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REVIEW

Small organic molecular imprinted materials: their preparation and application

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Abstract Molecular imprinting is a technique for preparing polymeric materials that are capable of recognizing and binding the desired molecular target with a high affinity and selectivity. The materials can be applied to a wide range of target molecules, even those for which no natural binder exists or whose antibodies are difficult to raise. The imprinting of small organic molecules (e.g., pharmaceuticals, pesticides, amino acids, steroids, and sugars) is now almost routine. In this review, we pay special attention to the synthesis and application of molecular imprinted polymer (MIPs) imprinted with small organic molecules, including herbicides, pesticides, and drugs. The advantages, applications, and recent developments in small organic molecular imprinted technology are highlighted.

Keywords Molecular imprinting · Solid-phase extraction · High-performance liquid chromatography · Sensor

Introduction

In recent years, molecular imprinting has attracted considerable interest in many areas of chemistry, biochemistry, and biotechnology. Cameron Alexander defined molecular imprinting as: "The construction of ligand selective recognition sites in synthetic polymers where a template (atom, ion, molecule, complex or a molecular, ionic or macromolecular assembly, including micro-organisms) is employed in order to facilitate recognition site formation during the covalent assembly of the bulk phase by a polymerization or polycondensation process, with subsequent removal of some or all of the template being necessary for recognition to occur in the spaces vacated by the templating species" [1]. In the field of chemistry, especially in analysis and separation science, molecular imprinted materials are receiving increasing attention owing to their high selectivity and affinity for the target molecules [2]. Molecular imprinted materials, usually called molecular imprinted polymers (MIPs), are artificially synthesized macromolecular materials with prearrangement of structure and specific molecular recognition ability [3]. Molecular imprinted materials have a lot of advantages over biological receptors. Molecular imprinted materials differ from biological receptors in that they are large and rigid, whereas their natural counterparts are smaller and flexible. Depending on their size, molecular imprinted materials can have thousands or millions of binding sites, whereas biological receptors have a few or even just one [4]. In addition to these advantages, the molecular imprinted materials are intrinsically stable and robust.

MIPs can be synthesized by three different imprinting approaches: the non-covalent, the covalent, and the semi-covalent. In all these protocols, a template molecule interacts with an appropriate functional monomer to establish specific interactions [5]. In the imprinting techniques, the removal of the template results in a material that contains imprinted cavities with a favorable size, shape, and chemical environment to selectively rebind the target molecules. The synthesis of MIPs and the recognition process are represented in Fig. 1 [6].

However, MIPs possess many disadvantages: for example, it is hard to completely remove the template from MIPs; the imprinted polymer is insoluble; and the polymer contains many imprinted cavities of which only some are

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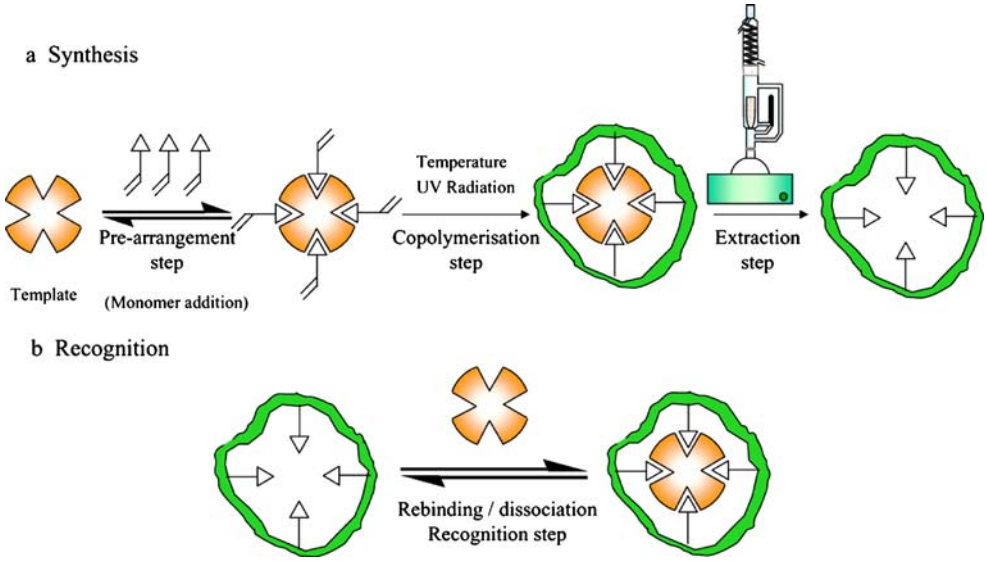


Fig. 1 Molecular imprinting process [6]

really good and match the template molecules. So, some new synthetical methods have been developed in the last few years [7].

Up to now, there have been numerous reviews summarizing the development of molecularly imprinted polymers [2, 8–10], but the preparation and application of small organic molecular imprinted materials have rarely been reviewed. In this article, the advantages, applications, and recent developments in small organic molecular imprinted technology are discussed in detail.

