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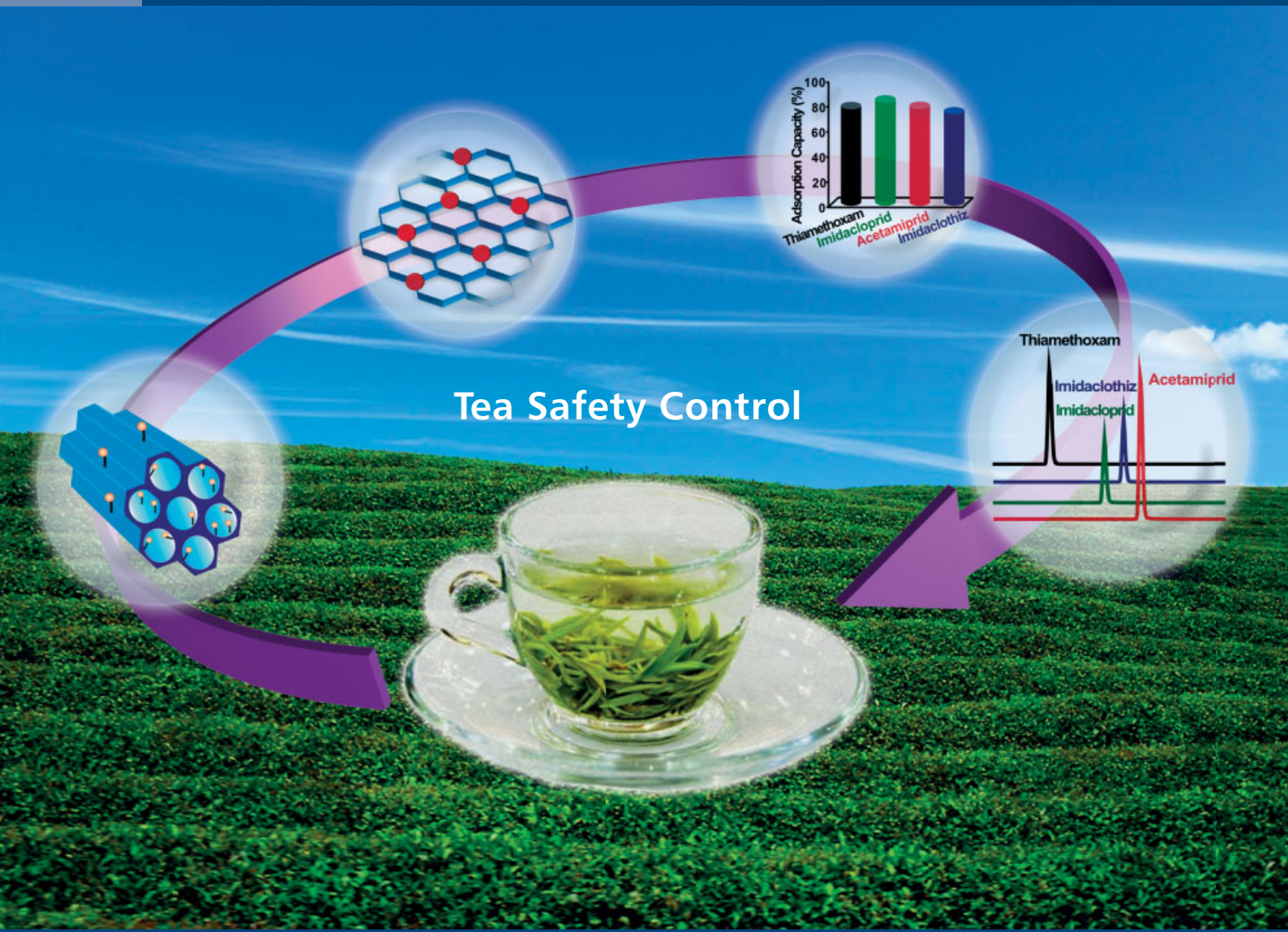


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Research Article

# 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**, non-imprinted polymer; **PCB**, polychlorinated biphenyl; **PVA**, polyvinyl alcohol; **XIP**, xylene-imprinted polymer; **4-VP**, 4-vinylpyridine; **μECD**, microcell electron capture detector

 

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.

