Lukas Baldauf

Molecularly imprinted polymers as selective sorbents for solid-phase extraction

Abstract

Molecularly imprinted polymers are tailor made materials with high affinities for specific molecules. The use of these materials as sorbents makes possible the selective extraction of analytes from complex samples, reducing matrix interferences and the use of more expensive sorbents or time-consuming calibration methods. The synthesis of molecularly imprinted polymers for the extraction of bisphenols and their applications as sorbents for solid-phase extraction are considered. The drawbacks that limit their success as extraction sorbents are discussed, and imprinting methodologies dealing with these drawbacks are presented using examples from the literature.

Contents

Abbreviations: BP, bisphenol; BPA, bisphenol A; BPF, Bisphenol F; BPS, bisphenol S; TBBA, tetrabromobisphenol A; HPLC, high performance liquid chromatography; MS, mass spectroscopy; UV, ultraviolet; DAD, diode array-detection; SPE, solid-phase extraction; LLE, liquid-liquid extraction; MIP, molecularly imprinted polymer; MISPE, molecularly imprinted solid-phase extraction; NIP, non - imprinted polymer; *IF*, imprinting factor; k_{MIP} retention factor on a molecularly imprinted polymer column; k_{NIP}, retention factor on non - imprinted polymer column; *Q*, specific binding capacity; EDMA, ethylene glycol dimethacrylate; TRIM, trimethylpropane trimethacrylate; 4-VyP, 4-vinyl pyridine; MAA, methacrylic acid; ACN, acetonitrile; AIBN, Azobisisobutyronitrile; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; CE, capillary electrophoresis; DMIP, dummy molecularly imprinted polymer; THPE, 1,1,1-Tris(4-hydroxyphenyl)ethane; DDBP, 4,4'-dihydroxybisphenol; BPAF, bisphenol AF; DADPM, 4,4'-diaminodiphenylmethane; HLB, hydrophilic-lipophilic balance; SCX, strong cation exchange; MAX, mixed-mode strong anion exchange.

1. Introduction

Bisphenols (BPs) are high production volume organic chemicals that are widely used in the plastic manufacturing industry. Because BPs are produced in such high volumes, they have been detected in several environmental matrices such as water and sediments, food products, especially canned food and beverages, and in human urine and serum. Bisphenol A (BPA) is the most abundant BP in environmental compartments and usually present in ng g^{-1} to μg g^{-1} concentrations, followed by bisphenol F (BPF) and bisphenol S (BPS), which are being detected at elevated frequencies and concentrations[1].

Several studies suggest that BPA exposure has significant effects on human health, such as reproductive impairment, metabolic disease and developmental effects[2]. Thus, structural analogs such as BPS, BPF, or tetrabromobisphenol A (TBBA) are increasingly used to produce "BPA – free" products. However, these compounds resemble BPA and may have similar adverse health effects, but their toxicological data are still limited[3].

Because of the widespread contamination of BPs in the environment and their detrimental health effects, numerous methods for the determination of BPs in a variety of matrices have been developed. (Ultra)-high performance liquid chromatography with tandem mass spectrometry ((U)HPLC–MS-MS), ultra-violet (UV) or diode-array detection (DAD) are most frequently encountered, coupled with various extraction methods for sample preparation. Most common is regular solid-phase extraction (SPE) both on-line[4, 5] and off-line[6, 7]. Other methods include liquid-liquid extraction (LLE)[8], dispersive-LLE[9] and magnetic-SPE[10].

As mentioned, bisphenols are often present in trace amounts in complex matrices, making their analysis difficult and prone to error when matrix components co-elute[5]. Even when co-eluting species are invisible to the detector when e.g. selected ion monitoring is used, they can interfere by enhancing or suppressing the ionization process in the detector, affecting the accuracy, precision and sensitivity of the assay (Figure 1a)[11].

Sample preparation methods such as SPE with regular sorbents or LLE may have difficulties with co-eluting species because they often have similar polarities, resulting in similar amounts being extracted (Figure 1b). To compensate for matrix effects, isotopically labelled standards, matrix-matched or standard addition calibration are required[12]. In contrast, using molecularly imprinted polymers (MIP) as sorbents makes possible the selective extraction of target analytes with relatively high recoveries compared to other components, which reduces matrix effects (Figure 1c).

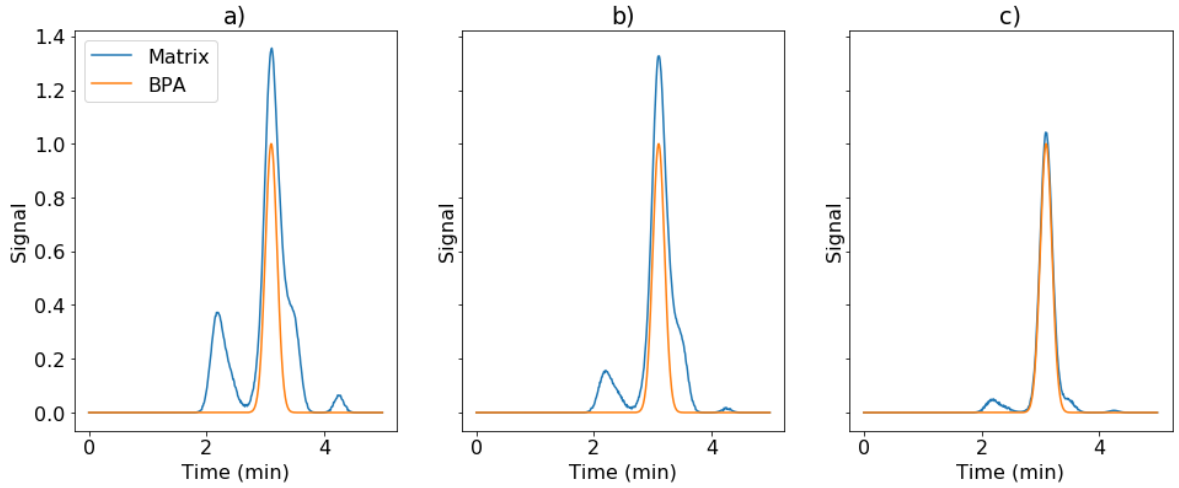

Figure 1: Illustrative chromatograms of a spiked sample containing BPA and other co-eluting matrix components obtained by a) direct injection, b) injection after SPE or LLE and c) injection after MISPE.

This text deals exclusively with the use of MIPs in the determination of BPs. Many BP imprinted MIPs have been developed in the literature, partly because they have suitable functional groups, are cheap materials and often present in complex matrices. Also, because of high ongoing research interests in BP contamination, molecularly imprinted solid-phase extraction (MISPE) cartridges for BPs have become commercially available, such as SupelMIP® or AFFINIMIP®.

Herein, important variables for synthesizing selective MIPs, and the inherent drawbacks of MIPs are considered. These drawbacks limit the success of MIPs as sorbents for SPE and stationary phases for chromatography. Different methods aiming to avoid these disadvantages are discussed, which make possible the successful determination of trace to ultra-trace BPs in complex matrices when traditional sorbents have difficulties.