Imprinting

In the past few years, various methods have been developed to synthesize MIPs [11–14]. Essentially two strategies for molecular imprinting have been established based on whether the template is associated with functional monomers using covalent bonds or non-covalent interactions [15]. The semi-covalent approach is a hybrid of the two previous strategies. There have been many excellent reviews on the topic, in which the covalent approach was summarized in detail [15–20]. In the present review, non-covalent and semi-covalent approaches are emphasized.

Advances in non-covalent molecular imprinting

The non-covalent approach, introduced by Arshady and Mosbach [21], is based on the formation of relatively weak non-covalent interactions between selected monomers and template molecule before polymerization. Non-covalent imprinting uses the typical intermolecular forces such as hydrogen bonds, ion pairs, dipole–dipole interactions, and van der Waals forces to generate adducts of template and functional monomers in solution [16]. The non-covalent approach is most widely used now because of the simple processes to remove the template and the resulting greater numbers of higher affinity sites.

Use of these materials can be attributed to their rather straightforward synthesis and the vast choice of available monomers. During the last few years, many different functional monomers have been tested in non-covalent imprinting: monomers may carry basic (e.g., vinylpyridine) or acidic (e.g., methacrylic acid) groups, or be permanently charged (e.g., 3-acrylamidopropyltrimethylammonium chloride), or be hydrophobic (e.g., styrene), or exhibit hydrogen bonding (e.g., acrylamide), and so on. Reference [16] summarizes the typical examples of monomers in non-covalent imprinting with their typical applications.

Molecularly imprinted polymeric nanospheres and nanowires

The common MIPs were prepared as a macroporous monolith, then ground and sieved to the required particle dimensions. Recent improvements in the morphology of MIP particles have been achieved using a precipitation polymerization procedure that allows one to obtain microspheres or nanospheres with regular size and shape. Some novel molecularly imprinted polymeric nanospheres (MIPNs) enhance the affinity, selectivity, capacity, and accessibility of the MIPs for recognizing target molecules owing to their high surface/volume ratio [22–35]. Zhao and the co-workers [22] prepared novel molecularly imprinted polymeric nanospheres by combining molecular imprinting and block copolymer self-assembly techniques. They synthesized a diblock copolymer with one block containing functional groups for both hydrogen bond formation and

cross-linking. After interacting with the template molecules to form hydrogen-bonding complexes, this block copolymer was allowed to self-assemble to form spherical micelles in a selective solvent. This desired structure was then locked in by cross-linking, and the cross-linked nanospheres were extracted to remove template molecules. The shape and size of the MIP particles are of critical importance for their performance. With the smaller sizes and well-defined structures of MedIP particles, the MIPs achieved higher affinity, selectivity, and better site accessibility. Figure 2 illustrates that MIPNs (100 nm) had higher capacities than common bigger MIPs (5 μm) and shape selectivity in rebinding the target molecules. Ciardelli and co-workers [23] demonstrated the formation of cholesterol-imprinted methacrylic acid nanospheres through a cross-linking polymerization starting from a dilute monomer solution. Henrik and co-workers [24] developed a straightforward route to prepare spherical MIP beads using a method that comprised the formation of droplets of pre-polymerization solution directly in mineral oil by vigorous mixing followed by transformation of the droplets into solid spherical beads by photoinduced free radical polymerization. They used this method to synthesize propranolol-imprinting polymer with methacrylic acid as monomer and trimethylopropane trimethacrylate as cross-linker. To contrast the traditional procedures of grinding polymer monoliths to irregularly shaped particles, the beads showed similar characteristics with regard to elemental composition, surface area, pore diameter, and pore volume. The binding capacity was higher for the beads than for the particles in most of the solvents investigated. This might be the result of a better accessibility of the recognition sites in the polymer network of the beads (i.e., the higher number of recognition sites). The main merits of the described procedure lie in the attractive format of the MIPs produced and the simple production method. Spherical beads are preferred over irregularly shaped particles for most applications and in particular for those involving chromatography, solid-phase extraction, and other flow-through techniques. During the synthesis, the suspension step was fast, typically less than 1 min using a homogenizer, and the polymerization was completed within 10 min under a high-efficiency UV lamp.

Li et al. [36] developed a convenient imprinting method for the preparation of theophylline magnetic molecularly imprinted nanowires within the pores of nanoporous alumina membrane. The nanopores were then filled with a pre-polymerization mixture containing the superparamagnetic $MnFe_2O_4$ nanocrystallites. After polymerization, the alumina membrane was subsequently removed by chemical dissolution, leaving behind magnetic polymer nanowires that contain theophylline binding sites uniquely residing at the surface and have a saturated magnetization of 1.97 emu g^{-1}. Figure 3 shows the scanning electron micrograph image of the magnetic imprinted nanowires. The resulting magnetic imprinted polymer nanowires were capable of binding theophylline more strongly than the non-imprinted nanowires. The use of theophylline-immobilized nanopores as the template resulted in imprinted nanowires having recognition sites on or close to the surface, making them more accessible for analytes to diffuse in. As a result, the commonly practiced porogen and polymer grind-up process used in making conventional molecularly imprinted nanowires is no longer needed. Furthermore, the size of these imprinted

