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Novel Molecularly Imprinted Polymeric Microspheres for Pre-concentration and Preservation of Polycyclic Aromatic Hydrocarbons from Environmental Samples

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Abstract

Molecularly imprinted polymer (MIP) microspheres with diameter in the range 60 – 500 µm were synthesized in a continuous segmented flow micro-fluidic reactor and used as packing material of microtraps for the selective separation of benzo[a]pyrene (BAP) from environmental aqueous samples.. The procedure for synthesis involved the pumping of mono-disperse droplets of acetonitrile containing methacrylic acid as the functional monomer, BAP as a template, and ethylene glycol dimethacrylate as the cross-linking monomer into the microchannels of the microfluidic reactor. The microspheres showed high adsorption capacity and selectivity for BAP in aqueous solutions, both important for the environmental monitoring and analysis of BAP. The adsorption capacity for BAP of the smallest MIP microspheres (size range 60 - 80 µm), prepared as part of this study, was 75 mg g^{-1} in aqueous solutions, furthermore, this adsorption capacity was close to 300% higher than that of commercially used activated carbon. Microtraps packed with MIP retained BAP intact for at least 30 days while the activated carbon packed microtraps for BAP showed 40% reduction in BAP concentration for the same period. The present study has demonstrated that the MIP microtraps have significant potential for the selective enrichment and preservation of targeted polycyclic aromatic hydrocarbons (PAHs) from complex environmental samples.

Keywords: environmental trace analysis; molecularly imprinted polymers; polycyclic aromatic hydrocarbons; benzo[a]pyrene (BAP)

1. Introduction

In recent years there has been an increasing public and scientific concern about chemicals which are known to cause cancer and affect normal hormonal functions in humans and wildlife. Treated industrial wastewater can induce carcinogenicity and estrogenic activity in recipient waters up to several kilometers from the source point , with feminization in male fish indicated by imposex condition and also development of cancer in wildlife and humans [1,2]. Carcinogenic contaminants are also introduced into the environment by fossil fuel combustion, discharge of recycled water used in industry and agriculture and domestic, industrial and agricultural effluents into waterways as well as leaching from industrial sludges. Polycyclic aromatic hydrocarbons (PAHs) are of major concern as environmental pollutants because they are persistent carcinogens originating from incomplete combustion of fossil fuels, industrial stack emissions, oil spills and in smaller quantities from forest fires and microbial synthesis [3]. U.S. Environmental Protection Agency (U.S. EPA) and the European Community have listed PAHs as priority pollutants [4,5]. A concerning issue is that even low concentrations of PAHs in air and water resources could have serious health impact on humans and wild life. The concentration of the PAH marker molecule, benzo[a]pyrene (BAP) in air and water ranges from 0.1 to 500 ng m^{-3} and needs to be monitored regularly as per the guidelines of environmental regulatory agencies. PAHs go undetected frequently because of inadequacy of the assays used. Another important issue is the complexity of environmental matrices. The concentration of target pollutants ranges between 0.001 and 0.1%, which is quite low [6] and therefore separation and preconcentration of these pollutants is required prior to their analytical measurement. Selectively adsorbing the targeted pollutants on adsorbents is one of the most widely used methods for sample preconcentration. The most commonly used adsorbents for analyte separation and preconcentration are activated carbon and polystyrene-divinylbenzene resins (XADs). The main problem associated with traditional adsorbents is the low selectivity of their retention mechanism [7-9]. A desired grade of selectivity can be obtained by using columns packed with materials based on well defined molecular recognition properties, which are able to bind the targeted analyte with a high degree of selectivity.

For the last few years molecular imprinting has emerged as a powerful technique for the preparation of highly selective polymeric substrates [10-14]. The technique entails polymerization around a template (analyte) using monomers with chemical functionalities complementary to those of the template. The interactions developed between complementary functionalities present in the template molecule and the monomer(s) prior to the initiation of polymerization are conserved in the resultant polymer. Subsequent removal of the template exposes recognition sites within the polymer which exhibit a "memory" for this template in terms of complementarity of both shape and chemical functionality. Molecularly imprinted polymers (MIPs) have several distinct merits that make them attractive for molecular recognition, i.e., stability of imprinted structures and wide variety of molecules amenable to imprinting. Several articles describe MIPs capable of metal ion uptake [15] and organic micropollutants [16-21] from environmental samples. Chen et al. [17] have developed imprinted polyethersulfone particles by using a phase inversion technique which exhibits selectivity for bisphenol-A versus biphenyl and phenol. More recently, a naphthalene derivative has been imprinted using a -traditionally formulated polymer, employing methacrylic acid (MAA) as the template interactive monomer and ethyleneglycol dimethacrylate (EGDMA) as the cross-linker. Competitive rebinding tests with an array of PAH compounds showed selective binding for naphthalene and even greater selectivity for the significantly larger phenanthrene [18]. In a series of recent articles, we have described MAA/EGDMA-based MIPs imprinted with PAHs, such as benzo[a]pyrene or PAH mixtures, that exhibit molecular recognition for PAHs in water samples [19-21]. Recently Song et al. (2012) prepared MIPs through non-covalent polymerization by using a mixture of 16 PAHs as a template and sol–gel surface imprinting [22]. Compared with the non-imprinted polymers (NIPs), these MIPs exhibited excellent affinity towards the 16 PAHs studied with a binding capacity of 111.0–195.0 $\mu g\ g^{-1}$, and imprinting factor of 1.50–3.12. High sensitivity was attained, characterized by limits of detection for the 16 PAHs in natural seawater ranging from 5.2–12.6 $ng\ L^{-1}$. MIPs with high affinity and excellent stereo-selectivity towards PAHs offer an attractive alternative to conventional sorbents in the solid-phase extraction (SPE) of these compounds from contaminated water. It has been largely demonstrated that MIPs offer the highest selectivity when

