ± 1.41	73.4 ± 4.18	74.2 ± 2.44
C_{18}	74.0 ± 10.5	80.5 ± 5.45	89.4 ± 7.75	80.1 ± 0.70	81.0 ± 6.31	81.4 ± 0.15

SD = standard deviation

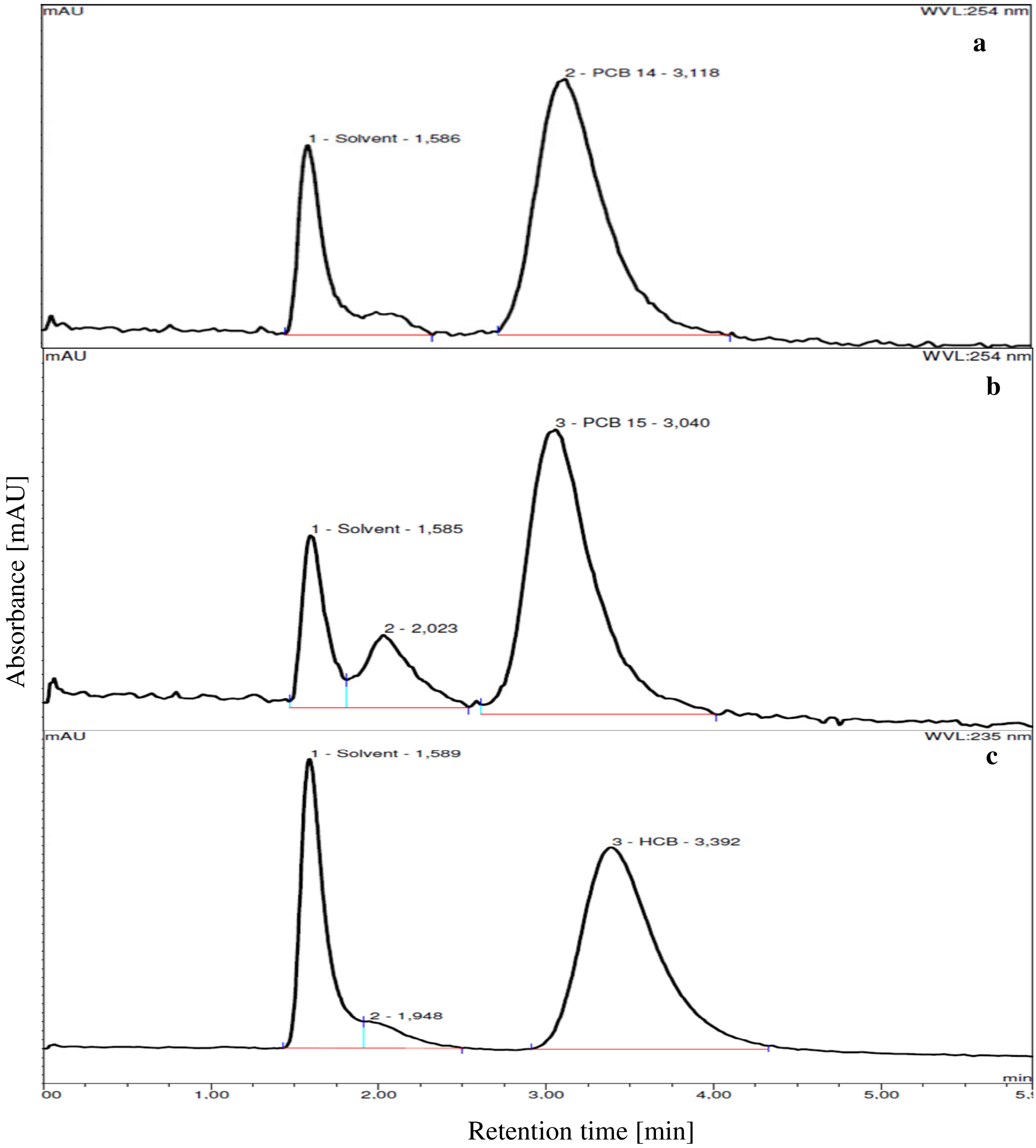

Figure S1: Chromatograms of a) PCB 14, b) 15, and c) HCB on XIP column showing a tailing effect which is associated with the heterogeneous binding site characteristics of imprinted polymers. Methanol (100%) was the mobile phase at a flow-rate of 1.5 mL min^{-1} with the detector wavelength at 254 nm for PCBs and 235 nm for the HCB.

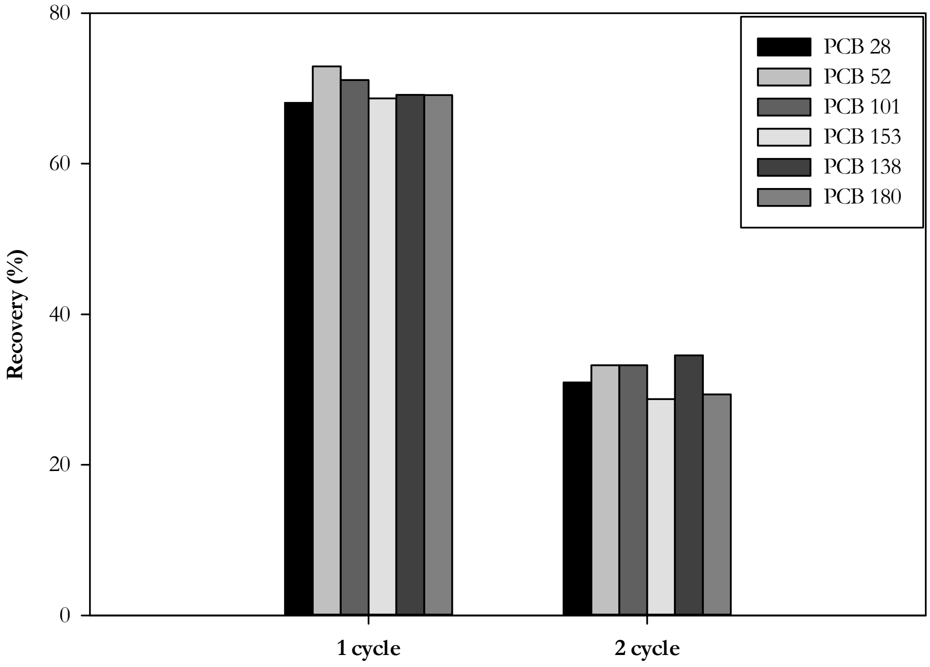

Figure S2 (a): Recoveries of PCBs on C_{18} after different cycles of regeneration and re-use.

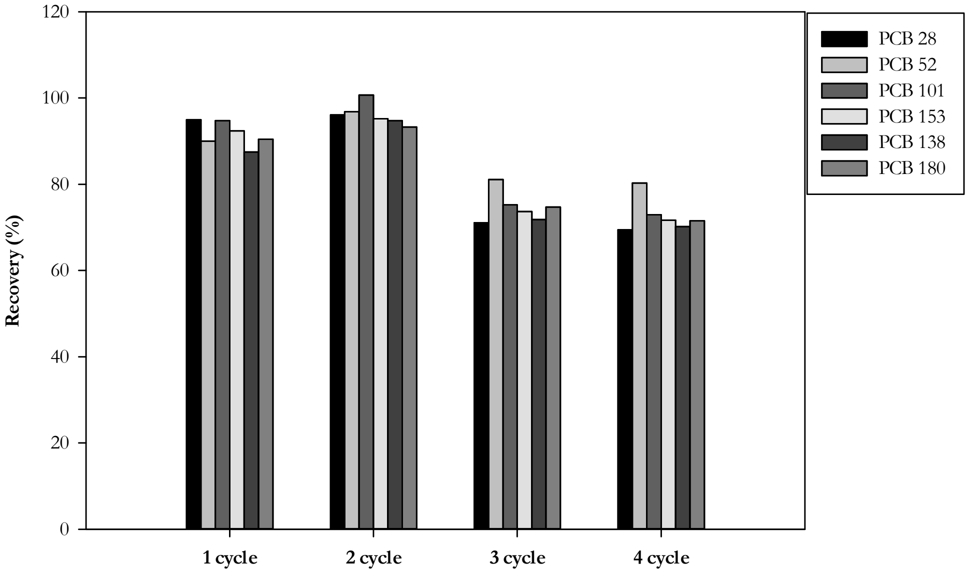

Fig S2 (b): Recoveries of PCBs on XIP after different cycles of regeneration and re-use.

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Multi-walled carbon nanotubes: innovative sorbents for pre-concentration of polychlorinated biphenyls in aqueous environments

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Carbon nanotubes (CNTs) have demonstrated outstanding chemical and mechanical stability, electrical properties, and strong interactions with aromatic compounds owing to the π-electron system on the graphene sheets. Taking advantage of these unique properties, we have developed a fully validated sample pre-concentration technique for determination of polychlorinated biphenyls (PCBs) in aqueous environments using gas chromatography combined with a micro-cell electron capture detector (GC-μECD). The optimized method using pristine MWCNTs gave recoveries in the range of 46.0–92.5%, 51.4–91.5%, 48.7–77.8% for tap water, river water, and lake water, respectively. Compared to conventional C18 adsorbent and oxidized MWCNTs (oMWCNTs), pristine MWCNTs provided the best recoveries, thereby confirming that MWCNTs are excellent alternatives for C18, with the ability to achieve high performance. The developed protocol achieved method detection limits in the range of 0.002–0.011 μg L^{-1} and relative standard deviation (RSD) < 15.5%.

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1. Introduction

Carbon nanotubes (CNTs) are materials of increasing interest since their discovery by Iijima in 1991.[1] The two forms of CNTs are the single-walled carbon nanotubes (SWCNTs) and the multi-walled carbon nanotubes (MWCNTs), which are formed from either a single roll or several cylindrical shells of graphene sheet, respectively. CNTs are characterized by their extraordinary chemical and mechanical stability, unique electrical properties,[2–4] and strong interactions with aromatic constituents due to the delocalized π electron system.[5]

However, especially in aqueous environments their application has been limited due to their strong van der Waals forces leading to agglomeration. Past studies have indicated that surface modification *via* covalent and non-covalent interactions improves the solubility of CNTs in organic and aqueous media.[2,3] The non-covalent modification is a physical process, which entails wrapping of polymers around the surface of the CNTs.[6,7] In contrast, covalent modification usually involves the introduction of hydroxyl groups or carboxyl groups at the surface of the CNTs, which then provides a chemical architecture for further functionalization of the surface.[2]

Surface-modified CNTs have therefore been used in biosensors,[8] electrochemical sensors,[9] diagnostics imaging,[10,11] drug-delivery systems,[12] nanocomposites,[13] and in general separation sciences.[14] Furthermore, CNTs have been proposed as new generation of adsorbents in solid-phase extraction (SPE), facilitating the analysis of environmental pollutants.[15] Particularly, CNTs have been applied as adsorbents in SPE for quantification of polycyclic aromatic compounds,[16] phthalate esters,[17] polyhalogenated compounds,[18] and tetracyclines.[19] Since studies on the adsorption of dioxins at CNTs indicated that they are excellent materials for the removal of dioxins,[20] it is anticipated that CNTs should be suitable materials for pre-concentrating polychlorinated biphenyls (PCBs) from aqueous samples.

PCBs are compounds that were majorly used as dielectric fluids in capacitors and transformers during their commercial production between the 1930s and the 1970s, resulting to subsequent releases into the environment. Hence, they have been detected in soil,[21,22] sediments,[23–25] water,[26] air,[27] and biota;[28] even in areas without any commercial production, PCBs are found to date. Consequently, their wide environmental distribution is directly attributed to their stability, their long-range transport, and their persistence ranking them among the most prevalent contaminants in environmental matrices.[29] Therefore, monitoring of these compounds is of substantial relevance for tracking either contemporary releases or for developing appropriate strategies towards their complete remediation and elimination from the environment, as is the long term goal of the Stockholm convention on persistent organic pollutants (POPs).

However, their determination remains challenged due to their occurrence at trace-to-ultra-trace levels, and in complex matrices, thus the need for a pre-treatment step to facilitate detection at such low concentrations. Conventional sample preparation steps usually include liquid–liquid extraction (LLE)

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followed by a clean-up procedure or solid phase extraction (SPE) based on C18 sorbents. While LLE is demanding in terms of the volume of solvent required and the extraction time using, *e.g.*, separatory funnels, C18-SPE is limited by the non-selective enrichment of various constituents leading to co-elution, which then may interfere with the quantitative determination.