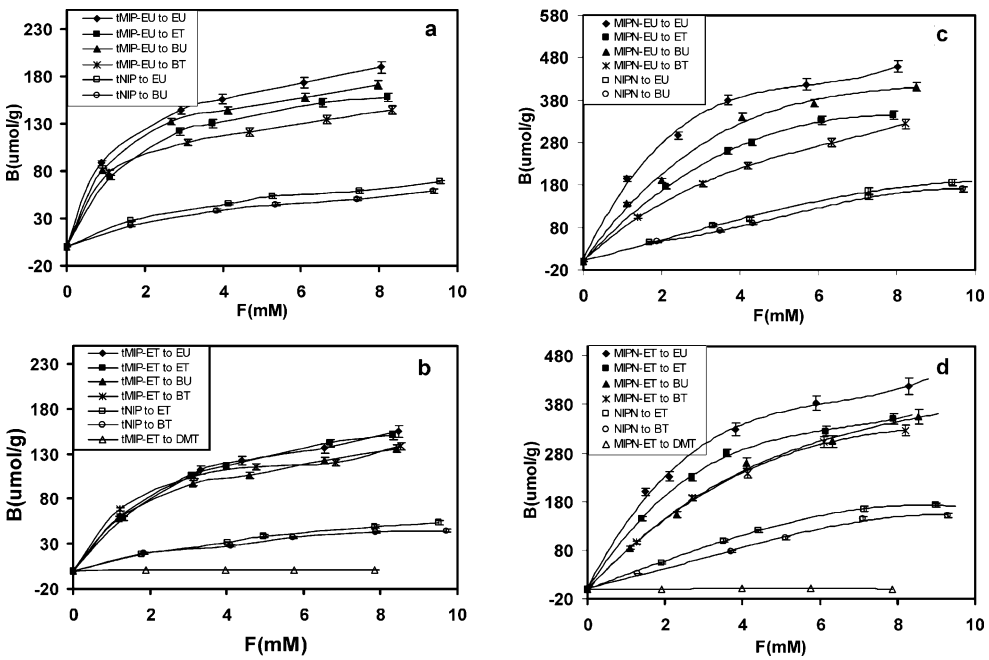


Fig. 2 Rebinding isotherm to analytes of (**a**) tMIP-EU, (**b**) tMIP-ET, (**c**) MIPN-EU, and (**d**) MIPN-ET. 1-Ethyluracil (EU), 1-ethylthymine (ET) [22]

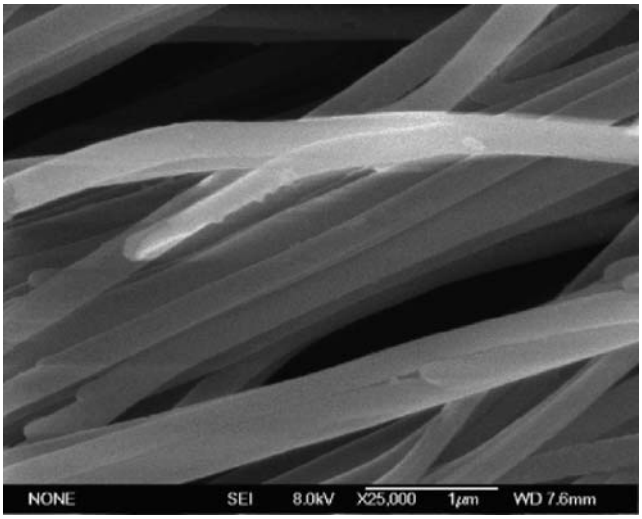


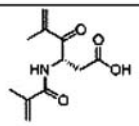
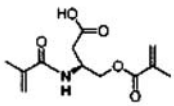
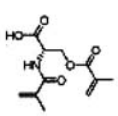
Fig. 3 Scanning electron micrograph (SEM) image of magnetic imprinted nanowires [36]

nanowires is in the nanometer range and they can therefore be well dispersed in solution; furthermore, their applications should be compatible with procedures where biological antibodies might otherwise be used. Chang et al. [37] prepared a molecularly imprinted nanocapsule, which showed excellent site accessibility and potential in delivery applications.

Molecularly imprinted new cross-linkable monomers

Some new monomers combined with cross-linkable groups were developed to synthesize MIPs effectively [38, 39]. Vazquez and co-workers [38] developed a new strategy to design monomers combining interactive monomer functionality with a cross-linking format and obtained the non-covalent MIPs with improved performance. They indicated that imprinted polymers formed using a non-covalent strategy relied on the solution concentration of functional monomer to form the pre-polymer complex. Increasing the concentration of functional monomer with a lower amount of cross-linker resulting in the MIP losing its recognition properties due to random motion of non-cross-linked polymer domains. Table 1 shows the binding studies for MIPs imprinted with (*S*)-nicotine and formulated using the new cross-linking functional monomers. Quantitative structure–selectivity relationship studies have verified that a key improvement to monomer design in these studies was to provide the template-interactive functional group in a

Table 1 Binding studies for MIPs imprinted with (*S*)-nicotine and formulated using the new cross-linking functional monomers [38]