samples are dissolved in a solvent used for the MIP preparation. This solvent is able to recreate the interactions that have taken place during the MIP polymerization. One of the drawbacks of the conventional molecular imprinting approach is that the polymer monolith produced from bulk polymerization must be crushed and ground to small particles. The mechanical crushing of the polymer monolith leads to the formation of irregular heterogeneous surfaces and loss of effective binding sites in the polymer [23]. The drawbacks outlined above can be overcome by manufacturing MIPs in the form of microspheres.

This study reports on the use of a microfluidic reactor for the manufacturing of MIP microspheres of defined size with high selectivity and capacity for BAP which are expected to be suitable for monitoring of PAH pollution in sediment near offshore oil and natural gas production facilities. The binding interactions between PAHs and MIPs require the use of apolar organic solvents, which complicates the use of MIPs for environmental monitoring and remediation applications. Hence, this issue was studied in detail and efficient procedures for PAHs' binding to MIPs in aqueous solutions were developed.

An important constraint in environmental trace analysis is the time gap between sample collection and laboratory analysis; which can range in some extreme cases between 15 and 30 days [24]. During this period, the trace amount of target analyte may undergoe microbial decomposition. The preservation potentials of microtraps packed with MIP microspheres, control polymer and XAD have been compared for periods of up to 30 days during marine sediment monitoring. A comparison of the binding properties of MIPs prepared by traditional methods and by a microfluidic reactor, proposed in this study, has been also conducted.

2. Experimental

2.1. Reagents and solution preparation

The polycyclic aromatic hydrocarbons - benzo[a]pyrene (BAP), chrysene (CHR), pyrene (PYR) (Fig.1) and polymer precursors - methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and 2,2'-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (Buchs, Switzerland). Methacrylic acid was distilled under vacuum to remove inhibitors before use. AIBN

was crystallized with ethanol. Acetonitrile and acetic acid of HPLC grade, sodium dodecylesulfate (SDS), and polyvinyl alcohol (PVA 4-88) were purchased from Merck (Darmstadt, Germany) and used as received. Deionized water (e.g. 18.2 MΩ cm, Millipore, France) was used for the preparation of the aqueous solutions. 100 mg of BAP was dissolved in 1 mL of acetonitrile and then made up to 100 mL with deionized water. This stock solution (1000 mg L^{-1}) was diluted to prepare the standard solutions for the adsorption experiments. The powdered activated carbon (PAC) of high purity and an average particle size of 150 μm used in this study was manufactured by A.B.Enterprises (Mumbai, India). The ion-exchange resin XAD-2 (Amberlite, Supelco, Bellefonte PA) was used as received.

2.2. Preparation of MIP microspheres

A 4% PVA solution in water was used as a continuous phase. The basic formulation of the dispersed phase was 10 mL of acetonitrile, 1.7 mL of methacrylic acid, 18.9 mL of ethylene glycol dimethacrylate, and 0.6 g SDS. The dispersed phase, surrounded and constricted by the continuous phase, formed droplets. The total volume of the dispersed phase was 30 mL. 126 mg of BAP were added as the template when MIPs were synthesized. Oil-in-water emulsions were generated in the micro-fluidic segmented flow process. The acrylate droplets that were continuously formed in the micro-reactor were cured by UV radiation once they had left the micro-fluidic unit. The ionic surfactant (SDS) and PVA, present in the continuous phase, prevented coalescence of the emulsion droplets and diffusion instability due to Ostwald ripening by stabilizing the interfacial surfaces. The diffusion instability is possible in the case of dispersed phase (acrylate) with low solubility in the continuous phase (water). As a result, the acrylate can diffuse from the smaller droplets across the aqueous phase towards larger droplets. The net effect is a reduction in total interfacial area, and consequently reduction in interfacial energy. When a droplet's diameter attains a critical low limit, the droplet disappears due to Kelvin instability. All fluids were pumped into the micro-fluidic reactor by syringe pumps to ensure even droplet formation and precise control of the flow rates and flow rate ratios. The schematic of the micro-fluidic reactor and its photograph are shown in Fig. 2. The pre-polymer droplets formed in the microfluidic system were collected in a beaker containing 10 mL of the continuous phase stirred at 250 rpm. Photo-curing of the droplets was performed for 30 min at

room temperature by employing a Bluewave 50 UV source (Dymax GmbH, Germany) which was submerged into the beaker. The microspheres produced from the micro-fluidic reactor were washed thoroughly with chloroform (thrice) followed by methanol (twice) and then the microspheres were dried in vacuum. The same procedure was followed for preparation of the non-imprinted polymer (NIP) without template (BAP).