MWCNTs provide an attractive option based on their chemical properties serving as adsorbents for aromatic constituents. However, to date they are still not considered analytically or commercially viable alternatives to conventional C18 sorbents, and only few studies have reported on the pre-concentration of PCBs, *i.e.*, enriching PCBs from aqueous samples using magnetic MWCNTs grafted with a hydrophilic layer,[30] and clean-up of fish extracts using magnetic molecularly imprinted polymers comprising MWCNTs as support material.[31]

Though modifications are necessary especially when dealing with complex matrices, the long procedures of synthesis and grafting are not only time consuming but the need for extra reagents makes it costly for continuous monitoring as is required for PCBs. Therefore, we report herein an affordable and fully validated MWCNTs-SPE protocol for determination of 6 indicator PCBs (*i.e.*, IUPAC no. 28, 52, 101, 153, 138, 180) in aqueous environments using pristine MWCNTs. We also prove that pristine MWCNTs are excellent pre-concentration materials compared to their oxidized forms and conventional C18, thereby resulting in a ready-to-use SPE adsorbent.

2. Materials and methods

2.1 Chemicals

SPE cartridges (6 mL) and frits (20 μm porosity), pesticides grade methanol, *n*-hexane, and dichloromethane (DCM) were purchased from Carl Roth Chemicals (Karlsruhe, Germany). The two types of MWCNTs used were: MWCNTs with outer diameter 110–170 nm, length 5–9 μm, and purity > 90% carbon basis, and MWCNTs with outer diameter 10 nm, length 3–6 μm, and purity ≥ 98% carbon basis synthesized *via* catalytic chemical vapour decomposition (Sigma-Aldrich, Steinheim, Germany). PCB standard mixtures (no. 28, 52, 101, 138, 153, 180), PCB 15 & 209, and Supelclean™ LC-18 SPE cartridges (6 mL, 500 g, 51.7 μm, 490 $m^2\ g^{-1}$) were purchased from Sigma-Aldrich (Steinheim, Germany). MWCNTs were dried at 120 °C for 2 h before use. Nitric acid (65%) and sulphuric acid (95–97%) were bought from Merck KGaA (Darmstadt, Germany). Water used in this study was purified using a Milli-Q filter system from Millipore (Billerica, USA). Nitrogen (99.999%) for gas chromatography was supplied by MTI IndustrieGase AG (Neu-Ulm, Germany).

2.2 Instrumentation

Gas chromatography was performed using an Agilent 6890 (Agilent Technologies) system coupled to a micro-cell electron capture detector (GC-μECD) for all the analysis discussed herein. The column used for the separation of PCBs was a ZB5-MS capillary column of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness with a 1 m silica-coated deactivated guard column (0.32 mm i.d.), which was connected to the analytical column *via* a glass capillary connector. The two temperature programs applied were: (i) initial temperature of 60 °C (hold time 2 min), ramp at 20 °C min^{-1} to 260 °C (hold time 5 min) for PCB 15, and (ii) initial temperature of 60 °C (hold time 2 min), ramp at 15 °C min^{-1} to 210 °C (hold time 2 min), and final ramp at 15 °C min^{-1} to 275 °C (hold time 5 min) for the PCB mixtures. The detector temperature was set at 280 °C.

1 μL of standards and samples was manually injected using the on-column injection mode. Nitrogen (purity > 99.999%) was used as both carrier gas at a flow rate of 2 mL min^{-1}, and as the detector make-up at 30 mL min^{-1}. Quantification was based on internal standard calibration using PCB 209, while the identification was performed by comparing the retention times with those of standards. Data was processed using the ChemStation software version A.03.08 supplied by Agilent Technologies.

2.3 Oxidation of MWCNTs

An amount of 0.5 g of pristine MWCNTs (10 nm) were placed in 100 mL flask, and 100 mL of H_2SO_4/HNO_3 (3 : 1; v/v) was added. The mixture was sonicated at 40 °C for 4 h (60 W, 35 kHz) and the resultant oxidized MWCNTs (oMWCNTs) were diluted with 500 mL water, filtered under vacuum, and washed with ultra-pure water until neutral pH; thereafter, they were air dried overnight.

2.4 Characterization of pristine MWCNTs and oMWCNTs

The surface morphology was investigated using a DualBeam Helios Nanolab 600 focused ion beam (FIB) – scanning electron microscopy (SEM) system (Hillsboro, OR, USA). Nitrogen adsorption–desorption experiments were performed using QuadraSorb Station SI from Quantachrome GmbH and Co. KG (Odelzhausen, Germany). Samples were degassed at 100 °C under vacuum for 3 h before data collection, and the specific surface area was calculated by the Brunauer–Emmett–Teller (BET) method. Studies on the oxygen-containing functional groups at the surface of MWCNTs were done by X-ray photoelectron spectroscopy (XPS).

2.5 Validation of MWCNTs-SPE

One hundred milligrams of the MWCNTs was weighed into a 6 mL empty polypropylene cartridge with a frit (20 μm porosity) at the bottom. Aliquots of methanol were passed through the column to ensure tight packing of the adsorbent. Thereafter, a second layer of frit was added on top of the packed adsorbent. The packed column was then mounted onto the Visiprep™ SPE vacuum manifold, and conditioned with 6 mL of methanol followed by equilibration with 6 mL Milli-Q water. During these steps, the column was not allowed to dry. Spiked water samples were loaded onto the column followed by washing with 2 mL of methanol. Prior to elution, the column was dried for 15 min by drawing air through the device under full vacuum. The analytes were desorbed using 10 mL *n*-hexane : DCM (1 : 1; v/v) into round bottomed flasks, and reduced to 0.5 mL using a rotary evaporator immersed in a water bath set at 30 °C. The eluate was

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then transferred into a glass vial, where the volume was further reduced to near dryness under a gentle flow of argon, and reconstituted in hexane into GC autosampler vials. 4 μL of PCB 209 (8.8 μg mL^{-1}) was added before GC-μECD analysis. Conventional C18 SPE columns were subject to the same processing steps (*i.e.*, conditioning and equilibration) as the MWCNT-SPE cartridges, however, were washed with 10% methanol in water.

2.6 Collection of water samples and analysis

Tap water was collected from the laboratory (Institute of Analytical and Bioanalytical Chemistry, University of Ulm, Germany). River water was obtained from the Danube river (Ulm), and lake water was collected from the Ludwigfelder See (Neu-Ulm). The water samples were collected into glass bottles, then filtered through 0.45 μm pore filters, and stored at 4 °C until analysis following the developed method.

2.7 Adsorption capacity

Twenty milligrams of MWCNTs were packed into Eppendorf tubes, and 1 mL of 0.4 μg mL^{-1} PCB 15 in hexane was added and equilibrated for 4 h. The extent of adsorption was followed by analyzing the supernatant every 30 min. The tubes were centrifuged at 3000 rpm for 5 min, and the amount of PCB in the supernatant was determined by GC-μECD analysis. After establishing the time required to reach equilibrium, 20 mg MWCNTs were incubated with 1 mL of PCB 15 at concentrations ranging from 0.4–4.8 μg mL^{-1}, and vortexed for 60 min. The amount of bound analyte was calculated using eqn (1) and binding characteristics determined by applying the binding data to Langmuir (2) and Freundlich (3) equations.

$$Q = \frac{(C_o - C_f)V}{m} \tag{1}$$

where Q is the binding capacity in μg g^{-1}, C_o is the initial concentration of the analyte in μg mL^{-1}, C_f is the concentration of the analyte in the supernatant in μg mL^{-1}, V is the volume of the solution in mL, and m is the mass of the MWCNTs in grams.

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{2}$$

q_e is the bound analyte at equilibrium (μg g^{-1}), C_e is the equilibrium concentration in μg mL^{-1}, q_m represents the monolayer coverage or maximum adsorption capacity (μg g^{-1}), and K_L is a constant related to the adsorption energy (mL μg^{-1}).

$$q_e = K_f C_e{}^n \tag{3}$$

K_f and n are Freundlich constants representing the adsorption capacity and heterogeneity index, and q_e and C_e are the concentrations of the bound and free analyte, respectively.

2.8 Quality control

The quality control measures included analysis of blank samples, rinsing of glassware before use, and the use of internal standards. The method performance was tested by determining accuracy, precision, linearity, and method detection limit (MDL). Standards were prepared from 10 μg mL^{-1} of PCB mixture stock standards by dilution with *n*-hexane. Quantification was based on the internal standard method with PCB 209 added to all samples and standards. The MDL was determined following the EPA method using blank samples (Milli-Q water) spiked at low concentrations (4-times the concentration providing a signal-noise-ratio of 3), and then taken through the validated analytical procedure.[32]

3. Results and discussion

3.1 Characterization of pristine MWCNTs and oMWCNTs

The SEM images revealed entangled fibres for both pristine MWCNTs and oMWCNTs. While oxidation is thought to affect both the structure and the physical properties of the MWCNTs, according to the SEM images the structure remained essentially intact, as no observable change in surface morphology were evident (Fig. 1a and b). MWCNTs (110–170 nm) revealed very short fibres and large diameters, as anticipated (Fig. 1c). Atomic force microscopy (AFM) studies have shown that there is an insignificant decrease in length following the oxidation process.[33] Raman spectroscopy of the oxidized MWCNTs compared to pristine MWCNTs has indicated that the graphene structure remains intact despite the curtailing of the fibre lengths.[34,35] However, the oxidation process induces defects (*i.e.*, amorphous carbon) with the degree of deformation at the graphene sheets characterized by the I_G/I_D ratio, which is the ratio of the G-band (*i.e.*, sp^2 hybridized carbon), and D-band (*i.e.*, sp^3 hybridized carbon) intensities.[5,36]

The increased dispersion of MWCNTs in water proved the successful oxidation of MWCNTs. Introduction of oxygen containing groups was also confirmed using XPS where increased intensity of oxygen (O1s) peak for the oMWCNTs (Fig. 2b), compared to the pristine MWCNTs (Fig. 2a) was observed. The total oxygen content is shown in Table 1. Specific surface areas determined *via* BET were 269.7, 244.8, and 17.4 m^2 g^{-1} for the pristine MWCNTs (10 nm), oMWCNTs, and pristine MWCNTs (110–170 nm), respectively. Therefore, no pronounced decrease in surface area was observed due to the oxidation process; likewise, recent studies confirm minute changes or no evident change in specific surface area after oxidation.[37–39]

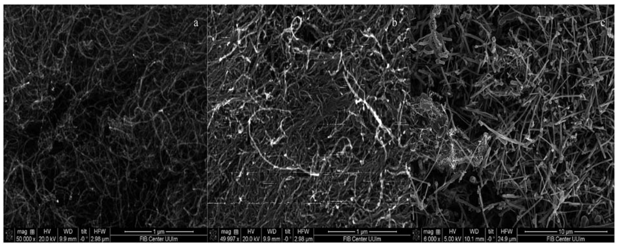

Fig. 1 SEM image of (a) pristine MWCNTs (10 nm), (b) oMWCNTs, and (c) pristine MWCNTs (110–170 nm).

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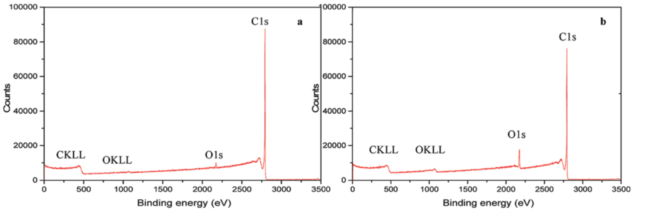

Fig. 2 XPS spectra showing change in C1s and O1s for (a) pristine MWCNTs, (b) oMWCNTs.

Table 1 Surface concentration (%) of carbon and oxygen at pristine MWCNTs and oMWCNTs

Peak	Pristine MWCNTs	oMWCNTs
C1s	98.0	91.69
O1s	2.0	8.31

3.2 Validation of MWCNTs-SPE

3.2.1 Determination of the elution solvent and volume. Non-polar *n*-hexane and polar dichloromethane – as a mixture and as pure solvents – were tested for the elution of the PCBs after enrichment. Pure *n*-hexane was not able to desorb all constituents and gave recoveries in the range of 22.1–89.1%. While pure DCM revealed adequate recoveries (60.2–100.3%), a mixture of *n*-hexane : DCM (1 : 1) gave the best results (>70%) for all the PCBs congeners, and was therefore adopted for all further analyses using 10 mL of volume. CNTs have been reported to strongly interact with aromatic compounds;[20] hence, the need for a strong elution solvent, which can efficiently and effectively disrupt the interactions with the CNT surface.

