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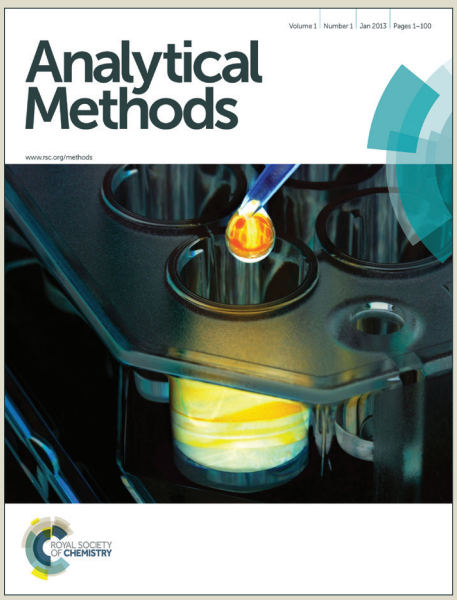

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ARTICLE TYPE

Selective extraction of light polycyclic aromatic hydrocarbons in environmental water samples with pseudo-template thin-film molecularly imprinted polymers

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An interesting approach to environmental analysis of trace level contaminants in the environment is molecular imprinting. This article describes the fast, low-tech preparation and evaluation of thin-film molecularly imprinted polymers (MIPs) for the specific uptake of light polycyclic aromatic hydrocarbons (PAHs) in complex aqueous matrices. Detection is carried out using an offline gas-chromatography-mass spectrometry (GC-MS). The use of toluene as a pseudo-template eliminates the problem of positive errors associated with incomplete template removal when the template and analyte are identical. These MIPs give a linear response in standard aqueous solutions and spiked wastewater over a concentration range of 10 to at least 100 µg L^{-1}, and superior selectivity over the non-imprinted materials. In MIP uptake studies for PAHs at environmentally relevant concentrations in spiked seawater samples, linearity was obtained over the range studied (0.5 − 5 µg L^{-1}) with R^2 values from 0.978 (naphthalene) to 0.997 (pyrene). Other than addition of standards for calibration, all environmental samples were used unmodified. Good recoveries owing to good selectivity, good response linearity and a good uptake trend overtime indicate these materials are suitable for *in situ* monitoring or remote monitoring.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) compounds represent a large class of non-polar organic compounds with a structure of two or more fused benzene rings. These compounds have been shown to possess carcinogenic and mutagenic effects such that monitoring guidelines for sixteen PAHs have been established by the United States Environmental Protection Agency (US-EPA). The PAHs with a smaller number of aromatic rings contained in the US-EPA list are mainly released into the environment in an unsubstituted or alkylated form from petrogenic sources (e.g. seeps), discharge of produced water, accidental spills or controlled release of petroleum products [1-4]. Depending on the solubility and hydrophobicity which vary with the molecular weight, PAHs ultimately end up in ground water, vegetation, soils and oceans [1,4,5]. Light PAHs in seawater could thus indicate crude oil or petroleum contamination in water, which is relevant in an era of deep sea oil extraction. An extensive range of articles and reviews have been dedicated to describing and proposing analytical methodologies for the determination of PAH in various matrices as reflected by a review [5]. Given the low concentrations of PAHs in the environment and the complexity of real samples, several requirements consistently appear, such as efficient sampling with no losses during storage (due to PAH hydrophobicity and volatility), effective pre-concentration and possibly clean-up steps, and methods that give efficient separations with selective detection and quantification [1]. Molecular imprinting technologies are useful in addressing most of these issues by selectively targeting PAHs in complex samples. Molecularly imprinted polymers (MIPs) are smart materials with remarkable molecular recognition properties that rely on the memory of shape, size and position of functional groups of the template molecule. The MIPs have a similar uptake mechanism to biological antibodies. The advantages of using MIPs are the chemical and physical stability, easy preparation and the ability to recognize small organic molecules [6]. Typically, the material is made by polymerization from a solution containing a functional monomer, a cross linking agent, an initiator, a porogen and a template (usually the target analyte). Since the template must be bound to the monomer either through covalent or non-covalent interactions prior to polymerization, the approaches for MIP preparation are often categorized accordingly. Non-covalent bonding occurs through hydrogen bonding, Van der Waals forces, π-π interactions, etc., and can be particularly useful because the template can be easily released and the analyte uptake is favoured by fast mass transfer [6].

For successful imprinting, the composition and all preparation steps – from identifying the most suitable components of the pre-polymerization complex to determination of the best template removal and analyte uptake conditions – must be optimized, most often through tedious experiments. For example, even a process as simple as template removal is key, where incomplete removal of the template can generate errors in accuracy (i.e. template bleeding) and may result in fewer cavities available for efficient re-binding. Template bleeding is an ongoing challenge and complete template extraction is hard to achieve [7]. Various template removal techniques have been reported, such as conventional solvent extraction, or Soxhlet extraction, sonication and supercritical solvent extraction, but most often the stability of the imprinted cavities is at risk when aggressive methods are used [7,8]. Alternatives to avoid these tedious steps have been proposed such as isotope molecular imprinting, parallel extraction on blank samples and pseudo-template imprinting, though these still have

drawbacks [9]. In this paper, pseudo-template imprinting was chosen based on toluene; its structure and functionality are similar to the target molecules and any residual template will not interfere with quantitation. In this case, the pseudo-template is smaller than the actual target analyte giving enhanced uptake of target analytes as reported [10,11], but is also in contrast to other work in which larger pseudo-templates were used [12].