2.3. Determination of the BAP adsorption capacity of the microtraps

Microtraps of 30 mm length and 5 mm width were packed loosely with MIP microspheres (10 mg) to reduce their hydraulic resistance. The adsorption capacity of the microtraps for BAP was determined by immersing a microtrap in a 10 mL glass vial containing a solution obtained by mixing 0.1 mL of a BAP standard (1 $\mu g\ L^{-1}$) and 5 mL of water:acetonitrile (99:1, v/v) solution. The glass vial was shaken in a water bath at 25^{o}C for 3 h after which the microtrap was removed and the concentration of BAP in the solution was measured using fluorescence spectrometry. The above experiment was repeated using different concentrations of BAP to find the effect of BAP concentration on the microtrap adsorption capacity. Each experiment was conducted in triplicate and the mean value was reported. Blank experiments were conducted witha microtrap packed with NIP microspheres to allow a reliable calculation of the amount of BAP adsorbed by the microtrap itself.

2.4. Selectivity studies

The selectivity of MIP microspheres for BAP was evaluated in batch mode adsorption experiments using other PAHs, i.e. pyrene and chrysene. In a series of disposable scintillation vials, 10 mg of MIP microspheres were suspended in 10 mL of solutions with BAP concentrations ranging from 1 to 5 $\mu g\ L^{-1}$. The mixture was agitated at room temperature for 3 h and then samples were centrifuged at 15 000 rpm for 5 min. The optimum contact time (i.e. 1 h) was determined by measuring the residual BAP concentration in solution at different contact times. Three replicate measurements were performed for every sample, and the mean concentration was calculated. The same procedure was repeated for chrysene and pyrene.

2.5. Application of the microtraps to sediment samples

One of the aims of this study was to determine the applicability of the proposed microtraps for selective binding of BAP from complex environmental samples such as marine sediment. The marine sediment samples were collected from Mumbai offshore region, where intensive oil and natural gas production facilities are located. 1 g of each sample was placed in a 10 mL glass vial containing 5 mL of water:acetonitrile (99:1, v/v), then the content of the glass vial was agitated in a water bath at 25^{o}C for 3 h. This was followed by centrifuging the glass vial and decanting the supernatant. To this supernatant solution, a microtrap was immersed and the content was agitated in a water bath. The adsorption experiments were carried out to determine the BAP adsorption capacity of MIP packed microtraps for marine sediment leachates. The concentration of BAP and dissolved organic matter (DOM) in the microtraps was monitored for up to 30 days. These experimental results allowed a better understanding of the interference of DOM on BAP adsorption onto MIP. Similar experiments were conducted using microtraps packed with a NIP, XAD or PAC to evaluate their selectivity, adsorption capacity and preservation potential for BAP during environmental monitoring.

2.6. Instrumental methods

The surface area and pore volume of the monolithic materials were measured by nitrogen adsorption with a JEOL JSM-6400 analyzer using the BET method [25]. The nitrogen adsorption-desorption data were recorded at liquid nitrogen temperature (77 K). Pore size distributions were measured by nitrogen desorption using the BJH method [26]. Scanning electron microscopy (SEM) imaging of the microspheres was performed using a Hitachi S-4700 instrument.

The Hitachi F-45000 fluorescence spectrometer utilizing CLASS-VP software was used for measuring BAP, PYR and CHR in the concentration range between 1 and 100 μg L^{-1} using the calibration curve method The limit of detection (LOD) of BAP, PYR and CHR calculated as three times the standard deviation of the baseline was determined as 0.01, 12 and 9 μg L^{-1}, respectively. In the case of BAP the LOD value corresponded to 1 μg g^{-1} BAP in marine sediment. All measurements were conducted in triplicate. Excellent linearity between peak area and concentration ($R^2 > 0.998$) was obtained.

2.7. Determination of DOM in sediment samples

DOM was determined by high temperature oxidation of 5 g sediment samples in a Shimadzu TOC Analyzer after sample acidification with 10 mL of 85% H_3PO_4 and purging with N_2 for 5 min. The repeatability of the method, expressed as relative standard deviation of five replicate measurements, was 10 – 12% for concentrations < 100 μg L^{-1} and below 8% in the concentration range of 100–4000 μg L^{-1}.