3.2.2 Effect of dimension on PCBs recovery. MWCNTs that are commercially available are usually of different dimensions in terms of outside diameter (o.d.), internal diameter (i.d.), and length. Therefore, the dimension of MWCNTs should to some extent affect the retention and recovery properties for the analytes of interest. El-Sheikh *et al.*,[40] have studied the effect of dimensions on the recoveries of three pesticides at oxidized MWCNTs. Using 10–20 nm, 10–30 nm, 20–40 nm, 40–60 nm, 60–100 nm, and 40–60 nm CNTs, they found that 40–60 nm provided superior recoveries. Decreased adsorption affinity for 1,2,4,5-tetrachlorobenzene[41] and dimethyl phthalate (DMP)[39] with increase in MWCNTs outside diameter (o.d.) has been reported before. In the present study, MWCNTs of two different dimensions were applied for pre-concentration of PCBs in Milli-Q water. MWCNTs with o.d. 110–170 nm showed decrease in recovery with increasing loading volume, while 10 nm ones maintained recoveries >80% for all loading volumes. These observations suggest that the dimension of CNTs is indeed important for adsorbents used in SPE; MWCNTs of 10 nm o.d. were applied for all further analyses herein.

3.2.3 Flow rate and breakthrough volume. The sample flow rate during the loading step is a crucial factor that affects the performance of SPE cartridges, as it influences the interaction time of the analytes with the adsorbent. While high flow rates are preferred, in cases where the loading volume is high they usually do not offer sufficient interaction time. On the other hand, low flow rates maximize the interaction time, yet, suffer drawbacks due to extended loading times. A compromise is therefore required to enable sufficient interaction while maintaining reasonable loading times. Flow rates of 1, 5, 8, and 10 mL min^{-1} were tested using 20 mL of spiked Milli-Q water. The recoveries were >80% for all flow rates and all PCB analytes except for PCB 28 (Fig. 3a). After optimization, a flow rate of 5 mL min^{-1} was applied for consecutive experiments. To determine the breakthrough volume, different volumes of water from 5–200 mL were loaded onto the columns following the validated method. Although a slight decrease in recoveries with increasing volume was observed, recoveries >70% were realized for all congeners in all volumes except PCB 28 (Fig. 3b). The method precision was acceptable with relative standard deviation (RSD) ranging from 2.5 to 10.7%.

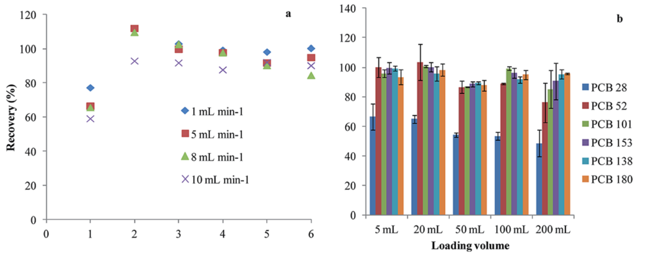

Fig. 3 (a) Recoveries of 6 indicator PCBs at different flow rates with water spiked at 1 μg mL^{-1} (*i.e.*, 1 ppb), where 1, 2, 3, 4, 5, and 6 are PCB 28, 52, 101, 153, 138, and 180, respectively; (b) recoveries achieved for different loading volumes.

3.3 Analysis of real world water samples

The pH of the water samples was determined as 7.69 ± 0.02, 8.42 ± 0.03, and 8.12 ± 0.00 for tap water, river water, and lake water, respectively. Water samples were analyzed before spiking to ascertain the presence or absence of any PCBs; no PCBs were detected in any of the samples. Then, the samples were spiked at a level of 0.2 ng mL^{-1}, and analyzed *via* the validated method. The recoveries ranged from 46.0–92.5% for tap water, 51.4–

Table 2 Recovery (% ± STD[a]) of 6 indicator PCBs after pre-concentration from a variety of real-world water samples

	Tap water		River water	Lake water
Analyte	MWCNTs	oMWCNTs	MWCNTs	MWCNTs
PCB 28	46.0 ± 4.94	42.9 ± 1.40	51.4 ± 4.34	48.7 ± 7.57
PCB 52	90.7 ± 4.23	54.4 ± 4.04	91.5 ± 2.30	77.7 ± 1.84
PCB 101	90.1 ± 3.61	48.9 ± 4.11	87.0 ± 6.06	76.7 ± 3.39
PCB 153	91.3 ± 3.48	46.6 ± 4.52	86.7 ± 6.01	77.8 ± 3.25
PCB 138	92.5 ± 3.46	44.9 ± 4.14	91.1 ± 8.37	74.1 ± 1.77
PCB 180	90.3 ± 5.19	43.4 ± 7.75	87.6 ± 6.92	76.5 ± 5.09

[a] STD is the standard deviation of triplicate analysis.

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Table 3 Adsorption isotherm parameters for pristine MWCNTs using PCB 15 as an exemplary constituent

	Langmuir				Freundlich		
	q_m (μg g^{-1})	K_L (mL μg^{-1})	R_L	R^2	K_f (μg g^{-1})	n	R^2
PCB 15	555.6	0.09	0.97	0.889	54.1	0.711	0.9972

91.5% for river water, and 47.8–77.8% for lake water (Table 2). Five out of six congeners had recoveries within 70–120%, yet again, PCB 28 gave poor recoveries (<70%). PCB 28 has the lowest number of chlorine atoms and is therefore expected to be highly soluble in water in contrast to the other five PCBs studied herein.

The aqueous solubility of PCB 28 is 0.16 mg L^{-1}, compared to 0.03, 0.01, 0.001 for PCB 52, 101, and 153, respectively.[42] It is therefore likely that PCB 28 already suffers from losses during the loading step. The same phenomenon was reported by Dahane *et al.*,[43] where the most soluble constituent methomyl gave recoveries in the range of 43–60%, compared to less soluble pesticides such as diazinon, whose recovery was between 58 and 106%. C18 adsorbent, which is the conventional adsorbent material used for most SPE applications recorded markedly lower recoveries (33.9–67.7%). Poor performance, *i.e.*, recoveries <60.0% for C18 cartridges, have been previously reported.[44–46] C18 is known to retain non-polar compounds *via* hydrophobic forces; yet, MWCNTs are subject to additional π–π interactions, and therefore strongly retain aromatic constituents.[47]

3.4 Effect of surface oxidation on the recovery of PCBs

To determine the effect of surface oxidation of MWCNTs on the recovery of PCBs, spiked Milli-Q water was pre-concentrated using oMWCNTs. Reduced recoveries were evident compared to pristine MWCNTs (Table 2). It should be noted that although the dispersion of functionalized MWCNTs in water increased due to the increase in hydrophilicity, the performance decreased. Similarly, a decrease in recovery of pentachlorophenol,[38] naphthalene,[33] phenanthrene,[48] toluene, ethylbenzene, xylene,[49] and triclosan[37] on oxidized MWCNTs as compared to pristine MWCNTs has been reported. The reason for the decreased performance of oxidized MWCNTs has been linked to the oxygen groups (*i.e.*, carboxyl and hydroxyl groups), which are hypothesized to localize the electrons at the surface of the MWCNTs, thus, removing them from the π-electron system.[50] In addition, the formation of water clusters due to hydrogen bonding between the water molecules and the oxidized MWCNT surface has been argued as a possible reason for the decrease in retention pertaining non-polar molecules.[33] Therefore, the retention of water molecules rather than non-polar constituents appears highly favoured.[33,51] Cho *et al.*,[33] suggested that oxidation could also prevent non-polar molecules from accessing the non-oxidized sites within CNT structures.

3.5 Effect of pH on the recovery of PCBs

Some studies have reported the isoelectric point of oxidized MWCNTs to be around pH 4.[37,39] Taking this into consideration, at lower pH oxidized CNTs are protonated, and no opportunity of hydrogen bond formation with water is provided. At higher pH, CNTs are deprotonated, and therefore, chances of hydrogen bonds with surrounding water molecules increase leading to the formation of water clusters. To determine whether the recovery of PCBs is improved at lower pH, tap water was spiked at a pH of 1 (*i.e.*, pH below the reported isoelectric point for oxidized MWCNTs). The recoveries ranged from 41.4 to 50.3%, which was within the same range of recoveries as the tap water at a pH of 7.69. Therefore, it may be concluded that the introduction of polar groups (*i.e.*, surface oxygen) generally increases the polarity of MWCNTs, which in turn leads to reduced interaction with aromatic constituents, and thus, reduced adsorption capacities.[52] These results are also backed by the earlier argument that oxygen-containing groups localize electrons at the surface of MWCNTs, thereby removing them from the π-electron system, which results in reduced adsorption.

3.6 Adsorption isotherms

The Langmuir adsorption isotherm given by eqn (2), and the Freundlich adsorption isotherm given by eqn (3) were applied for the binding characterization of the pristine MWCNTs (10 nm). Freundlich isotherms assume a heterogeneous surface with adsorption sites of different energies, while Langmuir isotherms are based on a homogeneous surface interaction. MWCNTs indicated a heterogeneous surface ($n = 0.71$) with the adsorption favouring the Freundlich isotherm, as confirmed by a correlation coefficient >0.99 (Table 3). Other studies on the adsorption of 1,2-dichlorobenzene, 1,2,4-trichlorobenzene,

Table 4 Analytical parameters derived for the developed MWCNT-SPE method

PCB congener	Retention time (min)	Linear range (ng mL^{-1})	Regression equation	Linear response (r^2)	Method detection limit MDL (μg L^{-1})
PCB 28	12.9	0.465–186	$y = 0.015x + 0.0217$	0.9997	0.011
PCB 52	13.6	0.465–186	$y = 0.0073x + 0.014$	0.9998	0.008
PCB 101	15.5	0.26–104	$y = 0.0102x + 0.0159$	0.9996	0.004
PCB 153	17.2	0.21–85	$y = 0.0153x + 0.0176$	0.9997	0.002
PCB 138	17.8	0.23–92	$y = 0.0254x + 0.0242$	0.9987	0.004
PCB 180	19.3	0.22–89	$y = 0.0279x + 0.0174$	0.9998	0.002

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1,2,4,5-tetrachlorobenzene, and trihalomethanes on CNTs have reported *n*-values of 0.52–0.65, 0.64, 0.75, and 0.58–0.75, respectively.[41,53,54]

3.7 Analytical performance of the proposed method

The obtained MDL values ranged from 0.002–0.011 μg L^{-1} with PCB 180 and PCB 138 providing the lowest MDL value, and PCB 28 the highest (Table 4). The RSD of replicate analyses ranged from 2.21–14.6%. The obtained calibrations were linear across the investigated concentration range of 0.465–186 ng mL^{-1} for lower chlorinated PCBs, and 0.21–104 ng mL^{-1} for the higher chlorinated ones at coefficients of linearity >0.99 (Table 4).

4. Conclusions

A fully validated MWCNT-SPE protocol for the pre-concentration of PCBs in aqueous environments has been presented. Pristine MWCNTs revealed higher recoveries for the investigated PCBs compared to conventional C18 adsorbents and oMWCNTs, confirming that pristine MWCNTs can be applied for studies of PCBs in aqueous media. In addition, only 100 mg of MWCNTs were necessary compared to 500 mg for conventional sorbent, thereby suggesting not only more reliable, but potentially also more affordable method for SPE. Compared to C18, the cost of MWCNTs – at this small quantity – is already now slightly less, thus offering an excellent alternative to conventional sorbent matrices. The developed method shows detection limits way below the maximum contamination level of 0.5 μg L^{-1} for PCBs in drinking water (as set by US environmental protection agency (EPA)), therefore suggesting that the established analytical method may be readily implemented for monitoring of drinking water quality and safety, which is of substantial societal relevance. However, despite the results achieved herein, the developed protocol may only be applicable for pre-concentrating sufficiently chlorinated constituents, as more soluble (*i.e.*, less chlorinated) constituents may be retained less due to the reduced interaction with MWCNTs.

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CRITICAL REVIEW

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Molecularly imprinted polymers for the analysis and removal of polychlorinated aromatic compounds in the environment: a review

Elizabeth N. Ndunda and Boris Mizaikoff*

Synthetic receptors and in particular molecularly imprinted polymers (MIPs) are gaining relevance as selective sorbent materials and biomimetic recognition elements for analyzing polychlorinated aromatic compounds (PACs) in the environment. PACs are still ubiquitous toxic pollutants requiring their continuous environmental assessment for protecting humans and animals from exposure. Since nowadays most PACs occur at ultra-trace concentration levels and in complex matrices, the selectivity of MIPs renders them ideally suited for facilitating either sample pre-treatment and quantitative enrichment, or acting as biomimetic recognition elements as an integral component of corresponding sensing schemes. Due to the diversity of PACs, imprinting polymers for these constituents appears particularly challenging. This review focuses on prevalent strategies towards successfully templating polymer materials towards polychlorinated biphenyls and their hydroxy forms, chlorophenols, dioxins and furans, and organochlorine pesticides, and successful applications of the polymer materials in monitoring of these compounds at trace-levels in real-world environmental matrices. Discussed are also group-selective sorbents for facilitating simultaneous detection and quantification of PACs.