MIPs can be polymerized as bulk polymers, suspension particles or as thin-films on an inert support or membrane. Thin-film MIPs eliminate long sample preparation steps, can be interfaced with sensors for on-site monitoring or can be coupled with offline detection [13]. There has been a steady interest in developing effective imprinted polymers for uptake of PAHs and the first report of a thin-film MIP for PAHs was published by Dickert and Tortschanoff, when polyurethane membranes were developed for use with a fluorescence detector, resulted in good sensitivity, and a prediction that a sensitivity of 30 ng L^{-1} should be achievable [14]. The versatility of these MIP membranes for various applications is demonstrated in the range of sensors to which they have been successfully coupled, e.g., the quartz microbalance, fluorescence and surface acoustic wave oscillator [14]. Although described mostly as proof of principle, Gonzalez *et al.* proposed a phosphorescence detector for thin-film MIPs for bisphenol A with sensitivities as low as 40 ng L^{-1} [15]. The most recent account of MIPs for PAHs is the work of Song *et al.*: MIP membranes were developed by sol-gel imprinting on a solid substrate of silica particles for traditional solid phase extraction coupled with GC-MS of PAHs in seawater [16]. Used as SPE packing, the large MIP surface area provided for detection limits ranging from 5.2 to 12.6 ng L^{-1} for a mass of polymer of 150 mg in extensive prior conditioning [16]. MIPs were also developed in bulk format for uptake of PAHs from air and water [17].

In this work the preparation of a novel thin-film MIP with a toluene pseudo-template is described for uptake of PAHs with detection by GC-MS in SIM mode and fluorescence. Binding assays carried out in aqueous standards or real influent wastewater samples show superior selectivity of the MIPs over the corresponding NIPs, though there is a linear response for both materials. The direct applicability of these MIPs to environmental samples at relevant concentrations with no prior sample preparation is demonstrated by linear calibration curves with excellent coefficients of variation obtained for analysis in raw seawater and wastewater and good recovery concentrations in wastewater samples. The method gives sensitive detection limits for real water samples, for example as low as 18 ng L^{-1} for naphthalene from spiked municipal wastewater.

Fig 1. MIP fabrication process with illustration of imprinting.

Experimental

Materials

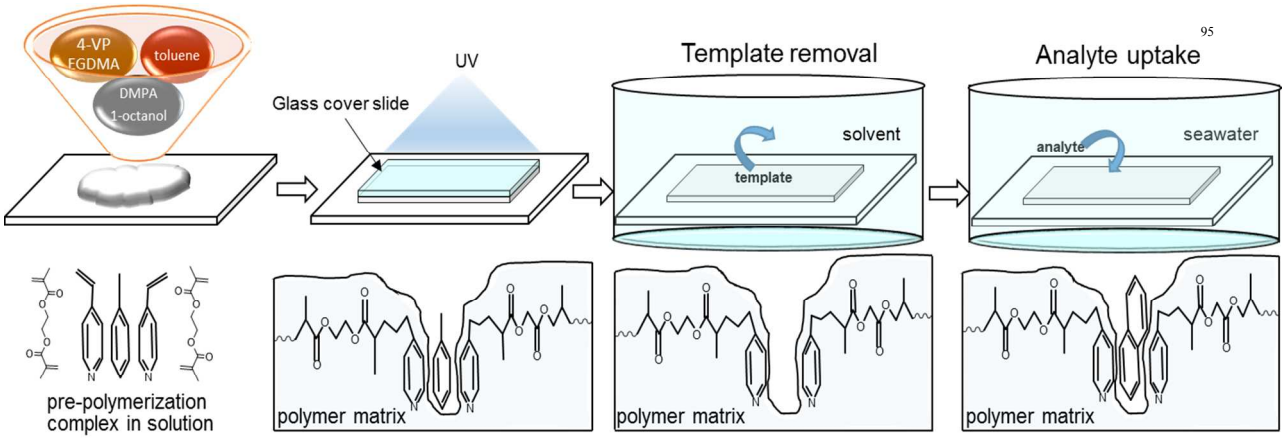
Naphthalene, fluorene, phenanthrene and pyrene were used as models for PAH classes with different ring numbers as shown in numbers S-Table 1 in Electronic Supplementary Material, ESM. Standards of naphthalene (99%), fluorene (99%), phenanthrene (99.5%) and pyrene (99%) were purchased from Sigma-Aldrich (St. Louis, Mo, USA) and were used without further purification. All organic solvents (toluene, ethyl ether, dichloromethane, acetonitrile and hexanes) were purchased with minimum 99.5% purity from ACP Chemicals. All compounds used in the preparation of the thin-film MIPs: 3-(trimethoxysylil) propyl methacrylate derivatizing agent, the 2,2-dimethoxy-2-phenylacetophenone (DMPA) initiator, ethylene glycol dimethacrylate (EGDMA) cross-linker, 4-vinyl pyridine (4-VP) monomer were purchased from Sigma-Aldrich in the best grade available and were used without further purification.