3. Results and Discussion

3.1. Preparation and characterization of the MIP microspheres

Uniformly sized MIP microspheres for selective BAP binding were prepared in the micro-fluidic reactor outlined earlier as a result of the formation of stable oil-in-water (O/W) emulsions using basic polymer formulation with BAP as the template. The flow rate of the continuous phase (Fig. 2a) was kept constant at 5 mL min^{-1}, whereas the flow rate of the dispersed phase was varied between 0.012 to 0.096 mL h^{-1}. Both phases were mixed at a T-junction in the micro-fluidic reactor. The maximum turnover of the reactor was restricted to 0.5 g of polymer microspheres per hour. The mean size of the MIP microspheres could be deliberately varied in a range between 60 and 500 μm by changing the flow rate ratio of the continuous and dispersed phases (Table 1). SEM images showed that the microspheres were of similar size (Fig. 3a) and had a porous structure (Fig.3b). Nitrogen sorption measurements performed with MIP microspheres displayed a type IV isotherm with large adsorption capacities of 0.41-0.33 cm^3 g^{-1}. The microspheres possess BET surface area of 460 m^2 g^{-1} and cumulative pore volume of 0.587cm^3 g^{-1}. About 90% of the total surface of the microspheres (410 m^2 g^{-1}) showed the presence of cavities with a diameter between 20 and 30 nm (Figs. 3c and 3d). The microspheres prepared by using different micro-fluidic reactor conditions had different surface and pore properties (specific surface area, cumulative pore volume and average pore diameter) (Table 1). The specific surface area decreased in the order of average microsphere size 70 μm > 100 μm > 260 μm > 490 μm. Therefore, smaller microspheres (average diameter of 70 and 100 μm) were expected to provide higher concentration of template specific sites than larger microspheres. This expectation was supported also by the nitrogen adsorption data which provided information on the pore structure

(average pore diameter and cumulative pore volume). The template-specific sites formed in the microspheres' matrix are responsible for the selective binding of BAP.

3.2 Adsorption capacity for BAP

The functional monomer MAA has a tendency to participate in weak π-π interactions and H-bonding with BAP [27]. The binding of BAP onto MIP microspheres is limited by the availability of active binding sites for BAP. It could be observed that under selected experimental conditions the MIP microspheres in the microtrap extracted ~98% of BAP from the aqueous solutions (Fig.4). The adsorption capacity of the MIP microspheres packed in traps for BAP was determined as 75 μg g^{-1} and was found to be independent of the initial BAP solution concentration in the concentration range 1-10 μg L^{-1}. Microtraps packed with powdered activated carbon (PAC) of similar particle size were used for BAP adsorption in aqueous solutions containing 1 μg L^{-1} BAP. It was found that the PAC packed microtraps showed about 60% lower adsorption capacity compared to the proposed MIP microtraps. These results confirmed the fact that MIPs show high adsorption capacity in aqueous solutions in general and that the proposed MIPs are suitable for the adsorption of BAP from environmental water samples in particular. The non-covalent interactions, mainly hydrogen bonding, that are employed for imprinting and subsequent recognition of the analytes of interest are highly dependent on the medium used for polymerization. For optimal efficiency of imprint formation, at least for the type of polymers used in this study, the polymerization reaction should be performed in a solvent as apolar as possible which can be used for the preparation of BAP solutions in the concentration range studied. The total analyte binding to a polymer can be viewed as consisting of two components, i.e. specific binding to the template sites and nonspecific binding to the polymer. If the nonspecific binding dominates, any selectivity shown by the imprints will be obscured. Most of all previous studies involving MIPs have focused on chromatographic separation for the analysis of pesticides, carcinogens and toxic metals [28]. Another important finding of the present study is that the microsphere size significantly affects the adsorption capacity for BAP. The microspheres with size 60-80 μm bound 75 mg g^{-1} of BAP while the microspheres in the size ranges 100-250 μm and 250-500 μm bound 14% and 8%, respectively, less BAP than the smallest size microspheres (Fig.5). This

trend of enhanced binding capacity of smaller MIP microspheres could be due to better mass transfer and high surface density of binding sites compared to larger microspheres (Table 1). Fig. 5 shows that the absorption capacity of MIP microspheres for BAP from aqueous solutions prepared by means of a micro-fluidic reactor was approximately 30% higher compared to MIP particles of similar size prepared by the conventional procedure based on mechanical grinding of polymer monolith formed during bulk polymerization for all three size ranges studied (i.e. 60-80, 100-250, and 250-500 μm).