2. Theory

2.1 Solid-phase extraction for sample preparation

Most assays do not respond well to target analytes in the sample matrix because of interfering components that complicate the final quantification step. This is especially true for trace level analysis in complex samples, and a preparation step is necessary. One of the most frequent used methods for sample clean-up and preconcentration in trace analysis is SPE[13]. In SPE, solutes

distribute between a solid-phase sorbent and the solvent. The general procedure consists of 4 steps (Figure 2):

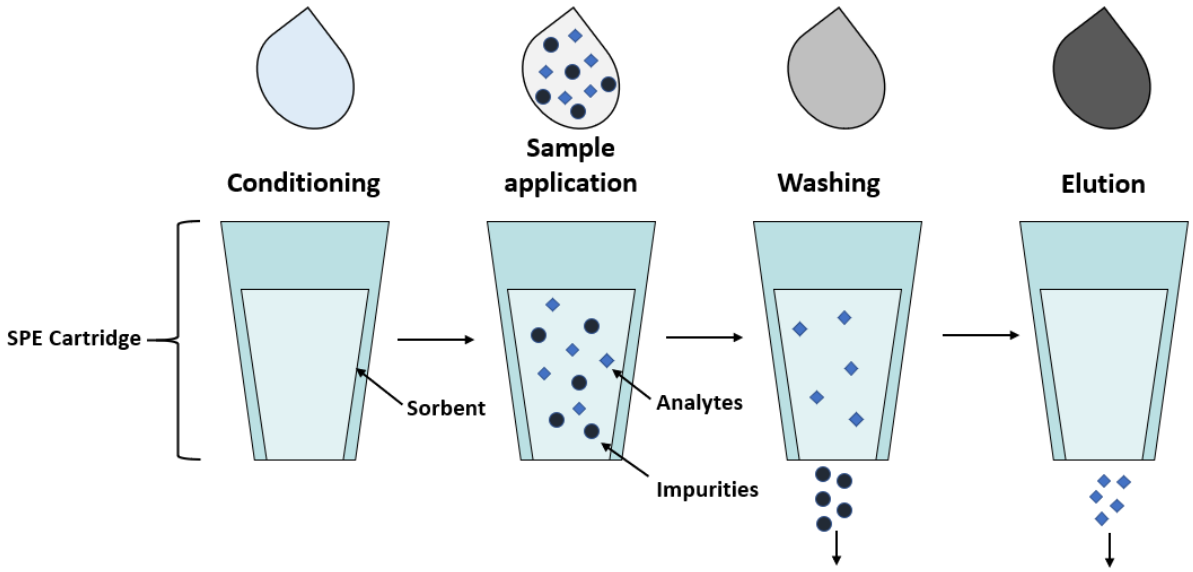

Figure 2: The 4 steps in the general solid-phase extraction procedure: conditioning the sorbent, sample application, washing, and elution of the target analytes from the sorbent. The solvent shading illustrates the elution strength.

A crucial conditioning step is to wet sorbent functional groups, fill void volumes containing air with solvent and remove impurities. What conditioning solvent to use depends on the chemical structure of the analytes and sorbent, but the elution strength is generally increasing after each step (shading in Figure 2). After conditioning, the sample is loaded onto the sorbent and analytes are retained. Application volumes can range from 1 mL to 1 L, depending on the sample and the breakthrough volume of the sorbent for the given solvent, i.e. the volume that can be applied without loss of analyte recovery. The recovery, *R*, of a SPE procedure can be defined as

$$R(\%) = \frac{A}{B} \cdot 100\% \qquad (2\text{-}1)$$

where *A* is the amount of compound in a pre-extraction spiked solution and *B* is the amount of compound in a post-extracted spiked solution, both spiked with the same amount.

The third step is a washing step using a solvent with appropriate strength to elute undesired matrix components. Analytes should be retained until the final elution step. In the elution step, the solvent should ideally displace target analytes whilst still retaining eventual remaining,

stronger bound matrix components[14]. The resulting sample is ideally free of interfering components and ready for analysis.

2.2 Molecularly imprinted polymers as selective sorbents

MIPs are synthetic materials with embedded functional groups that selectively recognize and bind certain molecules. In general, imprinting is achieved through co-polymerization of functional monomers and cross-linkers in the presence of a target molecule referred to as template (Figure 3). If no template is added to the polymerization mixture, the resulting polymer is referred to as a non-imprinted polymer (NIP).

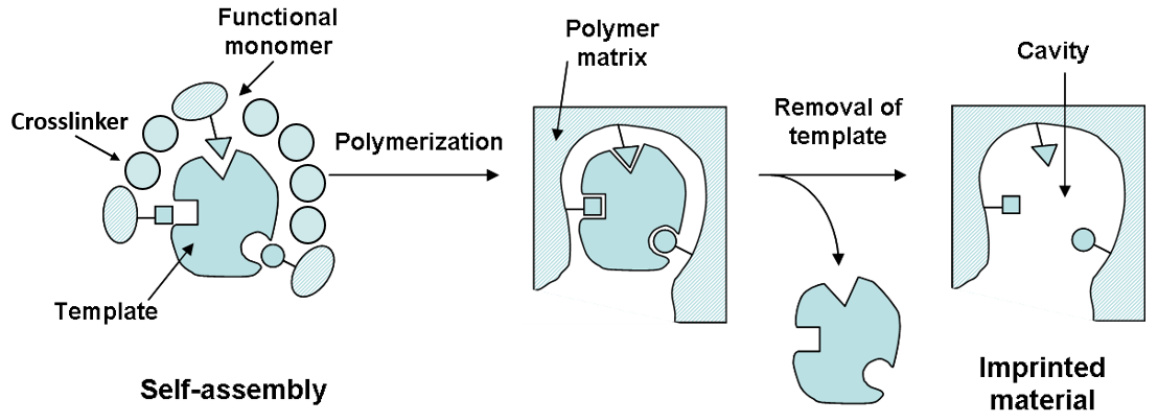

Figure 3: Schematic representation of the molecular imprinting process. Adapted from[15].

The mode of recognition can broadly be grouped into two categories; covalent and non-covalent[16]. The non-covalent approach has the most practical application and is most frequently encountered in the literature. It was first introduced by Mosbach *et al.*[17], using dyes as templates and acrylic-based monomers. Monomers can form complexes with the template through hydrogen bonds, electrostatic, van der Waals or π-π-interactions, i.e. non-covalent interactions. In the covalent approach, template molecules are covalently bound to the monomers. Following polymerization, the covalent bonds are cleaved, and template molecules removed. Binding is achieved by re-forming the covalent bonds between polymer and template. In either way, an imprinted material with functionalized cavities is left after polymerization and removal of the template[18]. These cavities have recognition abilities and affinities towards template molecules or similar compounds, making them highly selective sorbents suitable for SPE.