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1. Introduction

Sample pre-treatment (*i.e.*, clean-up, enrichment, *etc.*) remains among the predominant rate-limiting steps during the determination of pollutants in complex matrices, such as soil,

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sediment, wastewater, biological fluids, and foodstuffs. Selective sorbents, including restricted access materials (RAMs), immunosorbents, oligosorbents, and molecularly imprinted polymers (MIPs) have been suggested as potential alternatives to conventional sorbents (*i.e.*, non-selective sorbents such as C18) when handling such samples.

RAMs selectively separate small molecules from biomolecules *via* hydrophobic interactions and size exclusion effects,[1,2] while the latter sorbents provide selectivity based on actual molecular recognition mechanisms. By immobilizing antibodies and aptamers onto solid support materials for establishing immunosorbents and oligosorbents, respectively, pre-treatment of samples in a variety of matrices is enabled, as summarized in Table 1. However, RAMs indiscriminately retain small molecules, and are therefore mostly suited for applications comprising biofluids in clinical and toxicological analysis settings. While antibodies certainly entail the highest level of selectivity, their practical application is frequently limited by their high cost of production, and their potential instability outside optimized pH, ionic strength, temperature, and pressure conditions. Aptamers are certainly more robust alternative recognition elements, however, maybe equally costly. Hence, molecular imprints appear ideally suited to provide sorbent materials that are tailorable in their recognition properties to a wide range of environmentally relevant pollutants, are comparatively easy to synthesize and produce, and provide sufficient robustness ensuring storage endurance and applicability at harsh conditions. The few challenges that MIPs portray, such as distribution of binding site affinities, non-specific binding, template bleeding, and reduced performance in aqueous medium can be addressed through strategies like covalent or semi-covalent imprinting, dummy templating, polymerization in aqueous medium, among other strategies which are extensively discussed in a review by Chen *et al.*[3]

The ability of recognizing individual molecules or groups of molecules by MIPs relies on the co-polymerization of functional monomers in the presence of a target compound (*i.e.*, template) into a sorbent matrix entailing selective binding sites once the template is removed. Polymerization *via* an excess of cross-linker ideally facilitates the integrity of these binding cavities. The ultimate goal in molecular imprinting remains the synthesis of materials providing comparable binding properties as natural receptors. Although this may not be achieved in every templating scenario, recognition performance comparable to natural analogues has been demonstrated, *e.g.*, by Vlatakis *et al.*,[4] whereby molecularly imprinted sorbent assays (MIAs) achieved results comparable to enzyme-multiplied immunoassay techniques (EMIT). Without claiming completeness, MIPs have also been applied as enzyme mimics,[5] and for smart drug release,[6–8] and in separation science,[9–11] and recognition elements is sensors,[12,13] leading to studies of environmental contaminants, as captured within food safety,[12,14] analysis, removal of personal care products,[15] and MIPs for sample preparation reviews.[16]

The present review specifically looks at the application of MIPs in studies of polychlorinated aromatic compounds (PACs), which are some of the environmental contaminants of global concern. PACs are characterized by an exceptional environmental persistence (*i.e.*, half-lives ranging from 10 days to 22.9 years in soil),[43] long-range transport and bioaccumulation, thus rendering PACs ubiquitous trace environmental contaminants in soil,[44,45] sediment,[34,35,46] water,[47] air,[48] biota,[49] and remote areas such as the north and south pole,[50] *i.e.*, far from their initial release location. The documented evidence of adverse health effects[43] led to ban in production in many countries in 1970s and are currently regulated under the Stockholm convention on persistent organic pollutants (POPs),[50] which also calls for continuous environmental monitoring in order to minimize human and animal exposure.

However, the quantitative determination of PACs remains challenging due to their occurrence at trace-levels, and the frequent complexity of environmental matrices. Hence, prior to their quantification usually extraction, pre-concentration and/or clean-up procedures are required. Traditional liquid–liquid extraction, solid phase extraction (SPE) usually *via* octadecyl silica (*i.e.*, commonly known as C_{18}), and recently solid phase

Table 1 Some of the applications of RAMs, oligosorbents, and immunosorbents in sample pre-treatment

Sorbent	Analyte	Matrix	Application	LOD	Ref.
RAM	Atropine	Human plasma	Online-SPE	10 ng mL^{-1}	17
RAM	Tetracylines	Milk, water	SPE	15–30 ng mL^{-1}	1
RAM	Meloxicam	Plasma	Online SPE	3 ng mL^{-1}	18
RAM	Citalopram and its derivatives	Plasma	Online SPE		19
RAM	EDCs	Honey	SPE	5.3–31 ng g^{-1}	20
RAM-MIP	2-Methoxyestradiol	Plasma	RAM-MISPE	0.02 μg mL^{-1}	21
RAM	Acidic herbicides	Water	Online SPE		22
Oligosorbents	Ochratoxin A	Wheat	SPE		23
Oligosorbents	Ergot Alkaloids	Rye feed	SPE		24
Oligosorbents	Cocaine	Plasma	SPE	0.1 μg mL^{-1}	25 and 26
Oligosorbents	17β-Estradiol	Water	SPE		27
Immunosorbents	PAHs	Sludge, sediment	SPE	1.6–19.3 μg L^{-1}	28
Immunosorbents	Phenylurea herbicides	Plant material	SPE	5–20 ng g^{-1}	29
Immunosorbents	Phenylurea and triazine herbicides	Water	SPE		30
Immunosorbents	Haloanisols	Wine	SPE		31
Immunosorbents	Ractopamine	Meat	Immunoaffinity column		32

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Table 2 Conventional clean-up sorbents for the determination of PACs in various environmental matrices

Matrix	Analyte	Sorbent	Volume used	LOD	Ref.
Sediments and biota	PCBs and OCPs	Acidified silica (8 g)	15 mL *n*-hexane, 10 mL dichloromethane	0.1–1 ng g^{-1} (LOQ)	33
Sediments	PCBs	Florisil (2 g) + sodium sulphate (1.5 g)	8 mL *n*-hexane	0.0005–0.002 ng	34
Sediments	PCBs and OCPs	Acidified silica (8 g) + sodium sulphate (0.5 g)	15 mL *n*-hexane, 10 mL dichloromethane	0.1–0.4 ng g^{-1} (LOQ)	35
Water and sediments	PCBs and OCPs	Acidified silica (8 g) + sodium sulphate	15 mL *n*-hexane, 10 mL dichloromethane	0.03–0.09 ng g^{-1} (LOQ)	36
Sediments	OCPs and PCBs	Florisil (2 g) + sodium sulphate (1 cm)	18 mL petroleum-ether + ether for OCPs and 19 mL *n*-hexane + 1 mL petroleum ether for PCBs		37
Sediments and Fish	PCBs and HCHs	Silica (2 g) + silica gel-KOH (5 g) + silica gel (2 g) + silica gel-sulphuric acid (5 g) + silica gel-sulphuric acid (10 g) + sodium sulphate (5 g)	220 mL *n*-hexane	0.01–0.32 pg g^{-1} for PCBs and 4–20 pg g^{-1} for HCHs	38
Fish	PCBs and OCPs	Bio-Beads S-X3	28 mL cyclohexane-ethyl acetate		39
Beef	2,3,7,8-Tetrachlorodibenzodioxin	BioSil A silica gel (9.5 g) + Amoco PX-21carbon	15 mL dichloromethane + 10 mL benzene + 22 mL toluene + 3 mL *n*-hexane		40
Fish and water	PCDDs and PCDFs	Silica gel-sulphuric acid (10 g)	120 mL *n*-hexane		41
Plasma	OH-PCBs	Florisil (5 g)	60 mL DCM/*n*-hexane/methanol	1–50 pg g^{-1} (LOQ)	42

microextraction (SPME),[51] and star bar sorptive extraction.[52] are the common techniques for pre-concentration of these compounds from aqueous media. From solid matrices (*e.g.*, soil), PACs are usually extracted *via* soxhlet extraction, supercritical fluid extraction, microwave assisted extraction, pressurized liquid extraction, and ultrasonic assisted extraction.[53] As most extraction techniques are not inherently selective, the resulting extract is usually subject to one or more clean-up steps using materials such as silica gel, florisil (magnesia-silica gel), polystyrene-divinylbenzene, alumina, or a combination of these sorbents *via* a multi-layered column (Table 2). However, the non-discriminatory nature of these sorbents, and the rather high consumption of solvents and sorbents during these procedures demands for more efficient protocols and – ideally – more selective pre-concentration/clean-up sorbents enabling cost –efficient routine analysis and monitoring of these pollutants at low concentrations, as required by the international regulatory bodies, such as EU, WHO and EPA.[54,55]

Nowadays, MIPs have not only established themselves as suitable selective sorbents in environmental analysis, but have also innovatively contributed to advanced sample pre-treatment and SPE strategies. Accordingly, the number of papers discussing some form of molecularly imprinted solid phase extraction (MISPE) principle reveals an exponential increase as shown in Fig. 1. Consequently, a variety of synthesis routes have also been devised for designing MIPs specific to PACs, and for their application as sorbents in pre-concentration and clean-up procedures, which is the main focus of this review. Indeed, MIPs provide potential alternatives to conventional sample preparation techniques, as they facilitate the elimination of substantial matrix interferences, provide high recovery rates, and ensure reduced solvent usage.

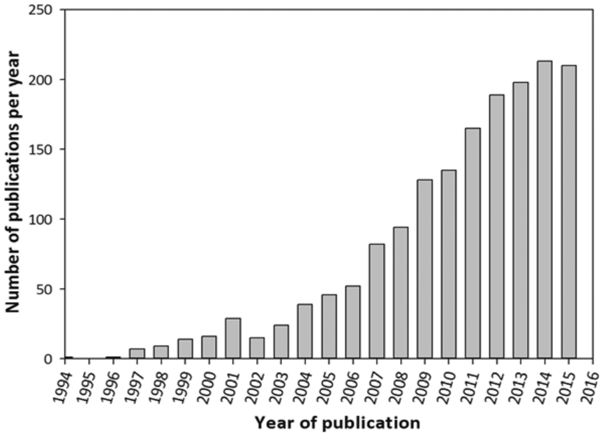

Fig. 1 Number of publication on molecularly imprinted solid phase extraction (MISPE) from 1994 to 2015 according to the SciFinder® search engine using the term "molecularly imprinted solid phase extraction".

Adequate sample pre-treatment is followed by quantitative analysis using instrumental analytical techniques suitable for the detection of PACs including conventional gas chromatography coupled to an electron capture detector, or – more selective and sensitive detection *via* high resolution mass spectrometry (GC-HRMS). While GC-HRMS provides the required figures-of-merit for the detection of PACs at trace and ultra-trace concentrations, the cost of instrumentation remains a concern. Therefore, robust analytical techniques that are affordable and facilitate low environmental impact are certainly in demand for efficient routine analysis of PACs at a global scale, in remote areas, and at harsh conditions.

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Complementary to such rather conventional analytical techniques, which usually remain confined to laboratory usage, increasing number of chemosensors and biosensors has been reported potentially enabling real-time and on-site detection of PACs.[56–58] Using appropriate molecular recognition elements combined with electrochemical, optical, or mass-sensitive transducers offers the opportunity for directly detecting analytes in complex matrices owing to their perceived specificity despite surrounding interferences. Due to the cost associated with biologically generated recognition elements (*e.g.*, enzymes, antibodies, *etc.*) and their limited robustness, so-called biomimetic recognition schemes including MIPs are increasingly proposed as viable alternative within such sensing concepts (*i.e.*, biomimetic sensors), and will be discussed in this review according to their suitability for monitoring PACs. Finally, a brief overview on the fundamental process of molecular imprinting and strategies towards group-selective MIPs will round off this feature.

2. Molecular imprinting technology

Molecular imprinting entails the creation of ideally molecule-specific binding moieties within or at the surface of a polymer matrix by co-polymerizing functional monomers in the presence of a target molecule. Subsequent removal of the template provides binding sites ideally complementary to the template in shape, size, and orientation of the functionalities. Thus, preferential rebinding of the template with high specificity is ensured. The process of molecular imprinting is based on three steps, as described below and illustrated in Fig. 2 for a non-covalent imprinting approach.

(a) Formation of a pre-polymerization complex between template and functional monomer *via* covalent (pre-organization) or non-covalent (self-assembly) interactions.