Derivatization of glass slides

Glass microscope slides were prepared for derivatization by cleaning in a solution of MeOH and HCl. The slides were then immersed overnight in a solution of 2.0% 3-(trimethoxysylil) propyl methacrylate in toluene. The resulting functionalized surface was rinsed with ethanol, dried under nitrogen and stored in the dark.

Preparation of the MIPs

The pre-polymerization mixture was prepared by pipetting or by weighing into a 2 mL vial the pseudo-template toluene, initiator DMPA, crosslinking agent EGDMA, monomer 4-VP and porogen 1-octanol (S-Table 2 in ESM). Figure 1 illustrates the MIP fabrication process. The pre-polymerization complex for the non-imprinted polymer was prepared similarly, but without the pseudo-template. The solutions were vortex-mixed until all components dissolved. Mixtures were degassed for five minutes to remove oxygen that may interfere with the polymerization process.



An 8.0 µL aliquot of the pre-polymerization complex was dispensed on the derivatized glass surface and covered with a glass microscope cover-slide. This "sandwich" was placed directly under a UVP handheld UV lamp (λ = 254 nm, 6 watt) at ambient temperature (~20 °C) for 30 min. Following removal of the cover slides, the imprinted polymers were soaked in ethyl ether for two hours to remove the pseudo-template and unbound polymer components.

Upload and analyte extraction

Stock multi-component solutions with each PAH at a concentration of 100 mg L^{-1} were prepared in acetonitrile daily. Working solutions were prepared by appropriate dilution of the stock solutions with distilled water. For binding tests, a thin-film MIP was immersed in 80.0 mL of aqueous PAH solution under continuous stirring at room temperature (approximately 20 °C) for specified time intervals.

The MIPs were removed from the upload solution, rinsed with a small volume of water and dried briefly with a stream of nitrogen to remove visible water. The analytes were extracted in 10.0 mL of ethyl ether under stirring for two hours. The solvent was removed from the extract under reduced pressure with a rotary evaporator and made to volume with DCM in a 1.00 mL volumetric flask. Concentrations of PAHs measured in these solutions are referred to as recovery concentrations in this paper. Depending on the type of test, other factors were considered, such as concentrations of PAHs or upload times were varied.

Characterization of MIPs

The physical characteristics of the MIPs and NIPs were established by studies of morphology by SEM and film thickness using a KLA-Tencor Alpha Step Development Series Stylus Profiler D-120 profilometer. SEM images were recorded using a FEI MLA 650F SEM, operating at an accelerating voltage of 10 kV and a magnification of 50,000 times. All samples were sputtered with gold prior to analysis.

Analytical performance criteria were characterized with GC-MS and fluorescence. GC-MS was performed with an Agilent Technologies 6890 gas chromatograph coupled to an Agilent 7683 Series Injector and Agilent 5973 inert Mass Selective Detector (MSD). Compounds were ionized by electron ionization at 70 eV and mass spectra were acquired in selected ion monitoring (SIM) mode (S-Table 3 in ESM). Separation was carried out on a DB-5MS column capillary column (0.25 mm x 30 m) with a 0.25 µm stationary film thickness. The oven temperature was programmed as follows: initially at 45 °C (held for 0.8 min), increased to 200 °C at a rate of 45 °C min^{-1}, then to 216 °C at a rate of 5 °C min^{-1}, and finally to 260 °C at a rate of 10 °C min^{-1}. The total time for all analytes to be detected was ~12 min. The injector temperature was set at 290 °C, and the injection was performed in splitless mode. The carrier gas, helium with a purity of 99.999% had a flow rate of 1.3 mL min^{-1}. The calibration curves for PAH determinations were obtained by linear regression of data from analysis of multi-component solutions made in three concentrations ranges for each PAH: 0.500–10.00 µg L^{-1}; 10.00–100.0 µg $L^{-1}$and 100.0–3000 µg L^{-1}. Fluorescence measurements were performed with a PTI (Photon Technology International) QuantaMaster 6000 Fluorometer. The excitation wavelength was 276 nm and data were collected at an emission wavelength of 303 nm.

Results and discussion

MIP preparation

Novel white opaque thin-film MIPs using toluene as a pseudo-template were prepared rapidly, using little organic solvent and low tech equipment: such as a vortex mixer, a sonicator and a hand held UV-lamp. SEM imaging of the imprinted polymer shown in Figure 2 suggested a high degree of porosity.