2.3 Characterization of MIPs

MIP selectivity is often reported as the imprinting factor, *IF*,

$$IF = \frac{k_{MIP}}{k_{NIP}} \tag{2-2}$$

where k_{MIP} and k_{NIP} is the retention factor of the template on a column using the MIP or NIP as stationary phase, respectively. MIPs and NIPs are also characterized by their specific binding capacities Q_{MIP} and Q_{NIP}, respectively, which is the amount of analyte bound to a given mass of polymer. This is calculated using

$$Q = \frac{(c_i - c_f)V}{m} \tag{2-3}$$

where c_i is the initial concentration of analyte in the solution, c_f the final concentration of analyte after extraction, V the volume of the solvent, and m the mass of the sorbent. The selective binding capacity of a MIP at a given concentration, $Q_S(c)$, can be expressed as

$$Q_S = Q_{MIP} - Q_{NIP} \tag{2-4}$$

The binding capacities of both NIPs and MIPs are frequently reported as plots against analyte concentration. Binding models can be fit to the data, yielding parameters that estimate the polymers properties. A simple model is the well-known Langmuir isotherm. However, this model assumes that the binding sites are equivalent, which is not the case for imprinted polymers. An alternative model that considers binding site heterogeneity is the Freundlich isotherm[19, 20]

$$Q = aF^m \tag{2-5}$$

where Q is the binding capacity, F is the concentration of unbound analyte, a is a measure of the number and affinity of the binding sites, and m is the heterogeneity index of the polymer. The heterogeneity index takes on values between 0 and 1. Polymers with m-values equal to 1 are homogenous, becoming increasingly heterogenous as m decreases. The parameters of the Freundlich isotherm can be estimated using least squares linear regression.

2.4 Polymerization methods

The structure of BPs and other structurally similar compounds considered in this text are given in Figure 4a.

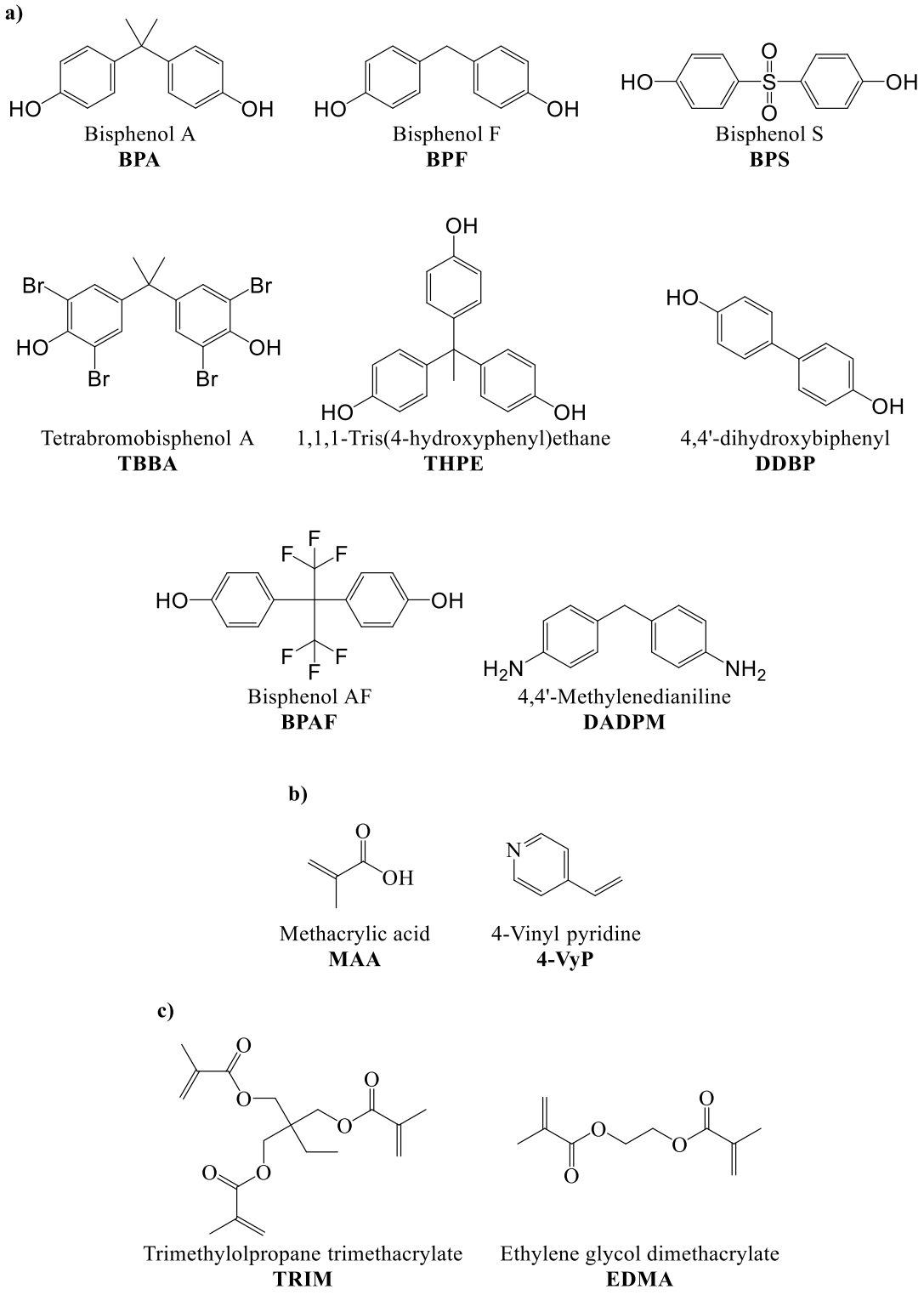

Figure 4: The structure of a) bisphenols and structurally similar compounds considered in this text, b) common functional monomers, and c) common cross-linkers used in the synthesis of MIPs.

Many of these can be considered as hydrophobic compounds which are present in aqueous environmental samples. MIPs for BPs are therefore often synthesized using free-radical polymerization[21, 22], with a rather scarce set of organic monomers and cross-linker (Figure 4b and c). 4-vinyl pyridine (4-VyP) and methacrylic acid (MAA) are almost exclusively encounter as monomers, and ethylene glycol dimethacrylate (EDMA) and trimethylpropane trimethacrylate (TRIM) as cross-linkers. Loading aqueous sample onto an organic based polymer retains hydrophobic molecules strongly, creating polymers with high breakthrough volumes when used as sorbents that can increase the sensitivity of an assay. In addition, organic MIPs are often stable over wider pH ranges than silica-based sorbents.

In the free-radical polymerization approach, monomers, cross-linker and template are solvated in the polymerization solvent, also called porogen, which is often acetonitrile (ACN). The polymerization is initiated using a radical creating compound. Azobisisobutyronitrile (AIBN) is frequently used for this purpose. The result is a bulk polymer, and this method is therefore referred to as bulk polymerization. An alternative method is precipitation polymerization, in which the polymerization can be tuned to yield uniform shaped polymer microparticles of a given size. The main difference between bulk and precipitation polymerization is the amount of solvent and template used. Yet another method is surface imprinting. Advantages and disadvantages of these methods are summarized in Table 1[23]

Table 1: Summary of the benefits and limitations of different MIP polymerization methods using free radical polymerization. Reprinted from[23].