Entry	Functional Monomer	Polymer	t_s (avg) min	t_R (avg) min	k'_s	k'_R	α
1	 **2**	Non Imprinted	0.63	0.63	1.03	1.03	1.00
		Imprinted	1.05	1.02	2.39	2.29	1.04
2	 **1**	Non Imprinted	0.88	0.88	1.75	1.75	1.00
		Imprinted	1.42	1.34	3.58	3.32	1.08
3	 **4**	Non Imprinted	0.51	0.51	0.28	0.28	1.00
		Imprinted	0.68	0.58	0.7	0.45	1.56
4	 **3**	Non Imprinted	1.88	1.86	3.7	3.65	1.01
		Imprinted	15.9	8.6	41.97	22.24	1.89

cross-linking monomer format. The origins of the improved selectivity were attributed to two factors of the cross-linking functional monomers. First, the degree of cross-linking is maximized without imposing restrictions on functional group concentrations. Second, covalently tethering the functional group to the binding site matrix reduces conformational entropy that would otherwise interfere with specific binding.

Zhao [39] and co-workers reported the synthesis and characterization of a range of novel multifunctional copolymers that possess both specific recognition groups and cross-linkable functionalities. Specifically, copolymers of 2-methacryloylethyl methacrylate and methacrylic acid P (MAEMA-*co*-MAA) with different compositions were synthesized and characterized with theophylline as a model template.

Template-analog approach

A few percent of the template molecules are trapped in the highly cross-linked polymer matrix and cannot easily be washed out from the synthesized MIPs. Some of these residual template molecules will inevitably leach out and falsify the results [3]. To overcome this drawback, some authors have used a "dummy template"—the imprint is created not with the target analyte itself, but with a structurally related molecule. The polymer can still bind the target analyte, but the leaching template does not coelute with the analyte during chromatographic analysis [40–45]. Graham et al. [40] used 4,4′-ethylidenebisphenol (EBP) as template to synthesize molecularly imprinting sol–gel materials for 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT) with the semi-covalent approach. Shugfart et al. [45] used 9,10-anthracenediol, which is structurally similar to 9-anthrol, as the template molecular to synthesize fluorescent MIPs to recognize 9-anthrol.

Semi-covalent molecular imprinting

The semi-covalent approach is a hybrid of the non-covalent and the covalent methods. Covalent bonds are established between the template and the functional monomer before polymerization. Once the template has been removed from the polymer matrix, the subsequent rebinding of the analyte to the MIP exploits non-covalent interactions, as per the non-covalent imprinting protocol [5]. References [15, 46–49] discuss several examples of semi-covalent molecular imprinting.

Ester et al. [46] synthesized two MIPs for the selective extraction of 4-nitrophenol. One polymer was synthesized via a non-covalent approach and the other via a semi-covalent approach. The selectivity of the polymers was evaluated in on-line molecular imprinted solid-phase extraction (MISPE). Figure 4 shows the differences in selectivity and recovery of the two polymers. Whereas the non-covalent MIP was more selective, the semi-covalent MIP showed slightly higher recoveries.

Figure 5 [45] illustrates the synthesis of semi-covalent fluorescent MIPs that recognize 9-anthrol using 9,10-anthracenediol as the template. In the polymerization step, two covalent (ester) bonds were formed between the template and the monomer. Following by the removal of the 9,10-anthracenediol with $LiAlH_4$, new amino-functionalized sites were formed. Consequently the target molecule, 9-anthrol, interacted with the functional polymer by hydrogen bonding. Cacho et al. [50] used propazine methacrylate as template molecule to prepare a semi-covalent imprinted polymer. Subsequently, the semi-covalent polymer was used to determine several triazinic herbicides in soil and vegetable samples by a molecular imprinted solid-phase extraction method. Table 2 shows a comparison between the non-covalent polymer and semi-covalent polymer [50]. The semi-covalent polymer presented a more homogeneous binding site distribution and higher capacities owing to the better-defined complex in the pre-polymerization step.

Applications

Selectivity is the predominant advantage of MIPs in separating and analyzing complicated samples, such as environmental and biological samples. For small organic molecules, MIPs have been exploited in several applications, e.g., solid-phase extraction (SPE), chromatography and electrochromatography, membrane separation, and sensors.

Molecular imprinted solid-phase extraction (MISPE)

Nowadays, solid-phase extraction (SPE) is being widely utilized for preconcentration or separation of organic molecular owing to its advantages [51, 52]. The essential requirements in selecting SPE sorbents are: 1) the possibility of extracting a large number of trace elements over a wide pH range; 2) quantitative sorption and elution; 3) kinetically faster sorption and desorption mechanism; 4) regenerability; 5) high retention capacity; 6) accessibility; 7) mechanical and chemical strength [53]. Trends in analytical extraction have been towards less solvent consumption, timesaving, higher recoveries, better reproducibility, lower method detection limits, and automation. Improved extraction selectivity is often achieved typically through subsequent separations methods, selective analytical methods, or both [54]. Development of new sorbents with high affinity, specific recognition, and high stability is