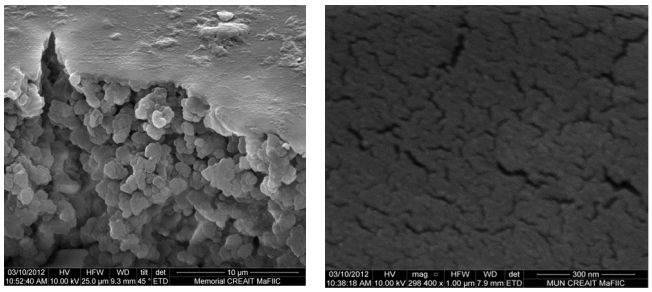


Fig. 2 SEM micrograph of the MIP at different scales

Thickness measurements of the MIP and NIP films showed a similar thickness for MIPs of 66.1 ± 0.90 µm as for NIPs of 65.6 ± 0.46 µm. A direct correlation was not found between the mass of the polymer, which varied from 2.10 - 3.71 mg for a constant surface area of 2.25 cm^2, and uptake of analyte. This is consistent with the idea that binding in these materials occurs near the surface, thus it was unnecessary to normalize the detector response to the mass of polymer in every instance. Because of the low mass of the polymer thin-film and the large relative mass of the glass slide limiting weighing to a four decimal place balance, data would have been limited to three significant figures when normalizing.

The main novelty of these MIPs lies in the use of a pseudo-template that is smaller than the target analytes. The pseudo-template approach has obvious advantages when it comes to template bleeding and for use with PAHs in particular, where even large PAH molecules will have substructures that are very similar to the structure and chemistry of toluene. Initially, we investigated the effect of the size of the various PAH templates on the uptake of key PAH targets. We noticed that use of phenanthrene as the template increased selectivity towards uptake of pyrene from a solution containing equal concentrations of pyrene and naphthalene. This was contrary to the expected "steric exclusion effect" anticipated for compounds larger than the template [12]. The reasoning behind it is that for some systems fast mass transfer kinetics favour small molecules with the optimum steric fit for accessible sites. Other authors reported that addition of 20% naphthalene to the pyrene template in the pre-polymerization solutions gave a significant improvement in the uptake of pyrene compared to pyrene or naphthalene templates alone [10,11]. To test the hypothesis that a smaller template can give MIPs with improved selectivity towards larger targets, PAH uptake from a 0.100 µg L^{-1} PAH multi-component standard solution was measured for a series of MIPs made with different templates (toluene, naphthalene, fluorene, phenanthrene and pyrene) in an octanol porogen (S-Figure 1 in Electronic Supplementary Material, ESM). The small template was selected from a range of compounds with one aromatic ring, with consideration given to lack of substitution by heterofunctionality and the toxicological profile.[18] Generally, all MIPs showed a good sensitivity and selectivity towards PAHs, with a higher uptake of the analyte by the MIP made with matching the template. However, since the analytes are analyzed as amount taken up by the polymer (as opposed to amount remaining in uptake solution), the

possibility of template bleed cannot be fully excluded and the final assessment of the performance of a given MIP was based on the extraction efficiency for non-template targets, for example, a fluorene MIP toward phenanthrene or naphthalene. The highest uptake for all PAHs (S-Figure 1 in ESM) was observed for the toluene MIP, thus, the results of the performance of this new thin-film pseudo-template PAH MIPs are reported and discussed here.

The selection of the other components of the pre-polymerization complex was guided by extensive trial and error experiments, but the options with respect to composition tested were based on those proposed by others for non-polar targets [3,17,19,20]. The final MIP composition used in the performance testing reported here was made from 4-VP as functional monomer, EGDMA as cross-linking agent and 1-octanol as porogen (S-Table 2 in ESM). Some special attention was given to the selection of the appropriate porogen, as it influences the physical and chemical characteristics of the MIP, and it must also solubilize all the components of the pre-polymerization mixture while ensuring a stable chemical environment during polymerization. As reported previously, the polarity and hydrogen bonding capacity of a porogen influence the growing polymer chain during the phase separation process, and thus affect the morphology of the imprinted polymer [21]. Therefore, for a non-covalent imprinted polymer, a non-polar porogen with a low dielectric constant can substantially increase the selectivity of an MIP [21]. A number of solvents were tested as porogens, DCM: MeOH: H_2O (1:3:1), toluene itself ($\varepsilon = 2.38$), ethyl ether ($\varepsilon = 4.3$), and octanol ($\varepsilon = 10.3$), with octanol giving the best analytical performance. We believe that octanol performed well because, although it is generally considered hydrophobic, it has some polar character. The result is a material with good wetting properties when in contact with water, but also appropriate non-polar characteristics for binding hydrophobic compounds like PAHs as reflected by their high log K_{ow} range values: naphthalene, log $K_{ow\ (naphthalene)} = 3.5$ to log $K_{ow\ (pyrene)} = 4.9$ (S-Table 4 in ESM).

To determine the optimum template and analyte extraction solvent, we looked for a compound with a similar polarity index to toluene ($P'_{toluene} = 2.4$) to effectively match the template but lacking aromaticity to avoid any binding to the polymer network through π-π interactions, that could possibly inhibit uptake of PAHs. We found that ethyl ether with $P'_{ethyl\ ether} = 2.8$ was effective, it is also volatile, which makes the analyte extracts easy to concentrate.