3.3 Selectivity of the MIP microspheres for BAP

~~It can be expected that PAHs with molecules larger than that of BAP are not likely to compete for the binding sites on the MIP microspheres developed as part of this study. Therefore, the selectivity of these microspheres for BAP was studied in the presence of the PAHs with molecules smaller than that of BAP. PYR and CHR were selected as representatives for such PAHs since both molecules incorporate 4 benzene rings (Fig.1). The selectivity of the MIP microspheres for the 3 PAHs mentioned above can be characterized by the corresponding cross-reactivity values.~~ ~~The percentage decrease in adsorption capacity of the BAP imprinted polymer for BAP in the presence of other PAHs (i.e. PYR and CHR) is defined as cross-selectivity. The BAP imprinted polymer cavities determined the selective adsorption of BAP in the presence of other PAHs and this difference in selectivity was attributed to a shape/size structural effect of the binding sites.~~ The cross-reactivity of different PAHs on BAP imprinted polymer microspheres was determined as the ratio of the corresponding imprinting factors (i.e. adsorption on MIP / adsorption on NIP) for BAP over other PAHs (i.e. PYR and CHR). The imprinting factor is used to evaluate the imprinting effect of a certain MIP, which is defined as the ratio of adsorption of the target template by the imprinted polymer and the non-imprinted polymer. The imprinting factor provides information about the selective adsorption of the target template due to the presence of template-specific binding sites in the polymer. These values were normalized by dividing all cross reactivity values by the lowest cross-reactivity factor at different initial concentrations of PAHs. The corresponding values (Table 2) suggest that the BAP imprinted polymeric microspheres exhibit excellent selectivity for BAP in aqueous solutions containing the other PAHs studied. In contrast, non-specific adsorption of the BAP imprinted polymer for PYR and CHR was 10% and 9% of the non-specific adsorption of BAP, respectively.Molecules

with a similar structure cross-react to a higher degree in water than in organic solvents [29, 30]. If the solvent has poor hydrogen bonding capacity, the interaction energy is influenced primarily by the solvent dielectric constant while if the solvent has a strong capacity to form hydrogen bonds, both the dielectric constant of the solvent and the hydrogen bonding interference affect the template–monomer complex formation, thereafter, the corresponding interaction energy. Aprotic porogen with low dielectric constant is likely to lead to high interaction energy between the template and the functional monomer, resulting in a MIP with better affinity and selectivity. Binding strength describes the affinity of PAHs to the polymer of interest in a given solvent system. Structurally similar molecules cross-react to a higher degree in water than in organic solvents [30]. This could be due to interference of the water molecules as a result of hydrogen-bonding interactions between PAHs and the MIP. However, it should be emphasized that despite such interferences, the BAP imprinted polymer still showed higher selectivity for BAP compared to those generally reported for resin XAD and activated carbon (Table 3).

3.4 Preservation of BAP in MIP microtraps

Sample contamination with reference to BAP will occur quite rarely, as long as trace-analysis standard procedures are followed; however, the preservation of samples could be one of the troublesome steps in BAP trace analysis. Changes induced by microbial activity or losses by volatilization or adsorption have to be avoided. Controlling the influence of critical factors for the stability of BAP such as temperature, dissolved organic matter, ions and container material is important for the preservation of sample integrity [31]. Freezing, cooling, and/or storage in the dark are the most preferable and simple methods for preservation of BAP. However, there is no general agreement on the suitability of these methods and reports sometimes contradict each other. This is especially true for complex solid matrices, such as soil and sediment. Nonetheless, for samples where bacteria will exist naturally, storage at low temperatures or even freeze-drying [32] is required to prevent biological activity from modifying the nature of the sample.

In sediment leachate, the DOM, responsible for microbial growth, was found to be 2450 mg L^{-1}. DOM is utilized by bacteria and is eventually oxidized to dissolved inorganic carbon (CO_2)

through terminal metabolism (respiration) [33]. PAHs, in particular, do not degrade easily under natural conditions. However, under anaearobic conditions the PAH-degrading microorganisms decompose organic compounds through biotransformation into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic), or CH_4 (anaerobic) [32]. The biodegradation of a pollutant and its rate depend on the environmental conditions, amount and type of the microorganisms, nature and chemical structure of the chemical compound being degraded. Thus, to monitor PAHs at trace levels, a number of factors are to be counted for. Both bacteria and fungi have been extensively studied for their ability to degrade PAHs [34, 35]. The extent and rate of PAHs biodegradation depends on many factors such as DOM, microbial population, degree of PAHs acclimation, accessibility of nutrients, and chemical partitioning in the environment. To minimize the biodegradation of PAHs accumulated in the microtraps it is essential to remove DOM from the microtraps before their further storage prior to analysis. In this study, a series of microtraps packed with 60-80 µm size microspheres were kept in contact with marine sediment leachate and then analyzed for BAP and DOM concentration over a period of time of up to 30 days at 20°C and kept frozen prior to PAH analysis. Similar experiments were conducted using PAC packed microtraps. The data showed that the concentration of BAP extracted into traps packed with MIP microspheres was reduced by <10% after 30 days (Fig.6a). This good preservation of BAP was due to the fact that bacteria could not attach to the MIP because of the polymer hydrophobicity. This hypothesis is supported by the lack of DOM biodegradation (Fig. 6b). About 50% of BAP was decomposed in traps packed with PAC after storage for 30 days (Fig. 6a). This result correlated well with the observed DOM biodegradation (Fig. 7b), which clearly indicated the presence of bacteria attached to PAC over this period of time. The experimental results discussed above clearly demonstrate the superiority of microtraps packed with MIP microspheres for pre-concentration and preservation of carcinogenic BAP over commercially used PAC microtraps.