Polymerization	**Benefits**	**Limitations**
Bulk	Simplicity and universality, No required particular skills or sophisticated instrumentation	Tedious procedures of grinding, sieving, and column packing, Irregular particle in size and shape, low performance.
Precipitation	Imprinted microspheres, Uniform size and high yields	Large amount of template, High dilution factor
Surface	Monodisperse product, Thin imprinted layers	Complicated system, Time consuming

Silica based MIPs can be synthesized with silane-based monomers and cross-linkers through sol-gel processes[24-27], but the details are not considered here.

2.5 Validation

Validation is the evaluation of an analytical method to establish if it is suitable for the intended use. Some general validation criteria are sensitivity, precision, and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) can describe sensitivity, and are calculated as some multiple of the signal-to-noise ratio (S/N), which can be defined as

$$\mathrm{S/N} = \frac{2H}{h} \tag{2-6}$$

where H is the peak height measured from the average baseline noise, which is obtained from a reference solution for the compound of interest. h is the noise range over an interval 20 times the width of the peak at half maximum around where the compound elutes(Figure 5)[28]. The multiple of the signal-to-noise ratio is often 3 and 10 for LOD and LOQ, respectively.

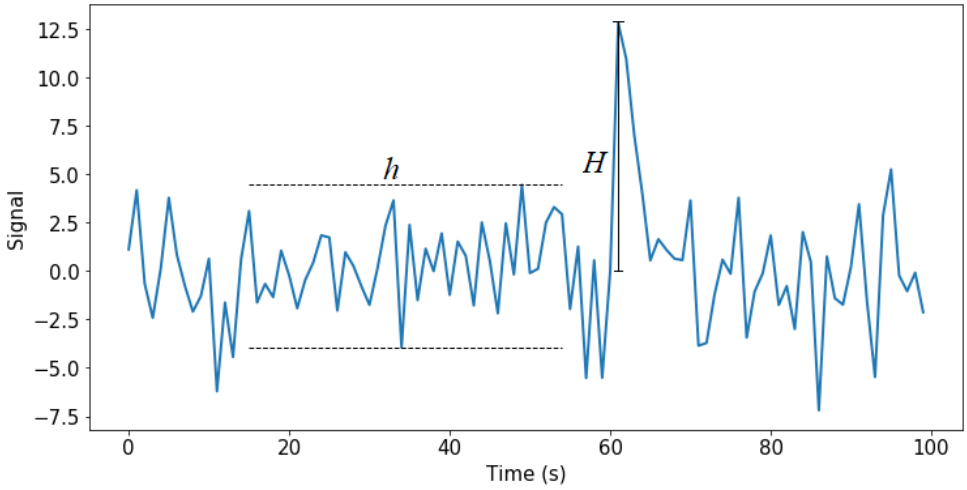

Figure 5: Illustration of the signal-to-noise ratio in the determination of LOD and LOQ.

The accuracy of an analytical method can be expressed as the absolute recovery, *R'*, often just referred to as recovery. It can be defined as

$$R'(\%) = \frac{A'}{C'} \cdot 100\% \tag{2-7}$$

where *A*' is the signal of a spiked sample treated according to the analytical procedure, and *C'* is the signal of the clean solvent spiked with the same amount. The relative recovery should not be confused with the recovery of a SPE-procedure defined in equation (2-1), and the context

implies which one is used. The precision of the method can be expressed as the relative standard deviation (RSD) of the absolute recovery[29].

Matrix effects can be present when analyzing real samples. They can be defined as a loss of signal due to interfering compounds present in the sample

$$ME(\%) = (1 - \frac{B'}{C'}) \cdot 100\% \quad (2\text{-}8)$$

where *B'* is the peak area of a background corrected post-extraction spiked sample, and *C'* is the peak area of the pure standard spiked with the same amount[30].

3. Discussion

As an introduction to MIPs in analytical contexts, the capillary electrophoresis (CE) -UV method developed by Ming *et al.*[31] demonstrates their potential as SPE sorbents in the determination of BPA from different sample matrices (tap water, river water, waste water, shrimp and human urine). CE is commonly coupled with UV detection, but the poor concentration sensitivity of this arrangement makes necessary several tedious pre-concentration or clean-up steps before the analysis. Therefore, using their in-house synthesized MIPs they developed a highly efficient method for sample preparation. Hydrophobic interactions retained BPA on both the NIP and MIP when loading aqueous samples. Applying even 2 mL of ACN completely eluted all compounds from the NIP sorbent, breaking the non-selective interactions. In contrast, the MIP still retained BPA and one very similar compound. Slightly less similar compounds were not retained on the MIP, indicating that the imprinted polymer contained selective binding sites. Compared to a C18 sorbent, the extraction recovery of BPA using MISPE was 10% higher, in addition to washing away interfering compounds with MISPE. Thus, MISPE provided selectivity to the high resolving power of CE. The MISPE-CE-UV method LOD ranged from 1.8 μg L^{-1} for shrimp samples to 84 μg L^{-1} for human urine, with absolute recoveries between 95% and 105% and RSDs less than 7.2%, demonstrating that accurate and precise results can be obtained in complex matrices with MISPE.

Many variables can be optimized in a MIP synthesis, and optimizing selectivity is not a trivial task since the success of an imprinting procedure depends delicately on the chemical parameters. Moreno-Bondi *et al.*[32] identified six variables with a large impact on MIP

performance, being the amount of functional monomer: 4-VyP or MAA, cross-linker: TRIM or EDMA, initiator: AIBN, and template: BPA, the type of polymerization solvent: tetrahydrofuran, toluene, chloroform or ACN, and polymerization initiation: UV or heat. The synthesized MIPs with MAA as functional monomer performed worse than the 4-VyP monomers. This was attributed to weak interactions between acidic carboxyl groups of MAA and the BPA hydroxyl groups before polymerization. Poor to moderate hydrogen bonding porogens did not influence the MIP binding capacity much, indicating that hydrogen bonding did not greatly contribute to selective interactions using this monomer. Instead, 4-VyP makes possible both hydrogen bonding and π-π interactions with the template, resulting in higher binding capacities. The amount of template and initiator had the lowest influence on binding, indicating that selective MIPs can be synthesized using even small amounts of template. Figure 6 (left) illustrates the variable importance, the most important being amount of functional monomer, cross-linker and polymerization initiation, in that order.