The polymerization and uptake conditions were optimized through a series of experiments that will not be presented in detail here. For example, we found that stirring is a factor that influences considerably the uptake. MIPs were left for upload in 80.0 mL of 10.0 µg L^{-1} multi-component PAH solutions for 18 hours in stirring and standing modes (S-Figure 2 in ESM). Analyte uptake was three times higher in dynamic mode than in standing mode. Stirring favoured replenishment at the surface of the diffusion layer with new analytes, which in turn favoured frequent interactions of PAHs with the cavities and a higher uptake.

Binding capacity of MIP

In non-covalent imprinted polymers the template-monomer interactions are weak such that equilibrium has to be shifted towards using all the template molecules by addition of monomer in excess. Despite this, only 10-15% of the template molecules are used to form high affinity binding sites in non-covalent imprinting, and cavities with varying structure and affinity are formed [21-23]. This results in heterogeneous MIP surfaces and non-linear response curves in sensors [23]. Optimization of the imprinting process could afford systems that yield linear response curves with varying slopes for distinct concentration ranges. Furthermore, the amount of excess non-complexed monomer is distributed randomly into the polymer network, adding functionality to the polymer surface outside of the binding cavities. This may be one of the factors responsible for non-specific binding in both MIPs and NIPs [24].

To evaluate the selectivity of the MIPs and NIPs, a series of two hour binding experiments in aqueous PAHs solutions with concentrations in the 10.0-120 µg L^{-1} were run. Figures 3 a) and b) show the uptake of PAHs by MIPs and NIPs in terms of the peak area with respect to the initial upload concentration. The slopes show that sensitivity is higher for the MIPs than for the NIPs.

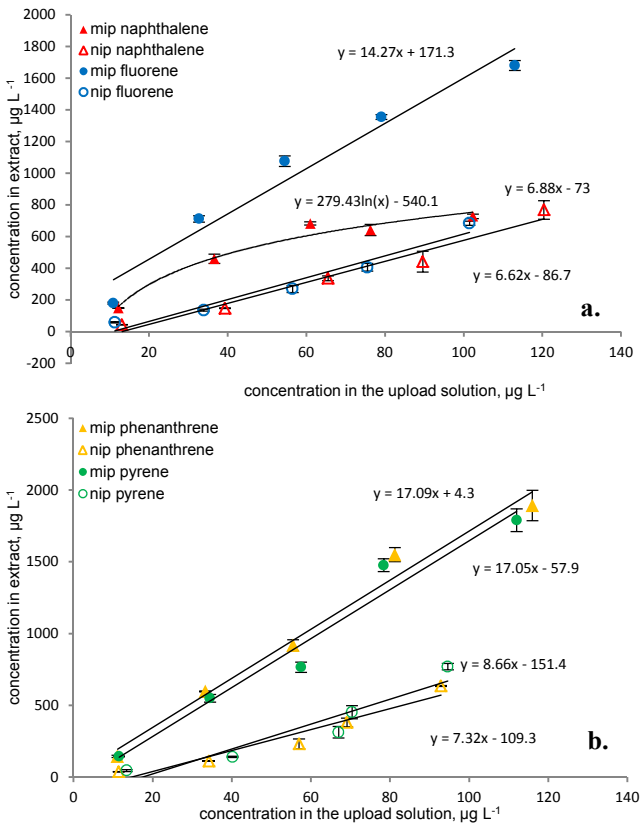


Fig. 3 Increase in concentration detected in the extract with the concentration in the initial upload solution; the concentration in the extract is determined by comparison to a calibration curve for MIPs and NIPs; experimental conditions of 80.0 mL sample for two hours, GC-MS in SIM mode. Bars represent standard deviation

The higher overall linear sensitivity of MIPs is indicative of a higher affinity of the MIPs for PAHs, which can be attributed to selective binding through π-π type interactions between the PAHs and the functionalized polymer network as well as conformational aspects of molecular recognition [14]. The MIPs showed the highest sensitivity toward pyrene and phenanthrene, followed by fluorene. All the curves show a clear linear relationship between uptake and concentration, except in the case of naphthalene (curved line in Figure 3a)), which starts to level off around 60.0 µg L^{-1}. It is likely that naphthalene uptake is inhibited by the increase in concentration of other larger, more hydrophobic PAHs; further data demonstrating this competitive effect will be presented in this paper.

Some PAHs, such as fluorene and phenanthrene have angled geometries (thus lower electron densities), while naphthalene and pyrene have planar structures and higher electron density [16]. A bent geometry can enhance binding into the cavities with appropriate shape and functionality, but changes in their conformations can also be distorted to fit in smaller cavities. These

aspects of geometry combined with the fact that hydrophobicity of PAHs increases with molecular mass, provide an MIP material with a higher selectivity towards phenanthrene, pyrene and fluorene. Similar conclusions were reported previously [16].