 

### 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.

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

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

 

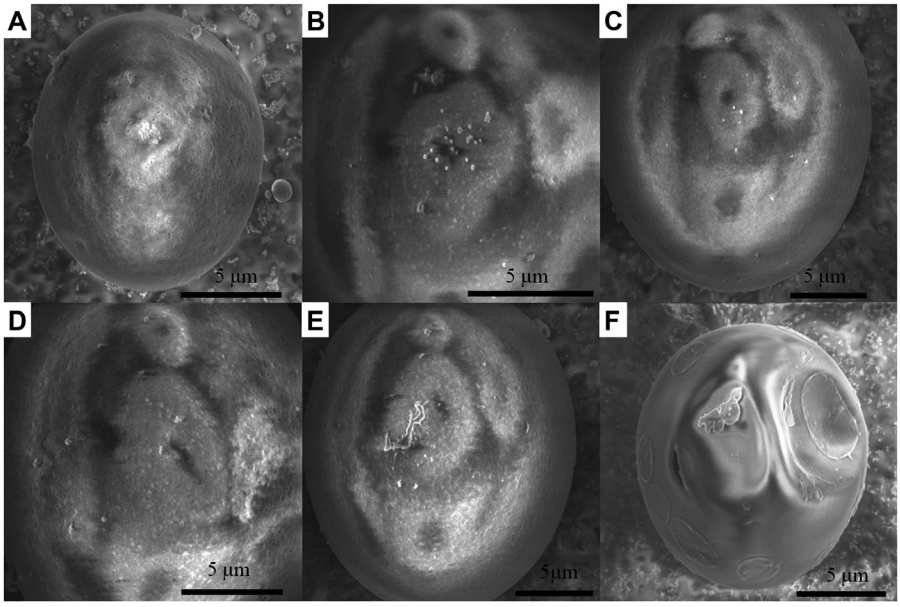


**Figure 1.** SEM images of (A) MIP1; (B) NIP1; (C) MIP2; (D) NIP2; (E) XIP; and (F) XIP-NIP).

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.

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

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \quad (3)$$

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 $\mu g^{-1}$) 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

 

**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.

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

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 $K_o = a^{1/m}$, 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).

$$k' = \frac{(t_r - t_0)}{t_0} \tag{5}$$

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

 

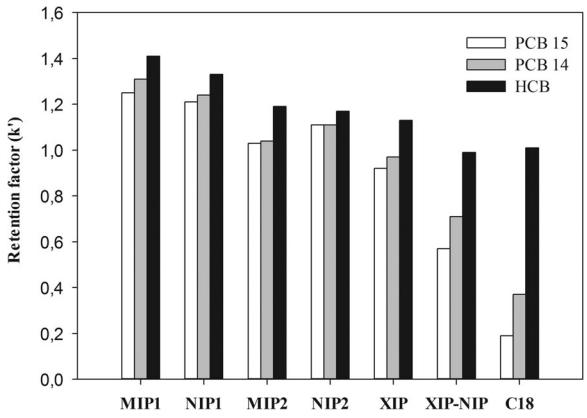


**Figure 2.** Retention factors of PCB 15, PCB 14, and HCB at the synthesized polymers and at a $C_{18}$ column using methanol (100%) as the mobile phase at a flow rate of 1.5 mL/min; detection was performed at a wavelength of 254 nm for PCBs and 235 nm for the HCB.

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 $k'_{XIP\text{-}NIP}$, 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

 

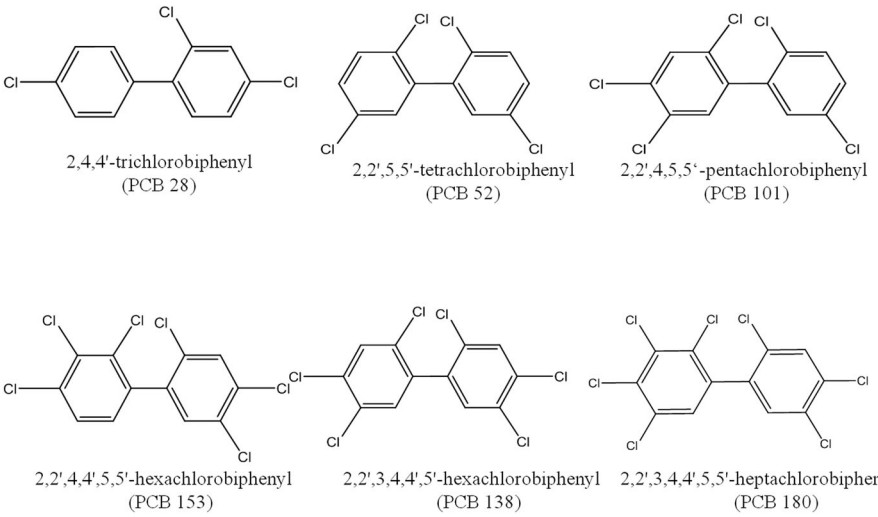


**Figure 3.** Structures of the six indicator PCBs studied herein.

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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