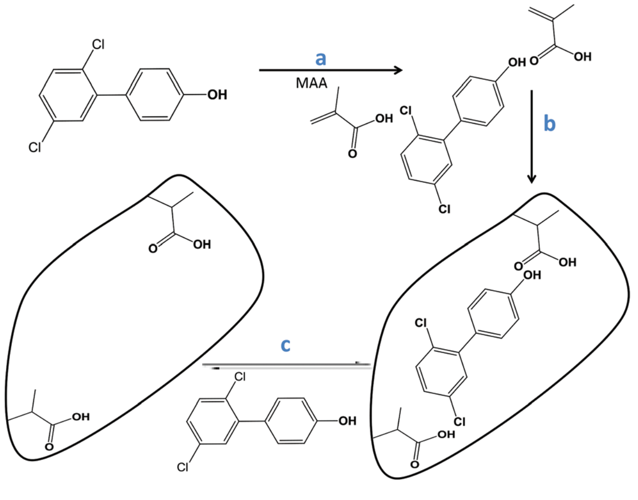

Fig. 2 Schematic illustration of non-covalent imprinting process for 4′-hydroxy-2,5-dichlorobiphenyl showing (a) the formation of a pre-polymerization complex, (b) polymerization in the presence of a cross-linker, (c) removal and rebinding of the template.

(b) Polymerization in the presence of cross-linker molecules usually activated *via* a radical initiator.

(c) Extraction of template leaving behind binding motifs with specific recognition properties for the template.

Self-assembly by formation of non-covalent bonds (*i.e.*, hydrogen bonding, π–π interactions, hydrophobic interactions, van der Waals forces, electrostatic forces, and metal coordination) are more prevalent due to their synthetic simplicity, and the fact that biological associations including antibody–antigen, receptor–ligand, and enzyme–substrate interactions follow similar principles. The merits of covalent imprinting – frequently also termed 'stoichiometric imprinting' – certainly comprise the usually superior specificity entailed *via* the covalent bond formation.

Though non-covalent imprinting appears straightforward, the first step during MIPs synthesis facilitating the formation of thermodynamically favorable and stable pre-polymerization complexes is crucial for successful imprinting procedures.[59] Optimizing this complex has in the past predominantly relied on a top-down approach, *i.e.*, trial-and-error based on synthesizing polymers with variations of the template: functional monomer: cross-linker ratio until a final MIP with favorable selectivity is obtained. Nowadays, combinatorial or high throughput screening approaches have largely replaced such efforts by synthesizing a range of MIPs at small scale (also called mini-MIPs) in order to establish the ones with the highest selectivity and binding capacity for subsequent up-scaling.[60–63] In contrast to such top-down strategies, the recent decade has also increasingly seen bottom-up approaches, which entail detailed studies of the chemical interactions at the molecular level combined with molecular modelling strategies.[64,65] For example, NMR titration,[66,67] UV-Vis studies, and infrared spectroscopies[65] have enabled careful selection of the functional monomer, porogen, and polymerization ratios, thereby resulting in the targeted synthesis of high quality MIPs. To complement these efforts, the research team of Mizaikoff has recently developed an advanced molecular modelling tool for studying the rebinding properties of imprinted polymers. Simulations of chromatographic studies using so-called virtually imprinted polymers (VIPs; a simulated molecular tube interacted with template molecules during a modeled imprinting procedure) have indicated that VIPs retain the template longer than the corresponding non-imprinted molecular tubes.[68] This approach will clearly revolutionize the synthesis strategies in molecular imprinting, as such advanced modeling studies facilitate understanding complex molecular interactions, and enable predicting the rebinding properties (*i.e.*, MIPs performance) toward rationally designed synthesis approaches.

MIPs have been traditionally synthesized by bulk polymerization; however, the demand for polymer formats with enhanced binding efficiency and mass transfer properties has seen synthesis evolving towards strategies that provide microspheres, nanospheres, and membranes. Bulk polymerization usually results in monolith blocks that are then ground and sieved for obtaining the desired particle size; this procedure

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not only lowers the overall yield,[69,70] but also leads to irregularly shaped particles and potential destruction of binding sites.[71] Consequently, *e.g.*, in chromatographic applications effects such as peak tailing, low column efficiency, and reduced loading capacity have been observed.[71,72]

An alternative to bulk polymerization is *in situ* polymerization, which is a direct approach for establishing MIPs within the chromatographic column, thus solving the tedious procedures of grinding, sieving, and column packing. Furthermore, the amount of template and functional monomer is minimized, as no losses have to be anticipated. Polymers synthesized *via* this approach have been successfully applied in column chromatography for the separation of enantiomers and diasteomers,[9] for the separation of xanthine derivatives (*i.e.*, achieving baseline separation compared to bulk polymers),[73] and for SPME procedures, *e.g.*, the pre-concentration of 4-nitrophenol.[74]

The ultimate alternatives to bulk polymerization are, however, suspension, precipitation, emulsion, mini-emulsion, multi-step swelling polymerization, and recently reported solid-phase template approach,[75] which inherently result in – ideally almost monodisperse – polymer beads for direct application. The optimization of parameters such as the amount of cross-linking agent, UV irradiation time, type of porogen, stabilizing agents, and stirring speed gives rise to monodisperse particles in the nanometer to micrometer size range,[69,70,76–80] which are ideally tailored for application in binding assays,[81] in column chromatography,[80] and for capillary electrochromatography.[82]

The most attractive approach for membrane synthesis entails phase inversion strategies using cellulose acetate, polyamide, polyacrylonitrile, polysulfone, polystyrene, and polyvinyl chloride (PVC) as membrane-forming matrices.[83,84] The resulting imprinted polymers (*i.e.*, in membrane or film format) may readily integrate into sensors *via in situ* polymerization, surface grafting, electropolymerization, physical entrapment or chemical coupling to the desired transducer surface.[85]

3. Synthesis of MIPs selective to PACs

The synthesis of MIPs selective to four main classes of PACs, including polychlorinated biphenyls, hydroxy-biphenyls, chlorophenols, dioxins and furans, and organochlorine pesticides is described in this section, and notable applications of the polymers for the determination of these compounds in different environment matrices summarized in Table 4.

3.1 Polychlorinated biphenyls and hydroxy-polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) were industrially used mainly as dielectric fluids in transformers and capacitors during their commercial production between the 1930s and the 1970s, which entailed approx. 1.5 million metric tons being produced. It is estimated that one-third of this amount has been released into the natural environment.[86,87] Evidence of toxicity and persistency led to a ban in production and use *via* the Stockholm convention on POPs, which has also instituted measures to prevent further releases, and calls for continued monitoring of these compounds.[50]

MIPs selective to PCBs would contribute positively to their monitoring and especially for selective pre-concentration at trace concentrations, however, imprinting PCBs is challenging. Firstly, some of their congeners are toxic and may therefore not be used as templates. Secondly, PCBs lack functional groups, and therefore do not fully satisfy the requirements for non-covalent imprinting, which favors multifunctional templates. Likewise, the functional monomer bears multiple functional groups facilitating the formation of stable pre-polymerization complexes *via* pronounced *e.g.*, hydrogen bonding or ion-pair interactions.[67,88,89] The only association opportunities for these constituents with functional monomers are rather weak π–π stacking, and hydrogen bonding with chlorine atoms. Thirdly, template bleeding is of substantial concern for application of PCB-imprinted polymers in environmental trace analysis. Two independent studies have indicated that in molecular imprinting 0.47–1.38% and 3 ng $\mathrm{mL^{-1}}$ of the template may remain incorporated in the polymer matrix even after extensive extraction procedures.[90,91] Relating this amount of potential residues with the maximum contaminant level (MCL) of 0.5 ng $\mathrm{mL^{-1}}$ for $\sum$PCBs in drinking water (as given by the USEPA), template bleeding would significantly contribute to erroneous results. These challenges have therefore demanded particularly innovative strategies for imprinting PCBs that address these issues, while achieving the desired selectivity and stability properties of the resulting MIPs.

The first attempt on imprinting polymers recognizing PCBs was reported by Hosoya *et al.*,[80] who introduced the rather promising, yet little explored porogenic template imprinting technique (PTIT), whereby a solvent is used as both a template and porogen. This technique is based on the fact that certain solvents capable of forming a macroporous structure can be imprinted within the cross-linked polymer network, thereby resulting into a polymer with recognition properties for the porogen.[92] In that study, *p*-xylene, *o*-xylene, and *m*-xylene were investigated as porogenic templates, and EGDMA as the cross-linker for synthesizing porogen-imprinted polymers *via* multi-step swelling polymerization. Upon application as stationary phases in HPLC, *p*-xylene imprinted polymers were highly selective towards PCB 15, which is chlorinated at the *para* position, while the ones synthesized using *m*-xylene were selective towards PCB 14, which is a *meta* chlorinated PCB; the same behavior was observed for PCBs 77, 80, 155, and 169 (see also Table 3). From these results, it is evident that alkybenzenes may indeed act as *dummy templates* for PCBs, thereby offering an attractive imprinting strategy that does not require explicit templates. This technique has also been reported to provide polymers with high loading capacity and stable recognition sites, as the porogen can be used at high ratios owing to its availability.[93]

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Table 3 Retention time and retention factors of PCBs on *o*-, *m*-, *p*-xylene imprinted polymers

PCB	*ortho*-Xylene	*meta*-Xylene	*para*-Xylene
Retention time (min)			
14		5.03	5.02
15		4.88	5.22
77		7.38	7.43
80		7.75	7.43
Retention factor (k')			
155	1.12	1.16	1.13
169	5.40	5.31	5.19

HPLC experiments performed using aqueous methanol (90%) as the mobile phase and UV detection at 254 nm. Reproduced from ref. 80. Copyright 1998, with permission from Elsevier.

Extending the benefits of porogen-imprinted polymers in environmental applications, the research team of Mizaikoff has recently demonstrated the applicability of such MIPs serving as pre-concentration materials during studies on 6 so-called *indicator PCBs* in both aqueous and organic media. A modified synthesis strategy was applied using in fact a mixture of *o*-, *p- and m*-xylene as porogenic templates, and 4-vinylpyridine (4-VP) as the polymerizable monomer additionally facilitating π-stacking interactions with electron-poor PCBs. The xylene imprinted polymers (XIPs) clearly outperformed conventional C_{18} stationary phase materials, while demonstrating enhanced recognition properties for PCBs with at least 2 chlorine atoms at the *meta*, and *para*-position.[94] This study also indicated that toluene could also serve as a potential dummy template for imprinting PCBs, as polymers synthesized using only toluene gave excellent results during pre-concentration studies reflected in enhanced retention factors compared to polymers synthesized using a template and a low amount of functional monomer. The imprinting effect realized by using alkylbenzenes was confirmed by the resulting imprinting factor of 1.56 *vs.* non-imprinted polymers (NIPs) synthesized using cyclohexane.

More advanced sorbent materials based on porogen imprinting and sulfoxide modification advancing the separation of PCBs from insulating oil have been reported by Tominaga *et al.*[95] As the sulfoxide modification of silica stationary phases has been shown to increase the retention of PCBs by availing electrophilic sulfur for interaction with aromatic rings of PCBs,[96,97] it was postulated that such modifications of porogen-imprinted polymers may potentially further increase the PCB recognition properties. True to that hypothesis, sulfoxide-modified polymers were synthesized using xylene as the porogen, divinylbenzene, and chloromethylstyrene as cross-linker and functional monomer, respectively. Using these materials, PCBs were preferentially separated from insulating oil compared to unmodified particles. In addition to being used for separation of PCBs from insulating oil, sulfoxide-modified silica is applicable for clean-up procedures of soil extracts in PCB analysis,[98] which in turn implies that sulfoxide-modified porogen imprinted polymers may also achieve corresponding enhancements. Coupled to their reusability, excellent materials for studying these pollutants in complex matrices are on the horizon.