The NIPs showed uptake even in absence of the selective cavities associated with the presence of template. To some extent this was anticipated; the PAHs can interact with the aromatic monomers through π-π interactions, as well as the bulk polymer through hydrophobic effects. This is supported by the consistent level of sensitivity for all PAHs as reflected by the regression data in Figure 3, which also highlights the non-selective uptake by the NIPs.

Characterization of heterogeneity

To assess the heterogeneity of the MIP surface several types of binding assay experiments can be used, for example, concentration in the uptake solution can be kept constant and the exposure intervals are varied, or concentration can be varied for a specific time period. In this work, an experimental binding isotherm was obtained by equilibrium binding experiments at a constant upload concentration of 10.0 μg L^{-1} over a range of time intervals that varied from 30 min to 23 h. The amount of analyte extracted was measured directly by GC-MS in SIM mode. We also measured the residual concentration by fluorescence, thereby indirectly determining the amount extracted by calculating the decrease from the initial concentration. We found that it was challenging to accurately determine by fluorescence low concentrations of individual PAHs in multi-component mixtures. Nevertheless, characterization of the MIP performance using fluorescence measurements for total PAHs were still quite useful and the uptake calculated displayed a similar trend to the results obtained by GC-MS.

In a first binding assay, where the upload time was varied for a constant concentration, a steady linear PAH upload was observed for the first three hours. With increase in the upload time, the amount of PAHs levelled out giving a typical adsorption isotherm (Figure 4). This could be useful for a rapid analysis of PAHs when a high uptake could be reached within the first three hours. For this reason, most uploads in the binding assays presented here were carried out within two hours. If time was not an easy factor to control, which is the case in environmental remote sensing, then uptake carried out in the 4 to 23 h range could ensure a constant maximum response.

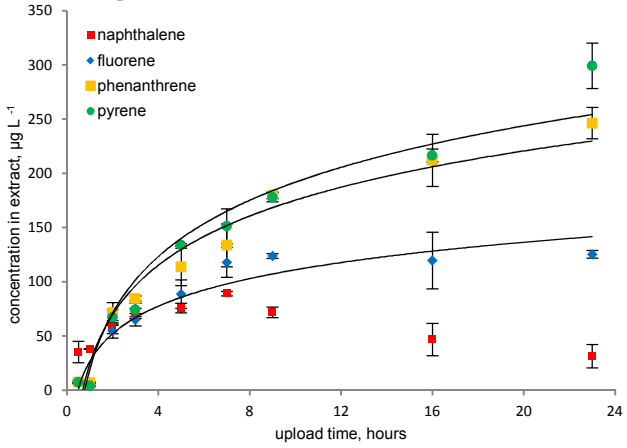


Fig. 4 Effect of upload time on the detected concentration in the extract for four PAHs; experimental conditions of 80.0 mL sample (10 μg L^{-1}), GC-MS in SIM mode. Bars represent standard deviation.

The shape of the response curve over time may be rationalized by understanding the chemistry of the MIP, where the material is thought to be made of both highly selective sites with appropriate geometry and chemistry, which should show more rapid binding behaviour. The uptake process reaches an equilibrium level after nine hours. All the PAHs fit this adsorption isotherm profile, except for naphthalene (Figure 4), which decreases over time. A possible explanation for this effect may be the semi-volatile nature of naphthalene. This has been observed and reported previously, where it was shown that storing a solution containing naphthalene in an opened container leads to loss of the analyte within very short time intervals [25]. Also, to a certain extent, adsorption into the glass surface could be considered.

Fluorescence was used to determine the residual concentration of PAHs in the above experiment. A low intensity signal was obtained for naphthalene and fluorene, such that the concentration of the PAHs was expressed as the overall amount of PAHs.

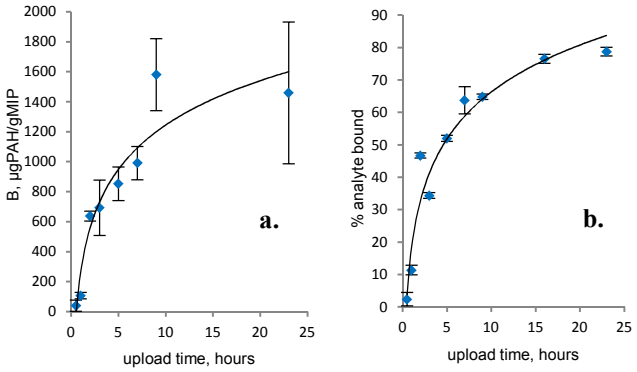


Fig. 5 Effect of upload time on the total concentration of four PAHs in the extract for a 80.0 mL sample (10 μg L^{-1}) **a.** overall binding capacity expressed as $B = \frac{\mu g\ PAH}{g\ MIP}$, measured by GC-MS in SIM mode **b.** percent recovery in aqueous PAH multicomponent solution expressed as $\% = \frac{m_{PAHs\ bound\ to\ the\ MIP}}{m_{PAHs\ in\ aqueous\ solution}} x\ 100$, fluorescence measurements. Bars represent standard deviation.