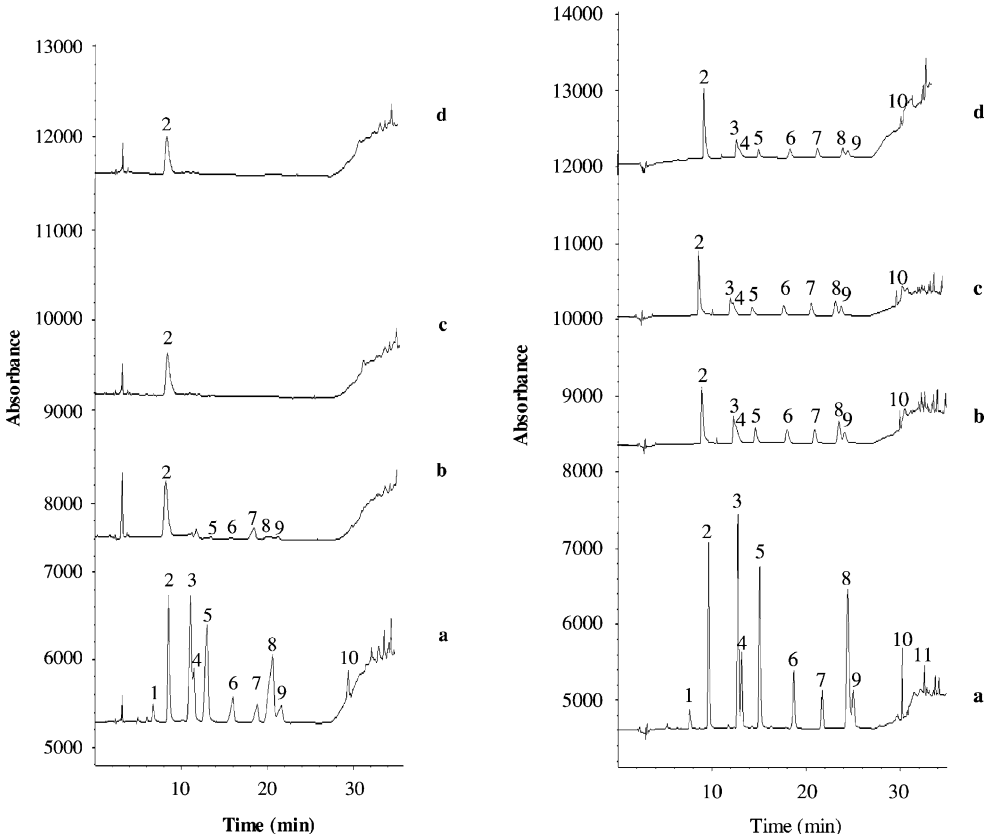


Fig. 4 Chromatograms obtained by on-line MISPE with the non-covalent 4-NP imprinted polymer (P1, *left*) and semi-covalent 4-NP imprinted polymer (P2, *right*) of 10 mL standard solution (pH 2.5) spiked at 10 mg L^{-1} with each phenolic compound. **a** Without washing step, and **b**–**d** with washing step using 0.1, 0.2, and 0.3 mL of dichloromethane, respectively. *1* Ph, *2* 4-NP, *3* 2,4-DNP, *4* 2-CP, *5* 2-NP, *6* 2,4-DMP, *7* 4-C-3-MP, *8* 2-M-4,6-DNP, *9* 2,4-DCP, *10* 2,4,6-TCP [46]

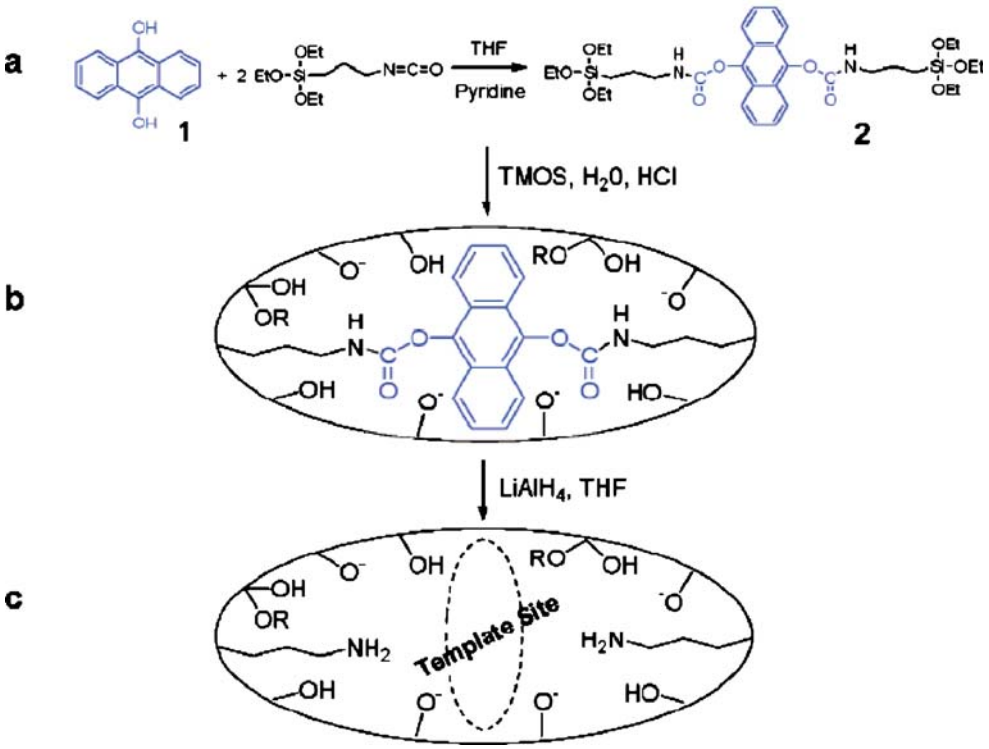


Fig. 5 Synthesis of fluorescent semi-covalent MIPs for 9-anthrol [45]