4. Conclusions

The results reported in this study demonstrate the suitability of BAP imprinted polymer microspheres as a highly selective adsorbent for environmental analysis of BAP. The advantage of

MIPs manufactured in a micro-fluidic reactor compared to traditional MIPs using bulk polymerization is that the former approach leads to the manufacturing of adsorbents with well defined binding sites ensuring better capacity and selectivity for preserving the analyte (i.e., BAP). The traditional method of MIPs preparation by bulk polymerization followed by mechanical crushing reduces the density of effective binding sites. The microtraps packed with MIP microspheres used in enrichment of BAP have exhibited better selectivity and adsorption capacity than commercially available adsorbents (e.g., XAD and PAC). On the basis of the results obtained in this study, it can be concluded that the analytical features of the MIP microtraps outlined above make them suitable for for the analysis of PAHs in environmental samples such as drinking water, wastewater, industrial effluents and marine water.

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Table 1. Size of microspheres as a function of flow rate of polymer precursors in the microfluidic reactor.

Continuous phase Flow rate, mL min^{-1}	Dispersed Phase Flow rate, mL h^{-1}	Continuous/dispersed flow rate ratio	Mean size of microspheres, µm	MIP			NIP		
				Surface area, $m^2 g^{-1}$ *	Total pore volume, $cm^3 g^{-1}$ #	Average Pore diameter, nm$	Surface area, $m^2 g^{-1}$ *	Total pore volume, $cm^3 g^{-1}$ #	Average Pore diameter, nm$
5	0.096	3125	60-80	460±13	0.587±0.015	20±2	270±10	0.392±0.017	240±7
5	0.048	1515	100-250	390±10	0.543±0.011	26±2	220±14	0.365±0.011	267±11
5	0.025	714	25-500	310±15	0.435±0.012	28±3	186±10	0.349±0.016	290±9
5	0.012	385	>500	286±11	0.402±0.013	31±2	170±13	0.312±0.015	315±11

**BJH determined using multi-point BET method; #cumulative desorption pore volume of pores between 5 and 100 nm; $BJH desorption average pore diameter of pores between 5 and 100 nm.*

Table 2. Normalized cross-reactivity factors for BAP, PYR and CHR on MIP with standard aqueous solutions and marine sediment leachates (MSL) containing these PAHs.

Initial concentration of PAHs ($\mu g\ L^{-1}$)	BAP	PYR	CHR
1	100	9	10
5	100	7	8
MSL (2.5)	100	14	12

Table 3. PAHs adsorption capacity of MIP and other adsorbents in aqueous solutions. PAHs standards contained 1 μg L^{-1} of each PAH; the sediment leachate was spiked with PAHs standard before the adsorption experiments. The experiments were conducted in triplicate and average values are reported in the table

	PAHs mix standard			**Sediment leachate**		
	BAP	**PYR**	**CHR**	**BAP**	**PYR**	**CHR**
MIP (μg g^{-1})	75.9	6.9	7.1	69.8	6.6	6.8
XAD (μg g^{-1})	23.4	14.2	16.1	18.2	11.3	13.6
PAC (μg g^{-1})	27.8	16.5	193	21.5	12.5	14.9
NIP (μg g^{-1})	14.8	12.6	13.9	14.1	11.6	12.9