The fluorescence data in Figure 5b) gives a similar adsorption curve to the overall amount of PAHs detected by GC-MS, in Figure 5a). This confirms that both the more selective and direct measurements by GC-MS are suitable to the purpose, and that in this instance the mass of the polymer is not relevant in assessing the binding properties of the thin-film MIPs, which may also indicate that uptake is mainly a surface or near-surface phenomenon.

Longer exposure times may be needed for *in situ* monitoring applications and longer experiments could also yield improved detection limits, thus binding assays for linearity and capacity (Figure 6) were carried out over a longer period of time. MIPs were equilibrated for 19 h in multi-component PAH aqueous standards at varying concentrations: 1.00; 4.00; 6.00; 10.0; 30.0; 50.0 and 80.0 μg L^{-1} with four replicates for each system. Direct analysis of the extracts by GC-MS showed a linear trend with high correlation coefficients for all the PAHs (Figure 6) even after exposure of the MIPs to concentrations higher than 80.0 μg L^{-1}.

Pyrene and phenanthrene showed highest upload, as with the previous study (Figure 3.b).

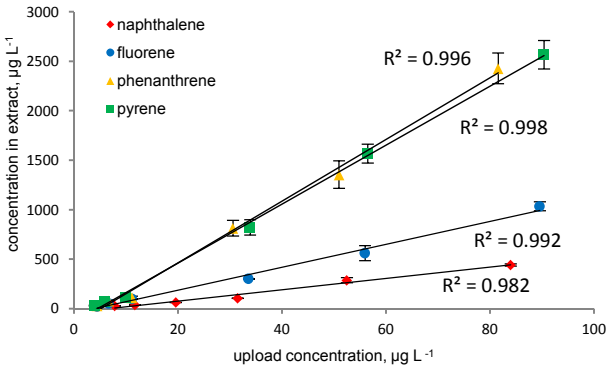


Fig. 6 Increase in the total detected concentration in the extract with increase in the upload concentration; experimental conditions: upload over 19 hrs; GC-MS measurements. Bars represent standard deviation.

Inhibition studies

Since applications to real-world samples involve highly complex mixtures of PAHs and other components of oil, it was interesting to assess the degree of competition for binding sites between the different PAHs in the uptake process. These interactions were evaluated by comparing the percent recovery when the MIP was exposed to pure solutions of 10 µg L^{-1} of naphthalene or 10 µg L^{-1} pyrene single PAH solutions versus a 10 µg L^{-1} naphthalene and pyrene multi-component solutions [10,12]. The MIP showed the most consistent uptake for pyrene, with similar results for both experiments involving pyrene: 11.9 % ± 1.52 recovery when in presence of naphthalene, versus 13.5 % ± 3.19 recovery from a single-component solution. This indicates a high selectivity of the MIP towards pyrene. The uptake of naphthalene was lower in presence of pyrene by a factor of two (from 12.5 % ± 1.15 recovery when singly in solution to 6.3 % ± 0.24 when in presence of pyrene), which indicates a high degree of competition for binding sites. This may be related to the higher hydrophobicity of pyrene that enhances its interactions with the polymer network.

The interference in the selective uptake of PAHs caused by a non-PAH compound, octanol, a mostly nonpolar alcohol with a chain like structure, was determined through binding experiments in 80.0 mL of 0.5 µg L^{-1} multi-component PAH solutions over 2 hours. As can be seen from percent recovery (Table 1) the presence of octanol does have an inhibitory effect, with the uptake of the analytes about half of what is seen with the interfering species.

Table 1. Interference of octanol in the adsorption of PAHs into MIPs; experimental conditions: concentration 0.50 µg L^{-1} for each compound in 80.0 mL; GC-MS measurements

	In presence of 1-octanol		In absence of 1-octanol	
PAH	Initial PAH concentration, µg L^{-1}	Percent recovery	Initial PAH concentration, µg L^{-1}	Percent recovery
naphthalene	0.555	27.6 %	0.555	47.0 %
fluorene	0.505	19.0 %	0.545	35.3 %
phenanthrene	0.520	21.8 %	0.560	34.2 %
pyrene	0.520	11.9 %	0.540	28.3 %

Given its relatively long alkyl chain, it is unlikely that octanol would compete for selective sites within the polymer network. Thus non-selective interactions are likely to be responsible for the difference in overall percent recovery. In addition to occupying potential non-selective binding sites, the hydrophobicity of octanol may also reduce the interactions of other PAHs with the polymer surface by inhibiting their approach to the polymer surface. This data, obtained at low upload concentrations, shows that the highest recovery is for naphthalene, which seems to contradict results presented earlier indicating higher affinity for the larger more hydrophobic molecules. This can be attributed to a higher diffusion rate for the smaller naphthalene and the presence of a limited number of sites with high affinity for the smaller aromatic compounds more similar to the template toluene. The data is consistent with the results presented in Figure 4 (uptake from a 10 µg L^{-1} multicomponent standard over time), where the naphthalene shows the highest uptake early in the experiment when the high affinity sites are being populated.