Table 2 Average recoveries of triazines after direct MISPE of spiked sample extracts on semi-covalent (sc-MIP) and non-covalent (nc-MIP) propazine-imprinted polymers [50]

	Potato		Corn		Soil	
	sc-MIP	nc-MIP [9]	sc-MIP	nc-MIP [9]	sc-MIP	nc-MIP
Desisopropylatrazine	83	n.d.[a]	n.d.[a]	n.d.[a]	91	95
Desethylatrazine	86	88	96	n.d.[a]	93	99
Simazine	81	91	85	72	89	92
Atrazine	85	93	92	93	95	105
Propazine	89	116	97	n.d.[a]	90	51

RSDs were below 10% in all cases
[a] Not detected due to the presence of matrix interfering components

of great significance. Consequently, in the last decade, SPE was the analytical technique in which MIPs have found most applications.

Reference [5] discussed the application of MISPE in environmental and biological samples in detail, e.g., ref. [6] discussed the application of MISPE to antibiotic determination.

Off-line MISPE

Herbicide and pesticide MISPE Many herbicide and pesticide MIPs were synthesized and applied as MISPE [55–69]. Yang et al. [56] used diniconazole, a triazole-based fungicide, as the template to synthesize MIPs. Moreover, a solid-phase extraction column packed with diniconazole-imprinted polymers was used to enrich diniconazole. It was proved that diniconazole-imprinted polymers have the potential applications in the enrichment, separation, and detection of diniconazole in biological fluids. Koeber et al. [61] developed a multidimensional on-line MISPE platform to analyze triazines in river water samples. The method comprises the combination of a restricted access material (RAM) and a MIP, allowing a selective sample preparation to be achieved in the on-line mode. The RAM column combines size exclusion and adsorption chromatography, and the MIP column selectively retains the triazine analytes, whereas the residual matrix is not retained and separated completely. Figure 6 shows the working principle of the RAM-MIP coupling procedure. The cleaned and enriched extract was subsequently eluted to an HPLC column and analyzed by LC-MS. A complete on-line analysis cycle including multidimensional solid-phase extraction, separation, and detection took less than 15 min.

Drug MISPE Many MISPE methods were developed to analyze drugs in biological samples [1, 70–85]. Caro and co-workers have done much research on drug MISPE methods [86–89]. They molecular imprinted polymers for drugs, including enrofloxacin, ibuprofen, and ciprofloxacin. For example, they used enrofloxacin as the template molecule to prepare a new molecularly imprinted polymer [86]. The molecularly imprinted polymer was then applied as a selective adsorbent in a two-step solid-phase

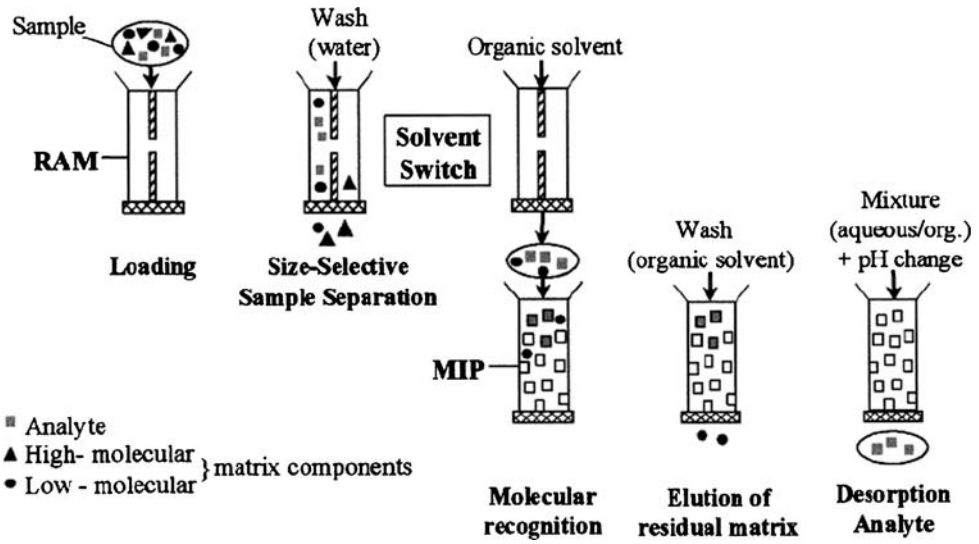


Fig. 6 Schematic of the working principle of the RAM-MIP coupling procedure. The *upper left row* shows the process in the RAM column; the *lower right row* shows the process in the MIP column [61]

extraction method focusing upon complex biological matrices, specifically human urine and pig liver. The research indicated that this efficient method suppressed the interfering peaks arising from the complex biological matrices and could determine enrofloxacin and ciprofloxacin at lower concentrations.