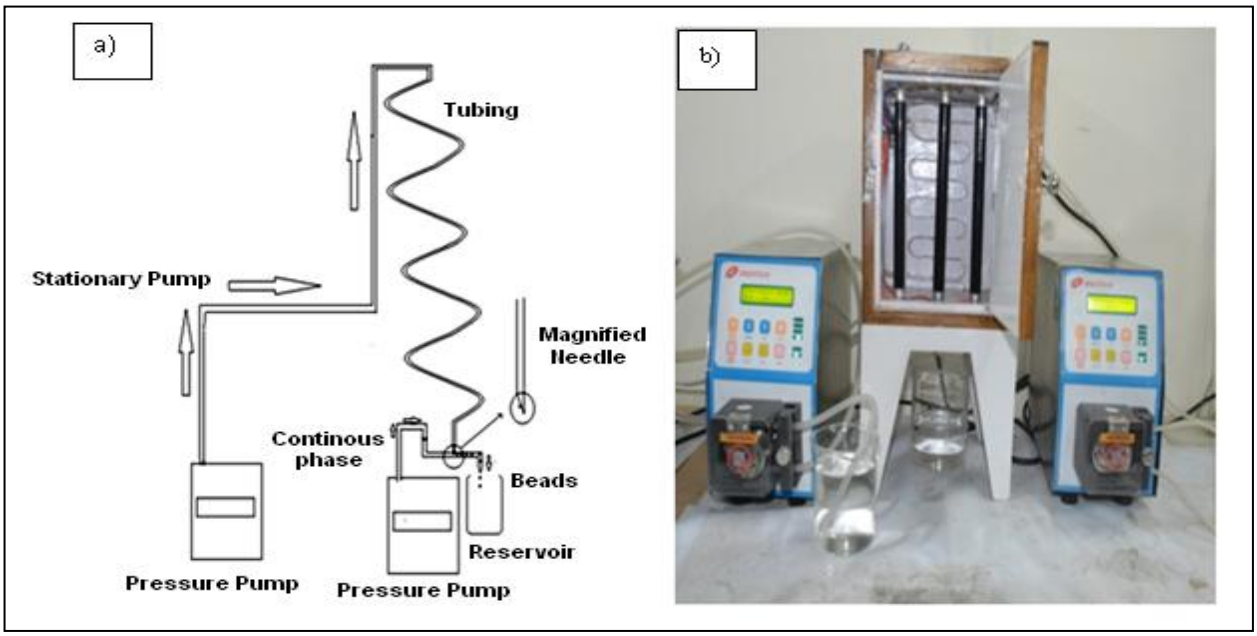

Fig.1. (a) Schematic of the micro-fluidic reactor and (b) a photograph showing the reactor set-up.

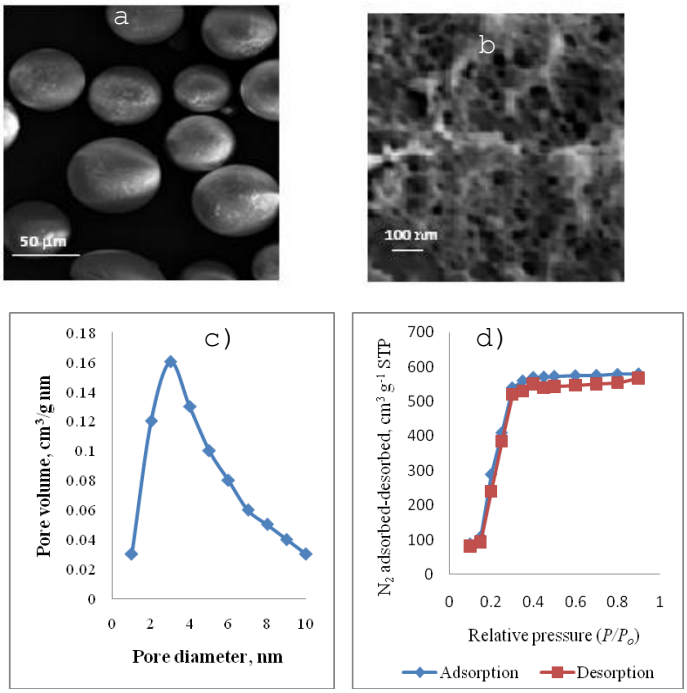

Fig.2. (a) SEM images of MIP microspheres prepared in the micro-fluidic reactor shown in Fig. 2; (b) SEM images showing the imprinting sites on the surface of 60-80 µm in diamater microspheres (x10000 magnification with 15 kV energy); (c) Pore-size distribution (cm^3 g^{-1}); and (d) Nitrogen adsorption-desorption isotherms of MIP microspheres in the size range 60-80 µm.

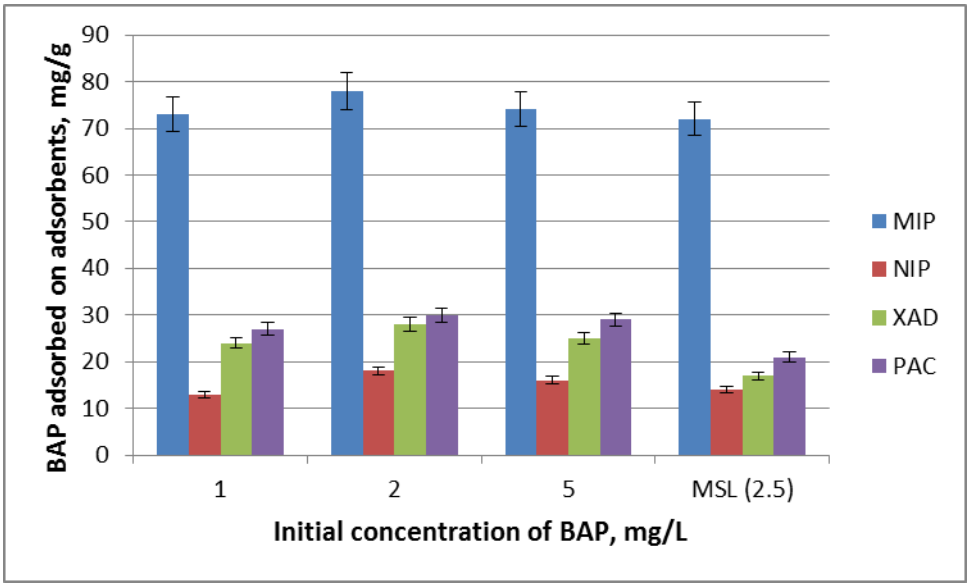

Fig.3. Binding capacity of MIP and other adsorbents for BAP in aqueous solutions with BAP concentrations of 1, 2 and 5 μg L^{-1} and marine sediment leachate (2.5 μg L^{-1}).