This conclusion is supported by the result for phenanthrene, where the impact on recovery due to octanol is lower and the hydrophobicity of phenanthrene is the highest among the group studied.

Real samples

The applicability of these novel systems for the detection of PAHs in aqueous samples was determined by MIP analysis in influent water samples from Riverhead Waste Water Treatment Facility and seawater samples collected from the St. John's harbour.

Assuming there is no response when no analyte PAH is present, MIPs and NIPs were exposed to wastewater samples with added 50.00 µg L^{-1} multi-component PAH standard for two hours (Table 2). Four replicates were used in each case. After recovery, the extracts were analysed by GC-MS, in SIM mode. The concentration of PAHs in the wastewater was determined by the standard addition method. A total concentration of 103.3 µg L^{-1} PAHs was detected with the MIPs in the wastewater samples. This is remarkable for a polymer with a mass close to 3 mg. Uptake by the NIPs was approximately five times lower at 19.07 µg L^{-1} total PAHs.

Table 2. Concentration detected (µg L^{-1}) in waste water samples for PAHs using MIP and NIP extraction coupled to off -line GC-MS

PAHs	NIP Concentration detected	MIP Concentration detected
naphthalene	4.17 ± 2.17	18.83 ± 0.46
fluorene	5.06 ± 2.51	28.70 ± 3.16
phenanthrene	6.84 ± 0.66	32.34 ± 1.61
pyrene	2.99 ± 0.0096	23.45 ± 1.57
total PAHs	19.07 ± 3.38	103.33 ± 3.90

Similar MIP and NIP studies to evaluate linearity to those illustrated in Figure 3 were carried out in wastewater samples in similar conditions and without any prior sample pretreatment (S-Figure 3a) and b) in ESM). The response of the MIPs was lower in the wastewater samples; however the MIPs versus NIPs uptake and the different individual PAHs uptake trends were similar in both studies.

The applicability of this MIP system to seawater was evaluated by carrying binding assays in samples spiked with low concentrations of PAHs in the 0 to 5.0 µg L^{-1}. The results (Figure 7) were remarkable considering the high linearity coefficient obtained for all the PAHs, the low concentration of PAHs present in solution and the lack of any preparative steps.

The limit of detection was calculated for the MIP from the standard deviation near the lowest detectable concentration. The detection limit was found to be 18 ng L^{-1} for naphthalene, 54 ng L^{-1} for fluorene, 270 ng L^{-1} for phenanthrene and 1.5 µg L^{-1} for pyrene. The detection limit was lower for almost all PAHs than the maximum allowable contaminant level in drinking water of 0.2 µg L^{-1} for PAHs as stipulated by the United States EPA [18].

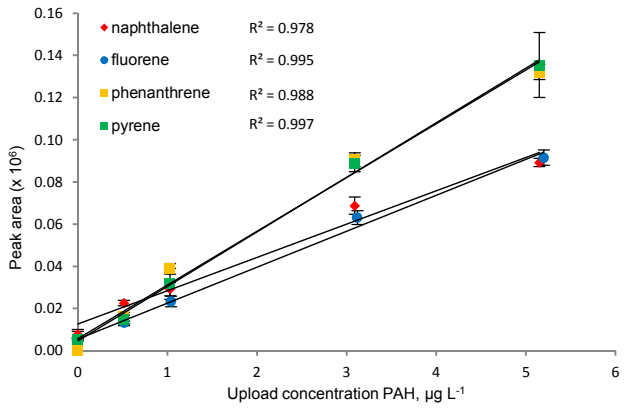


Fig. 7 Calibration curve constructed as the relative response against the upload concentration in raw sea water; experimental conditions: upload 2 h; GC-MS measurements. The response is the mean value of three measurements. Bars represent standard deviation.

Considering the recorded concentrations of light PAHs in produced water from various oil platform areas (Gulf of Mexico, North Sea and Grand Bank) (S-Table 4 in ESM), the polymers could be used for detection in complex matrices and to meet regulatory monitoring requirements [26].

Conclusions

A novel thin-film MIP with a toluene pseudo-template was developed for detection of PAHs. The suitability of this MIP for aqueous environmental monitoring has been demonstrated with selective uptake from various complex matrices. Surface and binding studies suggested that the MIPs are porous, but that selective adsorption occurs mainly at and near the surface. To a certain degree, NIPs were found to be sensitive toward PAHs which they owe to the optimized pre-polymerization composition. Binding assays for the MIPs showed a saturation of the selective cavities with increase in the upload time but a linear response with increase in concentration. Analysis of MIPs in real environmental samples showed very good recovery capacity and detection limits in wastewater, and excellent sensitive and linear response in seawater for individual PAHs. These findings indicate a great potential for use of the MIPs for in-situ real time environmental monitoring of light PAHs given the simple low tech method we proposed that includes the preparation of the polymer as well as the use of a simple detection system, a GC-MS with a simple quadrupole analyzer.

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