Yang and co-workers [72] investigated a method for MISPE of cotinine, which is the main metabolite of nicotine in the human body. The MIP obtained had good selectivity and affinity for cotinine and was successfully used as a selective sorbent in SPE. This MISPE-HPLC method was well suited for direct determination of cotinine in urine samples from both active and passive smokers. Figure 7 [72] proved the good selectivity of the MISPE method by the difference between the elution profiles obtained by RP-HPLC analysis before and after extraction on cotinine-imprinted polymer. Direct injection of the urine samples not only gave matrix peaks coeluting with cotinine, which also hindered accurate quantification and shortened the HPLC column lifetime because of uneluted matrix components.

Schirmer et al. [78] described a method to prepare a MISPE sorbent for chloramphenicol and its application to a honey sample. The authors optimized the methanol-to-water ratio carefully in the MISPE procedure to remove matrix components from the sample of honey and allow the satisfactory extraction of chloramphenicol. Recoveries of nearly 100% and approximately 90% were obtained from a standard solution and honey samples, respectively. In ref. [79], two MIPs were synthesized using tetracycline and oxytetracycline as template molecules in non-covalent molecular imprinting procedures. Oxytetracycline MISPE, which gave better MISPE results, was used to analyze tetracycline antibiotics in pig kidney tissue. It was demonstrated that a clean-up step of MISPE could disrupt the non-specific interactions between the MIPs and the compounds retained except tetracycline antibiotics. Lin et al. [1] prepared a sinomenine MIP as an SPE sorbent and utilized MISPE as the separation medium to extract sinomenine from herbs and plasma samples. The operating conditions of the MISPE method were optimized to clean up the impurity and the average recovery was 98%.

On-line MISPE

Some applications of MIPs to separation and extraction were novel and practical. A few of examples of MIP applications used the polymer as a pre-column sorbent [90–93]. Hantash and co-workers [90] prepared carbaryl and 1-naphthol molecular imprinted polymers, which were tested for selective and reversible binding to carbaryl and 1-naphthol, respectively. The imprinted polymer was then slurry-packed into a poly(ether ether ketone) (PEEK) column to prepare the pre-column. The pre-column was used to isolate carbaryl and its metabolites from complex matrices injected onto an HPLC system. The "dilute and shoot" approach for the rapid, inexpensive, and accurate determination of carbaryl and 1-naphthol in complex biological matrices (rat plasma and apple homogenate) with a detection limit of 1.00 ng mL^{-1} and a linear response (r^2>0.98) over the concentration range 1.00–10.0 ng mL^{-1} was validated in accordance with the Guidance for Industry Bioanalytical Method Validation as outlined by the US Food and Drug Administration (FDA). MIPs for the recognition of methyl-carbamate pesticide carbaryl in water have been synthesized [91] and used as the sorbent for on-line SPE combined with flow-injection and HPLC. This versatile platform for the determination of pesticide carbaryl is shown in Fig. 8 [91]. He et al. [92] developed a method for on-line MISPE combined with a flow-injection system to analyze metformin in human serum samples. The results showed that combining a flow-injection system with an MISPE column enables simple, convenient, and rapid separation of metformin from complex samples. Several experimental conditions which affected the extraction process were optimized and MIP was shown to have important practical advantages over other methods: in particular, the MIP column was placed before the flow cell and use of oxidant to elute the analyte

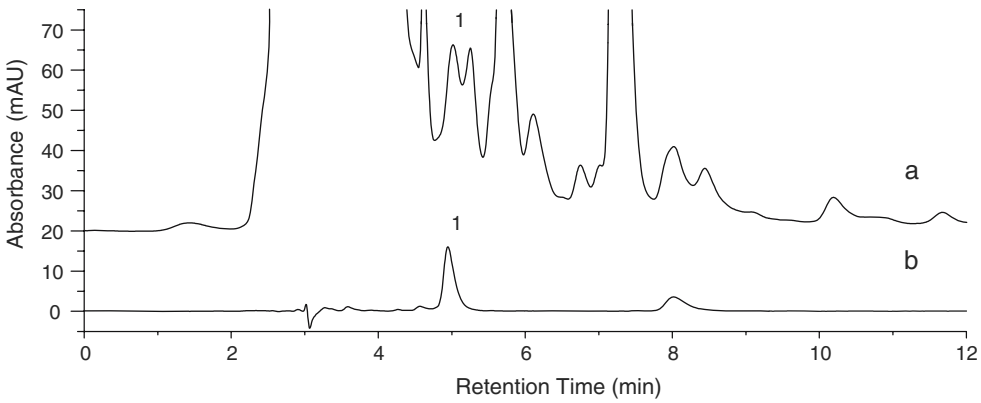


Fig. 7 Chromatograms obtained from an active smoker's urine sample before (**a**) and after (**b**) MISPE [72]