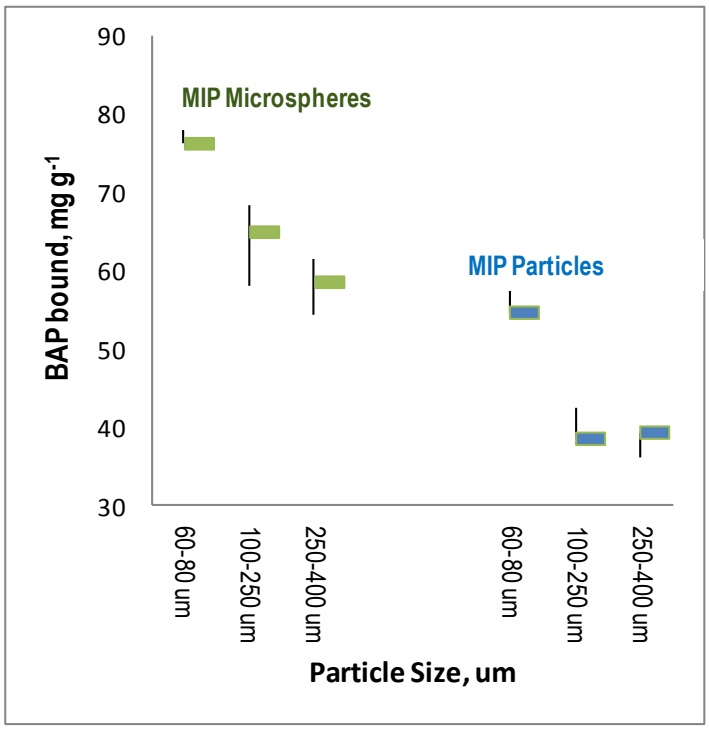

Fig.4. Effect of MIP microspheres/particles on BAP binding capacity. MIP microspheres were prepared using a microfluidic reactor and MIP particles were prepared by bulk polymerization followed by grinding of the polymer monolith.(Experimental conditions: initial BAP concentration, 1 μg L^{-1}; quantity of MIP microspheres/particles added, 10 mg; volume of the BAP solution, 10 mL contact time, 60 min; temparature, 25°C).

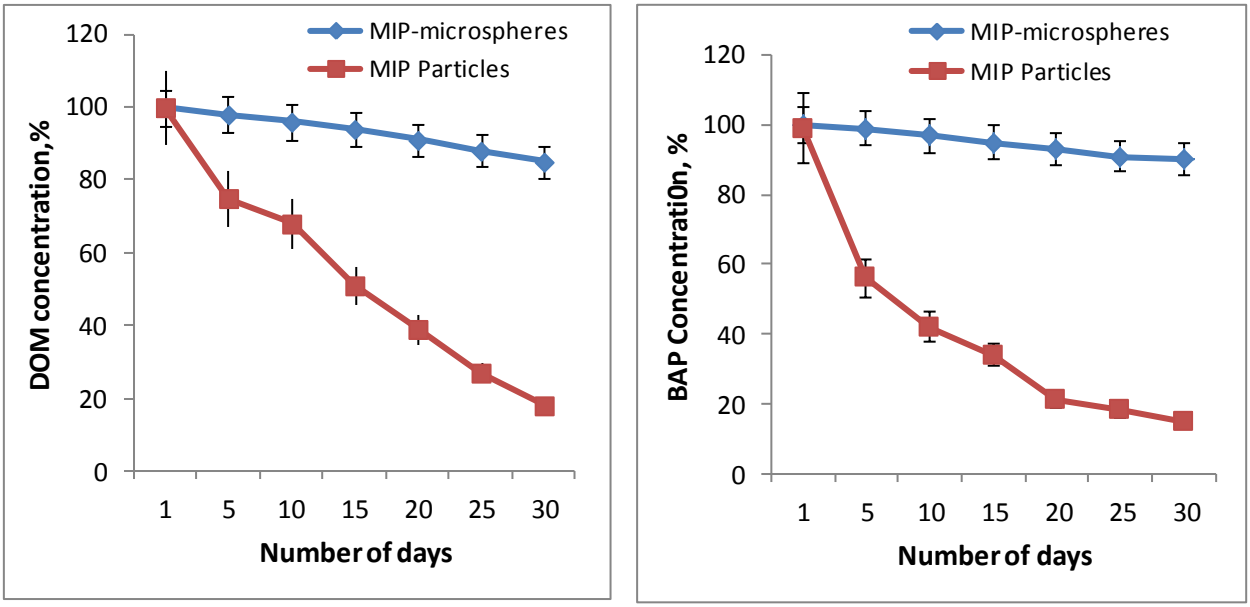

Fig.5. (a) Stability of BAP enriched from marine sediments in MIP and PAC microtraps over a period of 30 days (b) Percentage decrease in DOM concentration over a 30 day period in the case of MIP and PAC microtraps (initial concentration of DOM in sediment leachate sample was 2450 mg L^{-1}).