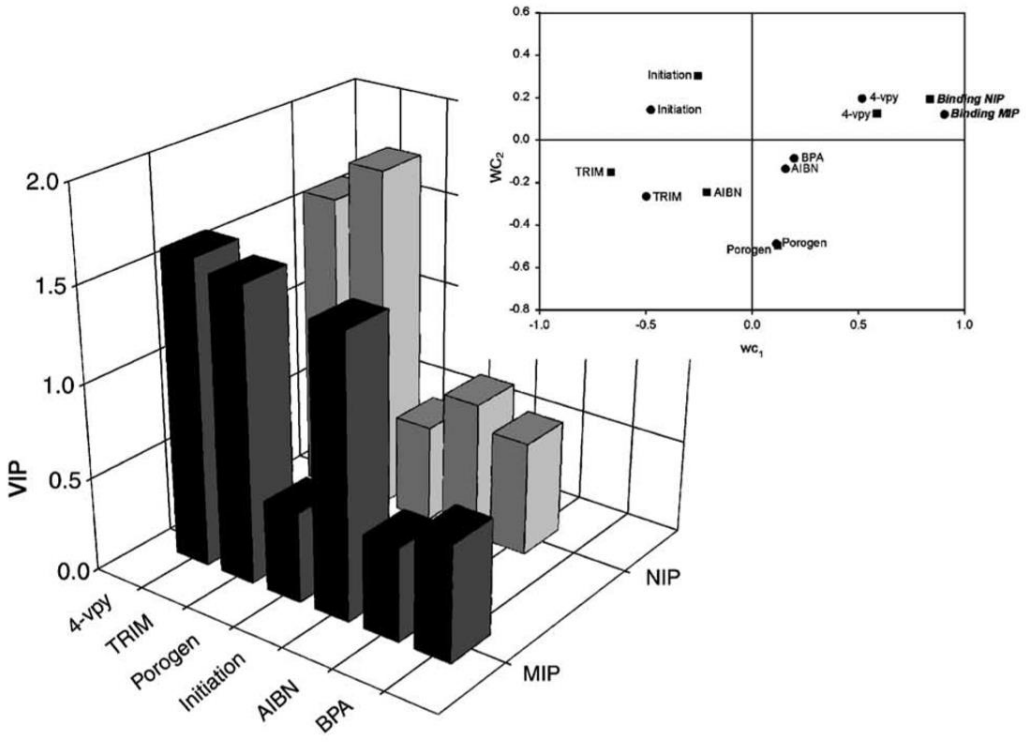

Figure 6: Left: the importance of variables on BPA binding capacity in the multivariate analysis of molecular imprinted polymers expressed as variable importance in projection (VIP) in the partial least squares regression model. Variables having values larger than one are considered important. Upper right: weighting plot for component 1 and 2 (wc_1, wc_2) of the regression model. Variables close together are positively correlated, and variables laying close to a straight line whilst going through origo are negatively correlated. Reprinted from[32] with permission from Elsevier.

Initiating polymerization with UV (4 °C) resulted in higher binding capacities compared to heat initiation (60 °C). This was attributed to the more stable and well-defined interacting complexes at low temperatures, and less stable and more disordered complexes at higher temperatures. Binding increased with increasing amount of functional monomer and decreasing amount of cross-linker (Figure 6, upper right). The amount of functional monomer influences the template-monomer equilibrium and thereby the number of binding sites, while the rigidity of the functional cavities depends on the cross-linking degree. However, a large excess of functional monomer can lead to the formation of non-selective binding sites, which will be discussed later. The optimal porogen was ACN, the same solvent that was used in rebinding experiments. In general, MIPs have better recognition properties when rebinding is performed in the same solvent as polymerization[33]. The optimal conditions were 1:6:6 (BPA: 4-VyP: TRIM) in ACN with UV initiation, yielding a selective binding capacity of 3.6 μmol g^{-1} (equation (2-4)).

As mentioned above, Ming *et al.*[34] previously developed a MIP synthesis procedure using precipitation polymerization to create uniform imprinted microspheres that were used in the MISPE-CE-UV method. Microspheres depend delicately on polymerization conditions, and therefore only the amount of template was optimized. Binding capacity increased with template amount, and an optimal ratio of 1:1:2 was used (same components as above). This was a rather large amount of template compared to those used by Moreno-Bondi *et al.*, but yielded a selective binding capacity of around 6.8 μmol g^{-1} under comparable conditions, almost twice as large. The reason for an optimum at large template amounts was attributed to the large solvent volume. As excess porogen is used in precipitation polymerization to synthesize uniform polymer microspheres, much of the template is dissolved in the solvent, rather than associated with the functional monomers. Initiating the polymerization by irradiation created very uniform shaped spheres with apparent diameters between 0.7 - 1.3 μm (Figure 7a). In contrast, Moreno-Bondi *et al.*[32] used bulk polymerized MIPs that were ground and sieved, and the 50 – 100 μm fractions selected for rebinding experiments. Thus, their lower selective binding capacity can partly be attributed to the lower specific surface area of their polymers, and the rupture or deformation of binding sites during grinding.

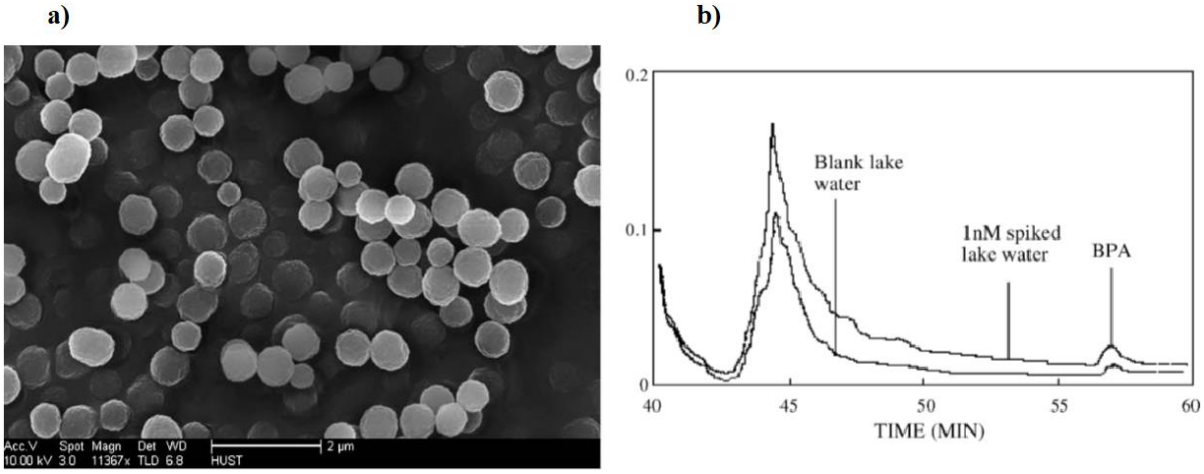

Figure 7: a) Scanning electron micrographs of MIP particles prepared by bulk polymerization. b) HPLC-UV chromatograms obtained from direct injection of 40 mL blank and spiked lake water for 40 min on a BPA-MIP column. Reprinted from[34] with permission from Elsevier.

The uniform synthesized MIP and NIP particles were further packed into chromatographic columns (50 mm x 4.6 mm, slurry packing) and coupled to a HPLC-UV system. The absence of obvious BPA peaks when injecting distilled water indicated that residual template did not leak from the polymers. With an *IF* of 6.53 (equation (2-2)) and a LOD of around 7 ng L^{-1}, the MIP column demonstrated excellent selectivity and sensitivity. Using this column, they also developed a method for the direct analysis of BPA in environmental water samples. Direct injection of 40 mL water samples made possible the enrichment, separation and determination of ultra-trace BPA in one, albeit long lasting, analysis process (Figure 7b). Recoveries ranged from 96% to 102% with RSDs lower than 10%.

Precipitation polymerization among others, makes possible the synthesis of uniform spherical particles of pre-determined size that can be used as stationary phases in liquid chromatography. However, MIPs are not routinely used as stationary phases because of binding site heterogeneity, resulting in broad and tailing peaks. The tailing of BPA can be observed in Figure 7b for the spiked sample. Heterogeneity is not easily avoided when synthesizing MIP because, in general, imprinting increases the heterogeneity of the MIP compared to the NIP, i.e. the imprinting process increases the number of binding sites and their binding energy irregularity (Figure 8).