In support of this approach, the fragment imprinting technique (FIT), whereby a segment of the target molecule is used as a pseudo-template[99] provides another route of overcoming template bleeding, and additionally entails a substitute (dummy) template, if the actual template is not available or toxic. Recently, Cleland *et al.*[100] introduced effective fragment potentials enabling a rational design strategy for imprinting polymers for PCBs. From the pre-polymerization complexes

Table 4 Applications of molecularly imprinted polymers in analysis of PACs in different environmental matrices

Analyte	Matrix	Synthetic protocol	Application	Recoveries	LOD/LOQ	Ref.
PCBs	Sediments and soil	Suspension polymerization	MISPE	70.2–102.5%	0.05–0.28 ng g^{-1}	98
	Water			83.5–103.1%	0.008–0.04 ng mL^{-1}	
PCBs	Insulating oil	Bulk polymerization	MISPE Chromatography			95
PCBs	Fish	Sol–gel	MISPE	73.4–114.2%	2.8–6.8 ng L^{-1}	101
OH-PCBs	Water	Precipitation polymerization	MISPE	89–110%	3–28 ng L^{-1}	102
2-Chlorophenol	Water	Bulk polymerization	MISPE	100.8–105.3%	0.05 ng L^{-1}	103
Pentachlorophenol		Precipitation polymerization	Removal of pentachlorophenol			104
2,4,5-Trichlorophenoxyacetic acid	Water	Bulk polymerization	MISPE	89–94%		105
2,4-Dichlorophenoxyacetic acid	Water		Potentiometric sensor	97.1–99.3%	1.2×10^{-9} M	106
2,4-Dichlorophenoxyacetic acid	Urine	Precipitation polymerization	MISPE	97.8%	1.8 μg L^{-1}	154
Hexachlorobenzene	Water		QCM sensor		1 pM	107
Lindane	Water		Potentiometric sensor		0.029 ng mL^{-1}	108
Lindane	Water	Electropolymerization	Photoelectrochemical sensor	98.3–103%	0.1–10 μmol L^{-1}	109
Endosulfan	Water		MISPME	108–124%	2.09 ng mL^{-1}	169
Organochlorine pesticides	Water, soil, rice, tea-leaves	Sol–gel	MISPE	65.7–121.6%		110

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formed by fragment templates: 1,2,3,4,5-pentachlorobenzene and 1,2,3-trichlorobenzene, and functional monomers: styrene, 2,3,4,5,6-pentafluorostyrene (PFS), 4-VP, and 2,4,6-trimethylstyrene (TMS), they identified PFS and TMS as forming the most stable complexes. Consequently, polymers synthesized using 1,2,3,4,5-pentachlorobenzene and TMS yielded imprinting factors of 1.38, 1.38, and 1.41, while those synthesized using 1,2,3-trichlorobenzene and PFS gave imprinting factors of 6.57, 3.46, 5.80 for PCB 44, 105, and 174, respectively. The optimized imprinting ratios were 1 : 2 : 10 (template : functional monomer : cross-linker) and 15 : 6 : 29, respectively, using methanol as the porogen, since the perceived application was in aqueous media. Surprisingly, PFS which is electron poor compared to TMS – resulted in polymers with high imprinting factors, which may be attributed to the quantity of the template used. It has been well-established that due to the weak interaction forces in non-covalent imprinting, an excess of the functional monomers or template is required in order to push the equilibrium towards the formation of possibly stable pre-polymerization complexes during self-assembly.[111]

While the overall goal of synthesizing MIPs is to enable the detection of compounds in complex matrices, in some cases a simple MISPE is not sufficient to remove all the interferences, and therefore necessitates two-step clean-up procedures, *i.e.*, first *via* a pre-column packed with NIPs, RAM, or C_{18}, and finally using MISPE.[112–114] In this case, the sample is first loaded onto the pre-column, and the target analytes are then eluted into the MISPE column during the washing step for further clean-up, which results in a more efficient elimination of interferences. Recently, the team of Mizaikoff has reported a one-step dual-layer column comprising MISPE and an acidified silica gel for advancing clean-up of soil and sediment extracts for the quantification of PCBs.[98] These matrices are important for monitoring organics, which accumulate by binding to particulate matter owing to their high octanol–water partition coefficient (K_{ow}).[115,116] Therefore, an affordable column for treating these matrices is demanded. Hence, polymers were synthesized using the less chlorinated PCB 15 as the dummy template to impact group recognition of PCBs at the finally obtained polymer matrix. The validated specific MISPE enabled the determination of 6 indicator PCBs in soil and sediment maintaining recoveries of 79.8–111.8% after 30 cycles of cartridge regeneration and re-use. The protocol also achieved efficient sample clean-up and minimal solvent usage, as only 5 mL of *n*-hexane : DCM (9 : 1) was used for elution compared to conventional multi-sorbent layered columns, which reported elution volumes of up to 220 mL along with a large quantity of sorbent, which is not re-usable (Table 2). The limit of quantification was 0.05–0.28 ng g^{-1}, which was within similar results reported using other clean-up methods,[35,36] thus indicating that the developed cartridges are indeed suitable for quantitatively analyzing and monitoring PCBs.

To maximize on the performance of MIPs, incorporation of magnetic particles in MIP synthesis is gaining attention, as they are perceived to offer a large surface area, opportunities for surface modification, and magnetic properties for efficient particle collection.[117] Thus, magnetic particles can be separated from a solution after incubation using an external magnet without the need for centrifugation, filtration or packing into a column. Application of these particles especially in dispersive solid phase extraction or batch SPE is facilitated, because once target molecules are adsorbed, the particles can be readily separated from the solution using a simple magnet. Multiwalled carbon nanotubes (MWCNTs) are other materials, which are being introduced as support materials in MIPs synthesis owing to their large surface area and strong interaction with aromatic compounds *via* π-stacking. Taking advantage of these two systems, MWCNTs modified magnetic MIPs (MWCNTs@mMIPs) selective to PCBs have been synthesized using 3,4-dichlorobenzene acetic acid as the dummy template, achieving recoveries between 73.4–114.2% for spiked fish samples, and have obtained detection limit between 2.8–6.8 ng L^{-1}, which was significantly lower compared to earlier reported methods.[118] Moreover, thus imprinted polymer cartridges gave rise to clean extracts compared to the controls, as was shown by the associated chromatograms.

Related to PCBs are hydroxy-PCBs (OH-PCBs), which are *in vivo* products of cytochrome P450 mediated oxidation of PCBs, or reactions of PCBs with hydroxy radicals in the atmosphere, aquatic environments, and aerobic biodegradation of PCBs.[119–123] To date, there are only two reports on HO-PCB MIPs. Kubo *et al.*[124] applied FIT using fragment molecules *p-tert*-butylphenol, *p-tert*-butylbenzoic acid, 2,6-bis(trifluoromethyl)benzoic acid, biphenyl alcohol and biphenyl carboxylic acid (BCA). The MIP column gave higher retention factors for OH-PCBs compared to conventional inertsil-ODS-3 column. Furthermore, MIPs against BCA successfully achieved separation of three analogues of HO-PCBs from the mixture, which interfere with the thyroid hormone activity.

The most recent report by Liang *et al.*[102] discusses selective MIPs for OH-PCBs using 4,4′-hydroxybiphenyl as the dummy template, and acrylamide (AM) and TRIM as functional monomer and cross-linker, respectively. Selectivity experiments gave recoveries >80% for OH-PCBs and <45% for PCBs. From the structure of the two molecules, OH-PCBs are able to form strong hydrogen bond with AM matrix, while PCBs can only manage a weak hydrogen bond with the chlorine atoms, thus the poor selectivity. Compared to C18 and NIP, the imprinted polymer was superior in terms of recoveries yielding 98–110% and 89–109% for spiked sea water and waste water, respectively, and a limit of detection of 3–28 pg L^{-1}, which is satisfactory for trace level analysis. AM provided functional groups for formation of strong hydrogen bond with the template, and electron rich amine groups to enable charge transfer interaction with electron deficient aromatic rings of the biphenyl.

3.2 Chlorinated Bisphenol A and other chlorophenols

2,2-Bis(4-hydroxyphenyl) propane (a.k.a., Bisphenol A; BPA) is widely used in the manufacture of polycarbonate plastics and epoxy resins, in thermal paper production, and as antioxidant or stabilizer in PVC plastics.[125] During thermal processes, BPA

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can leach from polycarbonate plastics, epoxy resin products, and PVC pipes into water where concentrations between 4.0–1730 μg L^{-1} in water from PVC tubes, and 0.05–2.47 μg g^{-1} in plastic products exposed for a maximum of 24 h have been reported.[126,127] Once in water, BPA can easily react with residual chlorine to form chlorinated BPA congeners, thus demanding for monitoring chlorinated derivatives for minimizing potential human exposure.

MIPs with recognition properties for homologues of chlorinated Bisphenol A (cl-BPA) applying 2,6-dimethylphenol and *p-tert*-butylphenol as fragment templates, and 4-VP and EGDMA as the functional monomer and cross-linker, respectively, have been reported.[128] 2,6-Dimethylphenol MIPs gave the highest capacity factors, with cl-BPA homologues having chlorine substitution at the 3rd and 5th positions being retained the most. Substitutions at 2nd and 6th positions on phenol ring correspond to 3rd and 5th positions on biphenyl ring; meaning that the recognition followed the substitution patterns of the template. However, comparatively low capacity factors have been recorded for BPA and the respective templates used. Another synthetic route, which has resulted in highly selective MIPs for BPA, and would be applicable for cl-BPA using BPA as a dummy template is use of gold nanoclusters as support materials for MIPs.[129]

Chlorophenols (CPs) are used as pesticides, antiseptics, and preservatives,[70] with most of these molecules listed among the priority pollutants by the US environmental protection agency. MIPs specific to 2,4-dichlorophenol, 2,4,6-trichlorophenol have been synthesized following conventional synthesis approaches,[70,130] reversible addition–fragmentation chain transfer (RAFT) for 2,4-dichlorophenol selective polymers,[131] and semi-covalent imprinting approach for MIPs selective to 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol.[132] Though these constituents are less functionalized, the hydroxyl group creates room for further modifications to increase the number of functional groups on the template so as to enhance the formation of stable pre-polymerization complexes. El-Sheikh *et al.*,[103] have demonstrated this strategy by derivatizing 2-chlorophenol with 4-amino-anti-pyrene to self-assemble with MAA. This approach resulted in highly selective polymers, which – when applied for pre-concentration in water samples – gave very low detection limits, *i.e.*, 0.05 ng L^{-1} *vs.* 7 ng L^{-1} for non-derivatized 2-CP. The 140-fold reduction in the detection limit reflects the increased selectivity, which enhances the elimination of matrix interferences.

For the removal of highly chlorinated pentachlorophenol (PCP) from the environment, selective sorbents using graphene and magnetic nanoparticles have been synthesized as schematically illustrated in Fig. 3. Magnetic graphene oxide MIPs (MGO@MIP) were selective for PCP with an imprinting factor of 4.36 compared to 1.02, 1.01, 1.01 for 2-chlorophenol, 2,4-dichlorophenol, and 2,3,4,6-trichlorophenol, respectively. The maximum adsorption capacity was 789.4 mg g^{-1}.[104] With respect to other sorbents reported for the removal of PCP, MGO@MIPs were by far the superior matrix. The composite MIP proved its stability by maintaining recoveries >90% after the 10th cycle of usage, thereby providing powerful adsorbents for the selective removal of PCP. Compared to core–shell magnetic MIPs prepared without graphene, a maximum adsorption capacity of 43.4 mg g^{-1} was achieved,[133] which corroborates the advantages of incorporating graphene into the MIP structure. It is therefore derived that graphene and related structures, *i.e.*, carbon nanotubes (CNTs) are excellent support materials for increasing the selectivity and loading capacity of MIPs in specific templating scenarios.

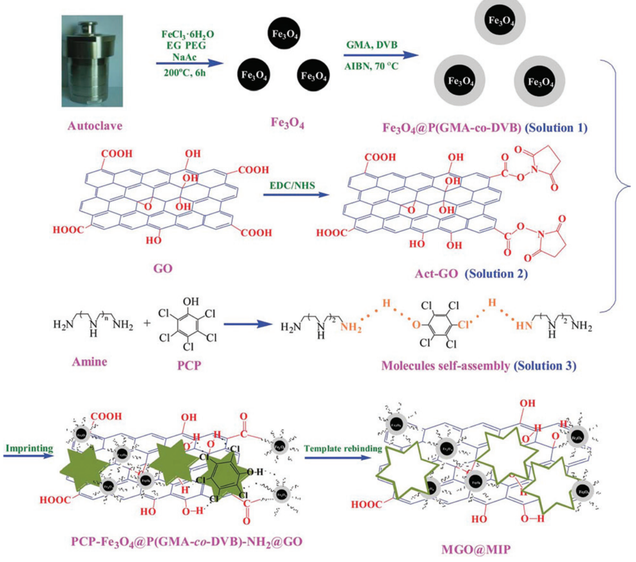

Fig. 3 Schematic illustration of MGO@MIP synthesis. Reproduced from ref. 104. Copyright 2014, with permission from the Royal Society of Chemistry.