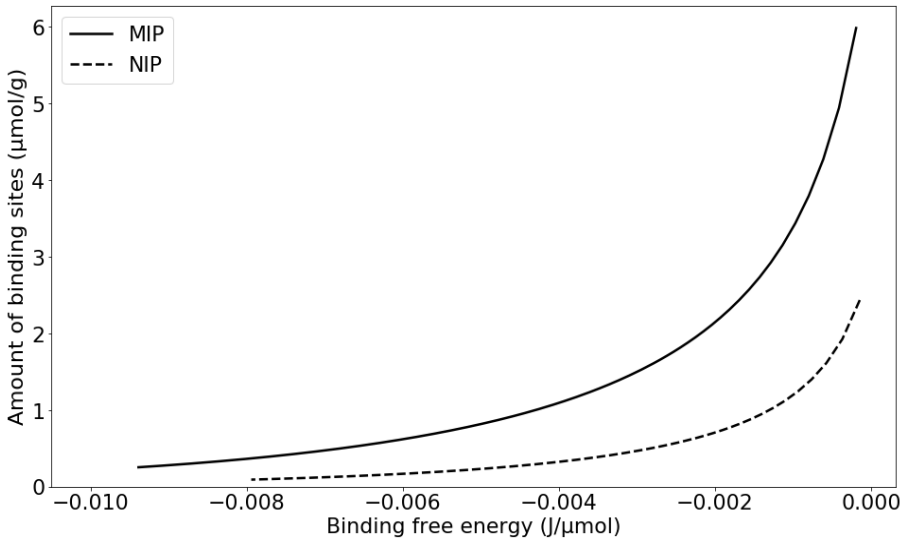

Figure 8: Affinity distribution illustrating the increase in heterogeneity during imprinting. Note the longer tail of the MIP extending into the high binding energy region with more numerous binding sites, which correspond to the selective binding sites in a MIP. Calculated using BPA binding data on a BPS-imprinted polymer from[35] according to equation 5 in[36].

In fact, Umpleby *et al.*[36] suggested that heterogeneity is not only an intrinsic property of imprinted polymers, but characteristic of the imprinting effect. They proposed that the heterogeneity index is a better figure of merit to compare MIPs, in contrast to the usually reported binding constants and number of binding sites obtained by a bi-Langmuir model. Figure 9 compares the heterogeneity of so-called dummy molecularly imprinted polymers (DMIPs) with the corresponding NIPs for all polymers with available binding data considered in this text. The name dummy stems from the use of the template molecule, which is no longer the analyte, but a structural similar compound. Templates in Figure 9 are 1,1,1-Tris(4-hydroxyphenyl)ethane (THPE), BPS, bisphenol AF (BPAF) and 4,4'-dihydroxybisphenol (DDBP). Polymer 6 seems to be an exception to the rule that imprinting increases heterogeneity. However, Gregory *et al.*[37] demonstrated that heterogeneity indexes calculated using the Freundlich isotherm are concentration dependent in some cases. Discrepancies arise in the calculated *m*-values when high concentrations relative to the mass of the polymer are used because the Freundlich isotherm cannot model saturation behavior. More drastic changes in *m*-values were observed for NIPs, attributed to the lower binding capacity and heterogeneity of NIPs. Because polymer 6 was synthesized by imprinting the surface of microparticles (particle diameters of 400 nm)[38], surface effects might also have influenced the heterogeneity.

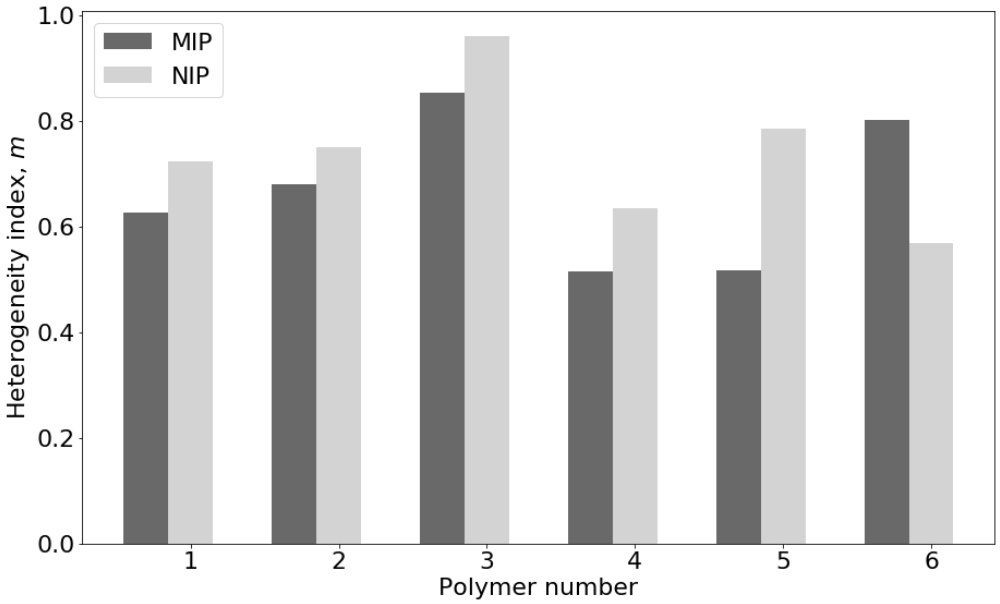

Figure 9: Heterogeneity index obtained by fitting the Freundlich isotherm (equation (2-5)) to binding data of BPA on DMIP and NIP with different templates. Low heterogeneity indexes indicate a more heterogenous material. Data are read directly from binding plots using WebPlotDigitizer[39]. Template molecules are 1: THPE[40], 2: BPS[35], 3-5: BPAF (3^{25},4^{24} and 5^{26}) and 6: DDBP[38].

In addition to their heterogeneous nature, MIPs are selectivity towards a limited number of compounds and have poor mass transfer properties. They have therefore found only few applications as stationary phases. However, they could in the future be useful for large scale enantiomeric separations[41, 42].

As mentioned earlier, preceding polymerization an equilibrium between free and complexed template governs the number of imprinted sites. To favor complex formation and thereby increase the number of imprinting sites, a large amount of functional monomer relative to template is needed to shift the equilibrium. Upon polymerization, unassociated functional monomers, now present in large amounts, are incorporated into the polymer matrix, and create non-selective binding sites. If the resulting material is used as a sorbent, the incorporated non-selective binding sites can co-extract matrix components[18, 43].

A hybrid of covalent and non-covalent imprinting, often called semi-covalent imprinting, seeks to combine the advantages of the two approaches. It is based on the use of templates that are covalently bound to the monomer. Upon polymerization and subsequent cleavage of template-monomer bonds, the functional groups will now be associated exclusively with binding site

cavities, creating more selective binding sites and less non-selective sites[21, 33], ideally creating more homogenous materials. This requires template molecules with suitable functional groups, making BPs with two -OH groups on opposing sides excellent candidates.