Imprinting of thin layers of polymers coated onto the surface of silica nanoparticles has been embraced as a strategy for providing surface-imprinted polymers with highly accessible binding cavities. This strategy not only facilitates removal of the template, but increases the diffusive mass transfer to surface binding sites provided by the imprinted polymer coating during rebinding.[134]

By combining surface imprinting with a sol–gel process, a PCP-imprinted amino-functionalized silica sorbent bound 80.4 mg g^{-1} of PCP, compared to 46.6 mg g^{-1} for the NIP. The sorbent was applicable for the trace-level analysis of PCP; in an online-SPE-HPLC configuration, detection limits of 6 ng L^{-1} within a linear range of 0.05–500 μg L^{-1} were achieved. Compared to structurally similar compounds (*i.e.*, phenol and 2,4-DCP), PCP was the only compound released during the elution step, *i.e.*, other constituents that had been non-selectively bound were eluted during the washing step.[135] Silica particles can also be functionalized with silane coupling agents (*e.g.*, KH-570), as imprinted polymers for 3-chlorophenol with MAA, EDGMA as functional and cross-linker resulted in maximum binding capacities of 163.7 mg g^{-1} and 143.1 mg g^{-1} for the MIP and NIP, respectively.[136] Moreover, cyclodextrin (β-CD) and

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attapulgite (ATP) have also acted as support material for chlorophenol-imprinted polymers giving rise to highly selective sorbents.[137–139] In this case, a composite formed from β-CD and ATP was used as the functional monomer to form a pre-polymerization complex with the template. The use of modified and unmodified β-CD as a functional monomer owing to its hydrophobic and hydrophilic properties favors synthesis and application of the resulting MIPs in aqueous media.[140]

3.3 Dioxins and furans

Dioxins and furans are products of incomplete combustion of chlorine containing hydrocarbons, and by-products during manufacturing of certain chlorinated pesticides. Among the dioxins and furans, 2,3,7,8-tetrachloro-*p*-dibenzodioxin (2,3,7,8-TCDD) is considered the most toxic, and has been classified by IARC as a human carcinogen.[141] Contrary to other toxic chemicals, which are detected in parts per million and parts per billion, dioxins occur in parts per trillion and parts per quadrillion (ultra-trace levels). Their toxicity at these low concentrations has necessitated a forcible USEPA maximum contaminant level of 0.03 ng L^{-1} in drinking water. To achieve quantification at these low levels, state-of-the-art instrumentation such as high resolution gas chromatography coupled with high resolution mass spectrometry and specialized clean-up protocols are required.[40,142,143]

However, due to their toxicity and unavailability in large quantities, dioxins may not be used directly as templates in molecular imprinting, which has raised the need for tailor-made substitute molecules serving as dummy templates. As these molecules are also poorly functionalized, *i.e.*, they are unable to form pronounced interactions in non-covalent imprinting, innovative synthetic strategies are required. Semi-covalent or sacrificial spacer synthesis is suitable for addressing these challenges.[144] Semi-covalent imprinting exploits both covalent and non-covalent interactions,[145] *i.e.*, a template is covalently attached to a polymerizable functional monomer followed by hydrolytic cleavage of the covalent bond after polymerization in order to remove the template and create room for re-binding *via* non-covalent interactions. This approach has been reported to provide polymers with homogeneous binding sites and almost complete removal of the template, thereby resulting in high binding capacities, pronounced selectivity, and limited template bleeding.[69,132,146]

So far, two studies based on sacrificial spacers have revealed that weak intermolecular interactions such as hydrogen bonding with chlorine atoms at the aromatic moieties can be exploited specifically for poorly functionalized constituents. The pioneering work of Lübke *et al.*[147] used urea derivatives of 2,8-dichloro-3,7-diaminodibenzodioxin **1** and *N*-(2-(3,7,8-trichlorodibenzodioxinyl)-2-methacryloyloxybenzamide) **2** (Fig. 4) as templates to introduce amine and methacrylic acid groups into the polymer matrix enabling the formation of weak hydrogen bonds with the chlorine groups of 2,3,7,8-TCDD. In addition, they incorporated polymerizable electron rich co-monomers, *i.e.*, 1,4-bis(3/4 vinylbenzyloxy) benzene **5** and 1-methoxy-3,5-bis(4-vinylbenzyloxybenzene) **6**, and electron poor pentafluorostyrene **7** to enhance π–π interaction with the electron deficient 2,3,7,8-TCDD.

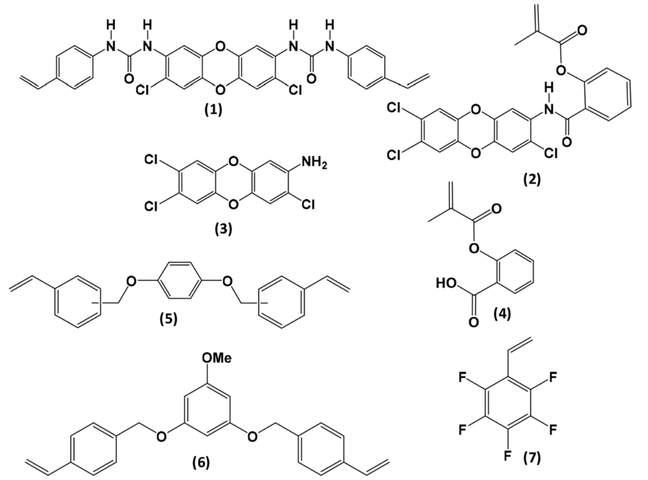

Fig. 4 Chemical structures of (1) urea derivative of 2,8-dichloro-3,7-diaminodibenzodioxin, (2) *N*-(2-(3,7,8-trichlorodibenzodioxinyl)-2-methacryloyloxybenzamide), (3) 2-amino-3,7,8-trichlorodibenzodioxin, (4) 2-methacryloyloxybenzoic, (5) 1,4-bis(3/4-vinylbenzyloxy) benzene, (6) 1-methoxy-3,5-bis (4-vinylbenzyloxybenzene), and (7) pentafluorostyrene.

Polymers synthesized using **1** as the template achieved imprinting factors of 1.14 (without co-monomer), 1.24 (with **5** as co-monomer), and 1.22 (with **7** as co-monomer) at a concentration in nM range for 2,3,7,8-TCDD.

The advantages of incorporating electron rich co-monomers was shown by increased binding capacities when **5** instead of **7** was used as the co-monomer (9.30 pmol g^{-1} *vs.* 6.37 pmol g^{-1}), thereby confirming the higher electron donating properties of **5**. The second template **2** gave imprinting factors of 1.20 (without co-monomer), 1.11 (with **6**), and 1.15 (with **7**). Though the imprinting factors seemed to decrease slightly compared to the first template, the polymers gave higher binding capacities. The second study sought to simplify the laborious process of synthesizing **2** by employing two separate molecules, *i.e.*, 2-amino-3,7,8-trichlorodibenzodioxin **3** and 2-methacryloyloxybenzoic acid **4**, thereby achieving an imprinting factor of 2.44 compared to the earlier reported 1.11,[148] thus introducing a facile and more reliable route for the synthesis of dioxin-selective sorbents.

A separate study following the rather elegant PTIT with *o*- and *p*-xylene as porogenic templates showed TCDDs having chlorine atoms at *ortho* positions being selectively retained on *o*-xylene imprinted stationary phase, while TCDDs having chlorine atoms at *para* positions were retained selectively on the stationary phase imprinted by *p*-xylene.[93] The selectivity realized for TCDDs further confirms the potential of the porogenic templates, *o*-, *m*-, *p*-xylenes to provide selective sorbents for PACs.

Recently, Khan *et al.*[149] – motivated by the toxicity of these compounds and the need for their continuous monitoring – have initiated a computational strategy for understanding the

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nature of the pre-polymerization complex formed by 2,3,7,8-TCDD and different functional monomers before actual polymerization. They established methacrylic acid and acetonitrile as compounds offering the most stable complex. To their advantage, the system could further determine virtual selectivity by analyzing the binding energy between the pre-polymerization complex and structural analogues of the template, where high selectivity for the template was observed. Successful introduction of molecular modelling for rationally designing MIPs for dioxins may be considered a remarkable milestone, since these compounds are highly toxic and trial-and-error methods may thus not be sustainably applied.

As mentioned earlier, MIPs in film or membrane format offer rapid mass transfer and binding kinetics, which widens the scope of application for MIPs as selective membranes in ultrafiltration, microfiltration, and reverse osmosis for water treatment and purification. A dibenzofuran (DBF) membrane synthesized *via* phase inversion is reported by Kobayashi *et al.*[150] and Reddy *et al.*[151] The polymer matrices used were polyacrylonitrile, polysulfone, polystyrene, and polyvinyl chloride, with DBF as the template, dimethyl acetamide or dimethylsulfoxide as the solvent, and water as the non-solvent. Rebinding studies in methanol resulted into binding capacities of 4.5 *vs.* 1.4 μmol g^{-1} for polysulfone-imprinted and non-imprinted membranes. While similarly structured dibenzo-*p*-dioxin was pronouncedly recognized, phenyl ether and benzodioxin gave low binding capacities. Membranes synthesized *via* phase inversion are highly applicable in aqueous media, therefore the present membrane is a promising adsorbent in water purification and real-time detection, *i.e.*, sensing of dioxin and dibenzofuran in water.

3.4 Organochlorine pesticides and herbicides

Due to the threats that pests and weeds pose to crops in agriculture, use of chemicals that can eliminate them and improve the yield during harvesting is inevitable. 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), and 2,4-dichlorophenoxyacetic acid (2,4-D) are two herbicides commonly used to kill weeds, and as plant growth hormones. Consequently, they percolate into the environment, which may have detrimental effects on human health and aquatic life upon exposure, thus requiring monitoring to comply with the USEPA set MCLs of 0.07 mg L^{-1} for 2,4-D in drinking water. Their determination in water is therefore of substantial interest to minimize human exposure. Thereby, the demand for MIPs that can be applied in aqueous environments, *i.e.*, water-compatible MIPs has led to fine-tuning of MIPs to incorporate a hydrophilic surface by using polar porogens, and hydrophilic monomers (*e.g.*, AM, HEMA) and cross-linkers (*e.g.*, methylenebis (acrylamide)).[16,140] More information on water-compatible MIPs can be found in a recent review by Zhang *et al.*[140] Moreover, it has been shown that MIPs work best when the same conditions applied during synthesis are replicated during their application, as a change in solvent affects the swelling behavior, and consequently, the recognition properties of the polymer.[64,152] Taking this into consideration, a 2,4,5-T selective MIP synthesized *via* bulk polymerization using 4-VP as a functional monomer, EGDMA as a cross-linker, and a mixture of water and methanol (1 : 3, v/v) as the porogen was reported to be superior *vs.* liquid–liquid extraction and C_{18} reversed phase extraction in terms of recoveries and elimination of interferences when applied for the pre-concentration of 2,4,5-T in spiked river water.[105,153] The enhanced performance in aqueous environment was probably due to polymerization in a polar environment. The utility of these imprinted polymers was also demonstrated in a separate study using 2,4-D imprinted polymers synthesized almost the same way as adsorbents in pre-concentration of 2,4-D in real-world water samples and urine yielding recoveries in the range of 97.8–99.8%.[154] Selective removal of these compounds from the environment is an important area of application, as water can be purified of 2,4-D using amino-functionalized silica gel sorbent with adsorption capacity of 30.4 mg g^{-1} prepared *via* sol–gel processes.[155]

Apart from using imprinted polymers as pre-concentration sorbents, another perceived applications is in immunoassays serving as antibody mimics, *i.e.*, so-called molecularly imprinted sorbent assays (MIAs) providing high selectivity for 2,4-D in radioligand binding assays and fluoroimmunoassays; with promises for group screening of similarly structured compounds like 2,4-dichlorophenoxybutyric which has shown 95% cross-reactivity.[156–158] Alternatively, due to the risk of template bleeding in trace-level analysis, and the toxicity of phenoxyacetic acids, the search for dummy templates for imprinting of chlorophenoxyacids has successfully identified Bisphenol A and 2-methylphenoxyacetic acid as being capable of producing polymers selective to 2,4-D, 2,4,5-T, and 2-(2,4,5-trichlorophenoxy)propionic acid; and 2,4-D, 4-chlorophenoxyacetic acid, and methylchlorophenoxyacetic acid, respectively.[159,160]

The contribution of molecular modelling and spectroscopic tools FTIR, NMR in design of high quality MIPs for these compounds has been clearly evidenced by Molinelli *et al.*,[66] who studied the pre-polymerization complex formed between 2,4-D and 4-VP for devising the development of a MIP that could distinguish between 2,4-D, 2,4-dichlorobenzoic acid, phenoxyacetic acid, 2,4-dichlorobenzyl alcohol, and 4-chlorophenol.

In addition, sensors for detection of organochlorine herbicides have been reported using MIPs as recognition elements. One such sensor is a novel potentiometric sensor based on imprinted polymer inclusion membrane for determination of 2,4-D in water.[106] The sensing element, *i.e.*, MWCNTs@MIP was dispersed in PVC matrix and plasticized with nitrophenyloctyl ether, which served to increase the mobility of 2,4-D and the overall electrochemical properties of the sensor. The sensitivity of the sensor to 2,4-D was evident from the detection limit of 1.2×10^{-9} M *vs.* 1.0×10^{-5} M for the control. Moreover, it was reusable for more than 30 times and stable for a period of 3 months without the need for conditioning, as it is the requirement for many conventional sensors. An equilibrium response time of 5 min, and recoveries >97.1% for ground and tap water spiked at trace-levels (*i.e.*, μg L^{-1}) confirmed the suitability of the sensor for the detection of 2,4-D in water.