Alexiadou *et* al.[21] applied non- and semi-covalent MISPE in the determination of BPA in milk and water samples. The non-covalent polymer yielded an *IF* of 4.10, higher than the two semi-covalent MIPs with *IF*s of 2.05 and 1.72. The low *IF*s of the semi-covalent MIPs were attributed to the harsh post-polymerization conditions, i.e. hydrolysis and subsequent grinding, making only a fraction of high energy binding sites available. Upon hydrolysis of the BPA-monomer covalent bond, only BPA should be present in the washing solution, but the presence of cleaved crosslinkers, release of oligomers and unreacted monomers complicated its use as a sorbent. In addition, so called template bleeding was encountered. Template molecules that are imprinted deep inside the polymer matrix will remain trapped even after exhaustive washing procedures and gradually release into solution[43]. This was observed by Mosbach *et al.*[17] using the non-covalent approach and dyes as templates, resulting in colored polymers even after several washings. Using sorbents that release target analytes into solution is problematic in trace analysis, and this so-called template bleeding is one of the main drawbacks of MISPE.

To avoid both non-selective binding sites and template bleeding, He *et al.*[24] synthesized a DMIP using BPAF (Figure 4a) and a semi-covalent sol-gel strategy. The result after gelation was a mesoporous material with template covalently bound to the surface of the silica. The covalent bonds were thermally cleaved, creating functional cavities with non-covalent binding sites. In a separate MIP using BPA as template they demonstrated that template bleeding could not be avoided. However, BPAF leaking into solution did not interfere with the determination of BPA because the two peaks were well separated.

Their group developed their existing procedure to improve binding kinetics[25], i.e. the time it takes for analytes to reach binding equilibrium with the sorbent. Using bulk polymerization creates materials with low accessibility and slow binding kinetics. If instead only the surface is imprinted, the sites would much more accessible and binding kinetics enhanced. In addition, problems with template removal are diminished because they are no longer trapped inside the polymer. Thus, He *et al*.[25] imprinted the surface of silica particles having 50 – 75 μm diameters. Figure 10 compares the binding capacity of BPA on the BPAF-DMIP as function of binding duration using the two techniques. The surface imprinting method successfully reduced the binding saturation time. However, BPAF leakage could not be avoided.

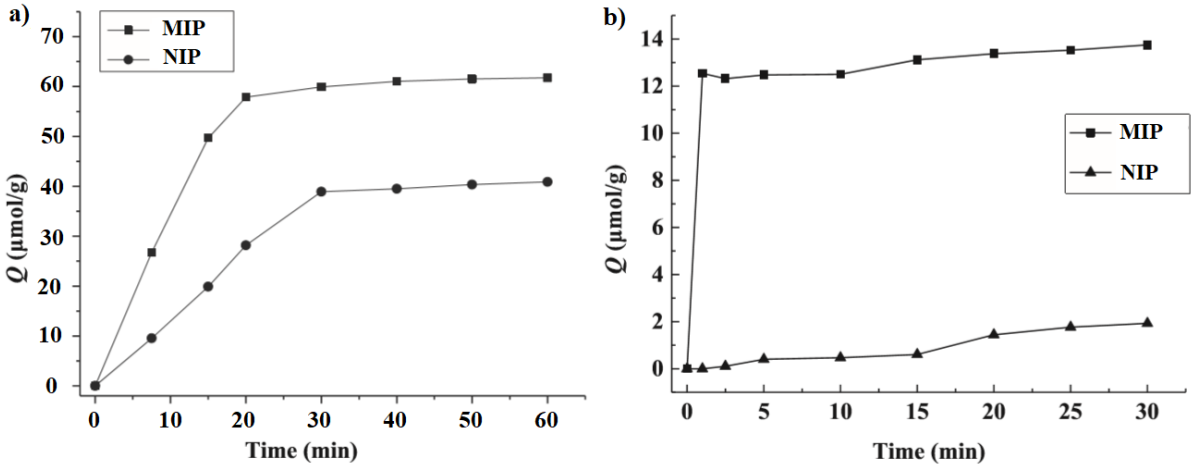

Figure 10: Binding capacity (*Q*) of BPA on BPAF-imprinted and non-imprinted polymers as a function of binding duration using a) bulk polymerization[24] and b) surface imprinting[25]. Note the different axis scales. Reprinted from[24, 25] with permission from Elsevier.

Their group further imprinted magnetic nanoparticles using their newly developed surface imprinting method, making possible the fast isolation of MIPs in dispersive-SPE by an external magnetic field[26]. With these three DMIPs they developed MISPE-HPLC-UV methods for the determination of BPA in different water samples and one orange sample, with recoveries in the range 93% - 106%, RSDs of 5% and lower, and LODs of 0.3 µg L^{-1}.

A similar DMIP surface imprinting technique with 4,4'-dihydroxy biphenyl (DDBP, Figure 4a) was developed using the non-covalent approach and monodisperse silica particles with 400 nm diameters[38]. The resulting polymers showed high breakthrough volumes and fast binding kinetics. In addition, surprisingly large selective binding capacities for BPA were achieved, attributed to the high surface-to-volume ratios of the microparticles. Using this DMIP as a sorbent, they developed a MISPE-HPLC-UV method for the determination of BPA in different water samples (rain, leachate, tap and lake water). Average recoveries ranged from 93% - 102%, with RSDs lower than 11% and LOD of 50.7 ng L^{-1}. The method would suffice for even lower BPA concentrations if higher volumes were loaded or a more sensitive detector used.

The choice of dummy template is not trivial. Because of template bleeding, the template should not be an analyte in trace analysis. It should have a similar structure compared to the analytes of interest, allowing strong and selective interactions with the functionalized cavities of the polymer. However, using dummies instead of analytes as templates generally leads to inferior

binding properties. Dummy templates are therefore often selected according to structural similarities to analytes, but the resulting selectivity can only be evaluated after the DMIP is synthesized and tested, which is time consuming. An interesting alternative approach to the selection of both dummy template and optimal polymerization conditions has been proposed by Sun *et al.*[40]. Instead of synthesizing multiple DMIPs with different templates and often with polymer compositions, only the corresponding NIP is synthesized. Because selective interactions of a non-covalent MIP are merely interactions of the polymer matrix with analytes formed according to a template, strong template-NIP interactions should yield high binding affinities towards analytes when instead the DMIP is used. The interaction strengths between templates and NIP can be estimated based on template retention factors on the NIP-column. To test the validity of the approach, they synthesized 10 DMIPs using 5 different templates and various polymerization conditions. The DMIPs, and corresponding NIPs, were packed into HPLC columns and k_{DMIP}, k_{NIP}, and *IF* were evaluated using porogen as mobile phase. A correlation coefficient of 0.9619 between k_{NIP} and *IF* for the template was found. That is, the *IF* of the template can be estimated by evaluating the retention of the template on the non-imprinted column, eliminating the need to synthesize several DMIPs and picking one with a high *IF*. When evaluating *IF*s of other compounds on the DMIP-columns, structural differences between template and analyte limited the applicability of the method, e.g. using TBBPA or 4,4'-diaminodiphenylmethane (DADPM) resulted in low *IF*s for BPs (Figure 11a).

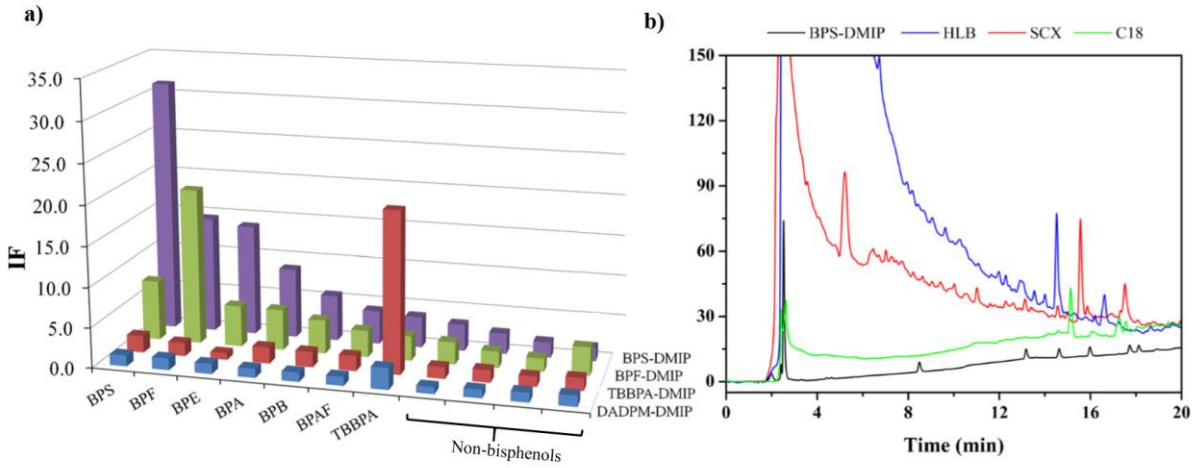

Figure 11*:* a) Imprinting factors of BPs and similar compounds on DMIPs. TC b) HPLC-DAD chromatogram of a river sample spiked with 200 ng L^{-1} of five bisphenols and extracted with BPS-DMIP, HLB, SCX, or a C18 sorbent. Adapted and reprinted from[40] with permission from Elsevier.

However, when structural similarities between template and analytes in addition to template k_{NIP} are considered, this method can successfully predict dummy templates that create high affinity DMIPs. The synthesized BPS-DMIP sorbent was compared with commercial sorbents such as a hydrophilic-lipophilic balance (HLB) sorbent, a strong cation exchange (SCX) sorbent and an octadecyl silica (C18) sorbent using SPE-HPLC-UV on a river sample. Figure 11b illustrates the superiority of the BPS-DMIP sorbent. Having breakthrough volumes greater than 500 mL resulted in a LOD of less than 3.6 ng L^{-1} for all five analytes.

BPS is an important substitute for BPA, and because of template bleeding (peak around 8 min in Figure 11b), it cannot be determined accurately in trace analysis when using a BPS-DMIP. An alternative template is therefore required when BPS is an analyte. Using their NIP column method, Sun *et al.*[35] found that THPE-DMIP yield highly class selective DMIP with superior *IF*s (Figure 12a).

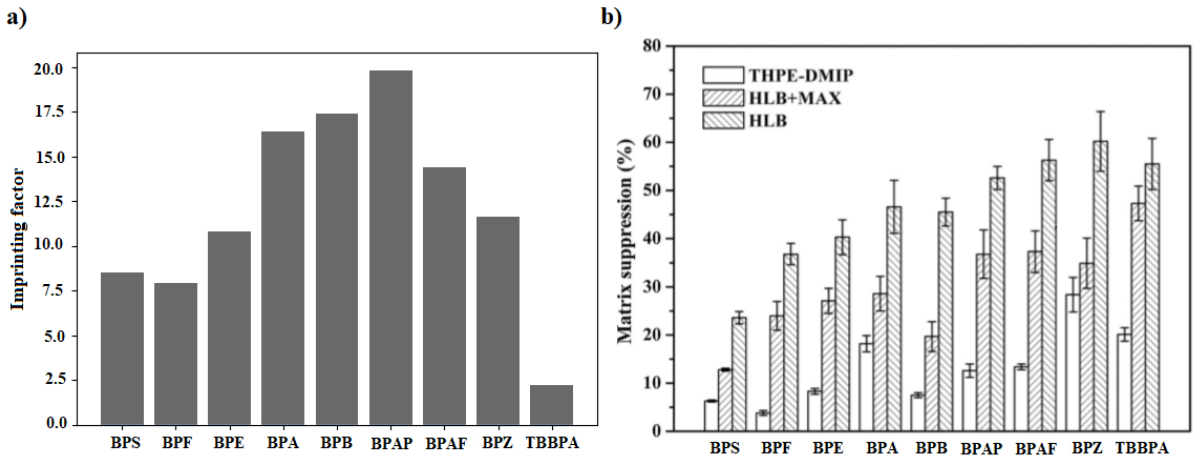

Figure 12: a) Imprinting factors of THPE-DMIP from[35]. b) Comparison of matrix suppression using a DMIP, HLB and HLB + MAX SPE for the determination of nine bisphenols in wastewater treatment plant influent[44]. Calculated using equation (2-8). Reprinted from[44] with permission from Elsevier.

This THPE-DMIP was used in the determination of BPs in sewage and sludge[44]. A common sorbent in these matrices is the HLB sorbent. In such complex matrices, ionization efficiencies can be influenced because matrix components can co-elute, reducing or enhancing the analyte signal. To diminish matrix effects, a subsequent extraction step by mixed-mode anion exchange (MAX) SPE can be used[4]. This is time consuming and expensive if many samples are analyzed. Thus, the highly class selective THPE-DMIP was used to replace the HLB + MAX sorbents in a method for the group extraction of nine bisphenols in sewage and sludge. The different

sorbents were compared in terms of matrix suppression (Figure 12b). Not only did MISPE reduce matrix interferences compared to HLB+MAX-SPE, but also the time and cost of analysis. This demonstrates the potential of MISPE in sample preparation. The ability to selectively extract analytes from complex matrices reduces matrix interferences and could eliminate the need for time consuming calibration methods. Owing to their ready synthesis and cheap starting materials, they can also reduce the cost of an analysis.

4. Conclusion

MIPs are materials that selectively bind their templates or structurally similar compounds. To synthesize MIPs with high binding capacities, strong interaction between template and functional monomer are required to establish ordered complexes which can be polymerized. In addition, polymerization temperature and cross-linker amount influence the order and rigidity of the polymer. Rigid and uniform shaped polymers are essential if they are used as stationary phases for chromatography, but their heterogenous binding sites, slow mass transfer and affinities for a limited number of compounds limit their use as stationary phases. Slow binding kinetics, in addition to template bleeding and non-selective binding sites are drawbacks that complicate the use of MIPs as extraction sorbents. This has led to the development of new methods such as surface imprinting, dummy imprinting and semi-covalent imprinting that aim to solve these inherent drawbacks and facilitate their use as sorbents. MIPs can then be considered as effective alternative sorbents for solid-phase extraction when readily available less selective sorbents are inadequate for the problem.

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