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The widespread use of organochlorine pesticides (OCPs) during their commercial production made them ubiquitous environmental contaminants, which together with their toxicity led to their ban in production and use in several countries, and recently through the Stockholm convention on persistent organic pollutants (POPs). Hexachlorobenzene (HCB) is one of the compounds that is no longer in use, though its presence in the environment cannot be ruled out as it is documented to be a possible by-product of industrial process like manufacturing of chlorinated solvents and other chlorine-containing constituents. Monitoring of HCB at ultra-trace level is feasible using a MIP-based quartz crystal microbalance (QCM-MIP) sensor designed by *in situ* polymerization of spin-coated mixtures of electron rich 1,4-diacryolyloxybenzene, 1,4-bis(2-acetyaminoacrylolyloxy) pentane to increase the adhesion at the gold surface, benzyl methacrylate, which serves as diluent by decreasing the degree of crosslinks, HCB, and Irgacure 369 as the photo-initiator.[107] The detection limits of the sensor was in the picomolar range (pM) with response times of 10 s, and significant selectivity of HCB *vs.* structurally similar cyclohexane, benzene, anisole, and chlorobenzene.

Moreover, for the rapid detection of lindane (λ-hexachlorocyclohexanes (λ-HCCH)), a highly selective and sensitive potentiometric sensor using MAA as the functional monomer, EGDMA as the cross-linker, HCCH as the template, and MWCNTs as the support material was reported by Anirudhan and Alexander.[108] MWCNTs-MIP was deposited at the working electrode by incorporating the particles in a supporting agarose gel with the sensor attaining selectivity for lindane over DDT, 2,4,6-T, 2,4-D, and chloropyrifos at detection limits of 1.0×10^{-10} M (0.029 ng mL^{-1}), and with a linear range of 1.0×10^{-10}–1.0×10^{-3} M. Electropolymerization stands out as an attractive way of preparing robust polymer films at (electrochemical) transducer surfaces offering advantages like control of film thickness, reproducibility, ease of preparation and template removal *via* overoxidation, and application in aqueous environments.[161] Therefore, a photoelectrochemical sensor generated by electropolymerization of *o*-phenylenediamine (*o*-PD) monomer and lindane on titanium dioxide TiO_2 nanotubes achieved selective detection of lindane in the presence of aldrin and heptachlor, and oxygen containing inorganic ions as interfering matrices.[109] With a detection limit of 0.03 μmol L^{-1} and linear range between 0.1–10 μmol L^{-1}, trace levels of lindane could be monitored.

Among the OCPs, 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT)) was widely applied to control pest, and continues to be used in the tropics albeit with restrictions for mosquito control. A sol–gel fluorescence MIP-based sensor for DDT following both non-covalent and semi-covalent imprinting approaches has been reported by Graham *et al.*[144] In the non-covalent approach, they used DDT and bis(trimethoxysilylethyl) benzene (BTEB), whereby the resulting polymer gel gave imprinting ratios of 1.46 and 1.50 for DDT and 4,4′-dibromobiphenyl, respectively, with other tested compounds giving almost similar values. They attributed the rather minimal selectivity to weak interactions between DDT and BTEB coupled to insufficient removal of template, thus availing minimal number of accessible imprinted sites for rebinding of DDT. To improve on the selectivity of DDT, they also applied the semi-covalent approach to introduce amine groups in the polymer gel, which can form weak hydrogen bonds with chlorine atoms. To this end, a sacrificial spacer synthesized from either ethylidenebisphenol (EBP) or 4,4′-ethylenedianiline (EDA) and 3-isocyanatopropyltriethoxysilane was polymerized with BTEB followed by cleavage of the spacer to form amine groups. The sol–gel matrix was selective to EBP and DDT, compared to other related compounds tested. By incorporating nitrobenzofurazan as a fluorescence reagent, a fluorescence sensor achieved detection limits of 50 ng mL^{-1} for DDT in aqueous environment, which is equal to the set MCL for $\sum$DDT, thus providing a real-time sensor for detection and monitoring of DDT.

Heptachlor – a chlorinated dicyclopentadiene – was applied for control of termites, and polymers selective for heptachlor were synthesized following conventional bulk-imprinting approaches with heptachlor as the template, MAA, 4-VP or styrene as functional monomers, and EGDMA or DVB as the cross-linkers.[162] Imprinting factors of 1.92, 1.51, and 1.49 for heptachlor, endosulfan, and heptachlor epoxide using heptachlor-*co*-MAA-*co*-EGDMA polymer indicates that the polymer is equally selective for the other organochlorines, and can therefore be applied for group selective studies.

Besides SPE, which is a well-established sample preparation technique, SPME, which does not require the laborious steps of preparing SPE columns, is free from solvent requirements. SPME involves partitioning of the analyte between the sample and the stationary phase, usually poly (dimethylsiloxane), polyacrylate, PDMS-divinylbenzene (PDMS/DVB), or carboxen-PDMS coated onto a fused silica fiber followed by thermal desorption of the enriched constituents into a gas chromatograph or solvent elution for further chromatographic analysis.[16,163,164] Since 2001, these non-selective polymer coatings are gradually being replacement with more selective sorbents leading to molecularly imprinted solid phase microextraction (MISPME) strategies, which have been tested in studies of brombuterol in human urine,[165] diacetylmophine,[166] atrazine,[167] and tetracyclines.[168]

To extend this technique to organochlorine pesticides, Shaikh *et al.*[169] designed an endosulfan specific MISPME by coating a stainless steel wire with endosulfan MIP prepared by copolymerizing Fe_3O_4@SiO_2-methacrylamide composite with *N*,*N*′-methylene-bis-acrylamide in the presence of the template as illustrated in Fig. 5. The MIP afforded imprinting factors of 10.1 and 9.1 for endosulfan I and II, compared to 3.3, 2.14, and 2.5 for organochlorines heptachlor, aldrin, and diedrin, with the MISPME giving recoveries of 97.7–104% and 108–124% for spiked waste and river water. Compared to commercial PDMS, MISPME outperformed conventional membranes, as evident by the high intensity peaks from spiked water samples. An earlier report using conventional PDMS/DVB gave limits of detection of 23 ng L^{-1} with extraction times of 45 min,[170] thus rendering endosulfan MISPME with detec-

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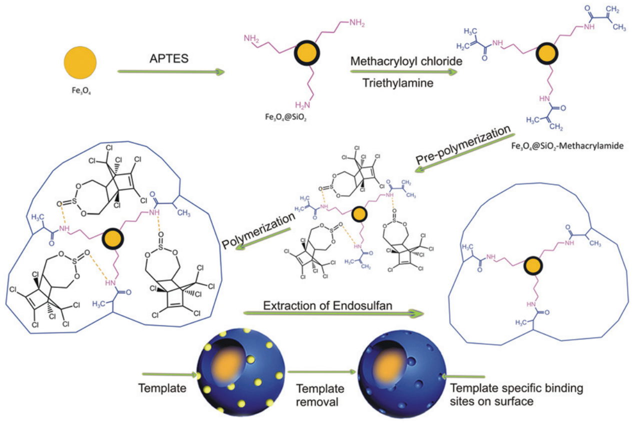

Fig. 5 Schematic representation on imprinting of endosulfan in poly (methacrylamide functionalized magnetic composites-*co*-*N*,*N*′-methylene-bis-acrylamide). Reproduced from ref. 169. Copyright 2014, with permission from Elsevier.

tion limits of 2.09 ng L^{-1}, a linear range of 7–5 × 10^{-3} ng L^{-1}, and extraction times of 2 min superior and a suitable option for the trace determination of endosulfan at a MCL of 74 μg L^{-1}.

As discussed in section 4, a promising and cost effective technique for monitoring PACs is the simultaneous detection of a group of compounds. Gao *et al.*[110] explored such techniques using an ionic liquid as the hydrophilic functional monomer in order to customize the MIPs for application in aquatic environment. Ionic liquids are reported to accelerate the synthesis process, and increase the selectivity and adsorption capacity of MIPs.[171] Ionic liquid 1-(triethoxysilyl) propyl-3-aminopropyl imidazole bromide was copolymerized with Bisphenol A as a dummy template *via* sol–gel processing giving rise to a MIP, which achieved simultaneous detection of 9 organochlorine pesticides. This MIP demonstrated rapid adsorption kinetics, and bound 30.01 mg g^{-1} of BPA, compared to 14.23 mg g^{-1} for the NIP. Application as both pre-concentration and clean-up adsorbents in SPE on spiked samples gave recoveries of 83.82–112.68% (water), 65.65–129.61% (soil), 74.95–121.64% (rice) and 66.31–119.49% (tea leaf) for the 9 organochlorine pesticides investigated,[110] thus establishing group selective sorbents based on this dummy template approach highly suitable for application in aqueous environments.

4. Group selective molecularly imprinted polymers: a promising strategy for monitoring of polychlorinated aromatic compounds

By far and large, synthesis of MIPs has predominantly focused on using a single template species to create binding sites. However, the emergence of 'group-selective MIPs' (gMIPs) or 'class selective MIPs' is increasingly prevalent, which enables molecular recognition for a group of closely related compounds rather than an individual constituent.[10] The two commonly applied approaches for the synthesis of such gMIPs are using template mixtures (a.k.a., multi-template approach), or a single compound as a generic structural analogue. Multi-template approach has successfully been demonstrated by Lieberzeit *et al.*,[172] Song *et al.*[11] and Duan *et al.*,[173] using a mixture of 2 PAHs, 16 PAHs and 5 acidic pharmaceuticals, respectively, to produce MIPs with recognition properties for these compounds. The polymers were successfully applied in fluorescence sensor, as SPE sorbents for the determination of 16 PAHs in sea water, and for the enrichment of pharmaceuticals in environmental samples. For further reference, Krupadam *et al.*[174–177] have executed extensive studies on the use of PAH template-mixtures during the synthesis of selective sorbents for environmental applications.

On the other hand, using a single template serving as surrogate for an entire group of compounds takes advantage of structural analogies among a group of compounds. This approach saves on the amount of template required, and limits the problem of template bleeding. The viability of this approach for environmental studies was evidenced by Ho *et al.*[178] and Ndunda *et al.*,[98] who used benzo[*a*]pyrene (BaP) and PCB 15 to synthesize MIPs for the determination of 16 PAHs in cigarette smoke, and for 6 PCBs in sediment and soil. The approach owes its success to taking advantage of the usually avoided cross-reactivity resulting from structural analogues. Rather than being a curse, this phenomenon is used as a blessing, especially for polychlorinated aromatic compounds which occur as congeners or homologues, whereby 'group selectivity' would lead to their simultaneous enrichment and detection. Tapping into the advantages of 'group selective MIPs', *i.e.*, being able to work on a number of compounds in one synthesis approach, and reduced template requirement for single compounds as structural analogues, a number of class-selective MISPE cartridges – though not yet for PACs – have been commercialized by Sigma-Aldrich.[179]

5. Conclusions and outlook

The ubiquitous and toxic nature of PACs renders them among the most relevant pollutants in environmental monitoring. The present review discusses the contributions, promises, and challenges using MIPs as selective synthetic sorbents and molecular recognition elements for the determination and quantification of PACs at environmentally relevant low concentration levels. While imprinting of these constituents is challenging due to their lack in functional groups, limited quantities, and toxicity, these limitations can be circumvented *via* particularly innovative imprinting strategies, including dummy template approaches, sacrificial spacer synthesis, and the incorporation of silica nanoparticles, magnetic nanoparticles, graphene, and carbon nanotubes as assistive materials for enhancing the selectivity and adsorption

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capacity. As an important aspect during the development of such challenging MIP matrices, the successful introduction of advanced molecular modelling schemes and spectroscopic tools for detailed analysis assist in designing high-quality MIPs for these constituents, and paves the way towards rational design strategies in lieu of trial-and-error experiments.

In addition, the elegant yet little explored technique using the xylenes and toluene as so-called porogenic templates for synthesizing polymers with selectivity for PCBs and PCDDs offers an attractive option for generating selective MIPs in absence of the actual target molecules. Furthermore, the opportunity to simultaneously determine several PACs *via* group-selective MIPs, which eliminates the need for synthesizing of a number of individual MIPs specific to one particular compound is highlighted.

Throughout this review it is evident that using dummy templates is prevalent for the synthesis of polymers selective to PACs, which appears to be advantageous for scaled-up synthesis of such MIPs at larger quantities. Last but not least, the commercialization of sensors and SPE cartridges based on such polymers would render the analysis of PACs in environmental monitoring scenarios more affordable given the reusability of these materials; furthermore, MIP-based sensors enable on-site detection, thereby simplifying sample pretreatment.

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