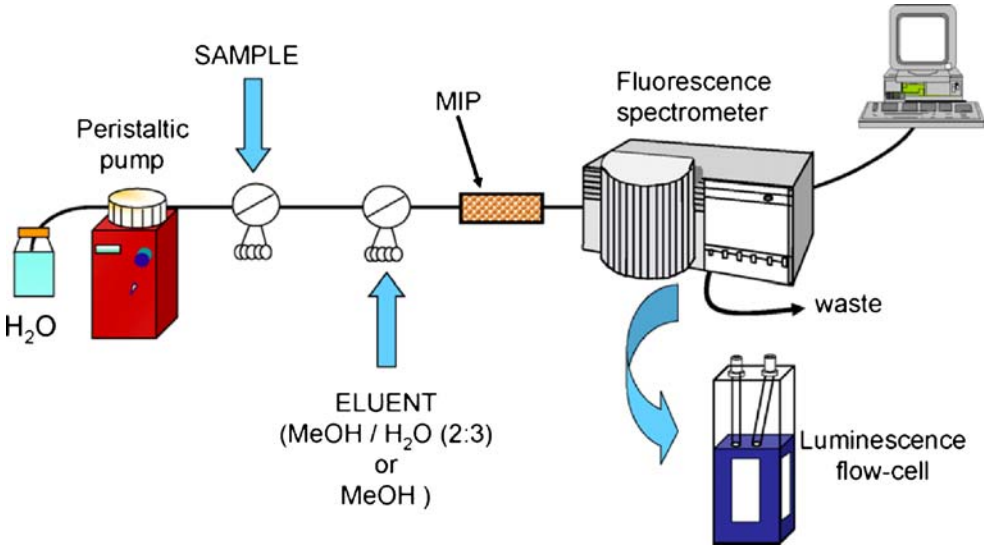


Fig. 8 On-line MISPE platform for the determination of the pesticide carbaryl [91]

simplified the elution procedure and saved reagents. A schematic of the analytical process is shown in Fig. 9. As a result, selective determination of metformin at low concentrations in human serum can be achieved in less than 15 min.

In situ synthetic MISPE

Natalia et al. [93] developed a method for the direct rapid synthesis of MIP beads in SPE cartridges. They prepared 36 polymers for the imprinting of propranolol and morphine. The polymers were directly polymerized under UV light in SPE cartridges, then washed and extracted in the same cartridges, resulting in a rapid and automatable process that requires no transfer or manipulation of the polymer particles. Figure 10 shows the preparation process of the polymers using rapid synthesis in SPE cartridges. The clear advantage of using this method is that the time for the synthesis and preparation of the imprinted polymers for their final application is reduced to a few hours without any need to process or transfer the polymer. Because the polymers are directly obtained in their final particulate form, there is no polymer waste or damage to cavity sites during processing.

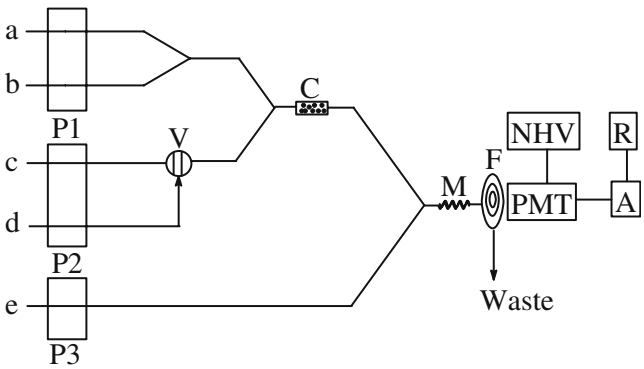


Fig. 9 Schematic of the CL-FIA system: **a** cupric polyphosphate, **b** hydrogen peroxide, **c** ultrapurified water, **d** sample, **e** RhB, *A* amplifier, *C* MIP column, *F* flow cell, *M* mixing tube, *NHV* negative high voltage, *P1–P3* peristaltic pumps, *PMT* photomultiplier tube, *R* recorder, *V* injection valve [92]

MIP columns for liquid chromatography

MIP sorbent in HPLC

Recognition skills of the polymers are usually magnified in HPLC owing to the characteristically high number of theoretical plates. For their application in separation techniques, MIPs are usually synthesized in bulk, ground and sieved to remove fine particles and packed in the chromatographic column [6]. Xia et al. [94] used quercetin as template, chitosan as functional matrix, and methacrylic acid as the functional monomer to prepare quercetin MIPs. An HPLC column was filled with the MIPs which were further evaluated. In this work, the authors discussed the optimal separation conditions and the retention mechanism. Liu et al. [95] developed a MIP stationary phase for an HPLC method for the antibiotic sulfamethoxazole. They prepared a stationary phase comprising monolithic sulfamethoxazole MIPs by an in situ polymerization method and applied it to the HPLC separation of sulfamethoxazole and its analogs. The authors demonstrated that the driving forces of the recognition process were mainly the shape of the molecule and the hydrophobic interactions between the template and the monolith.

MIP sorbent in capillary electrochromatography (CEC)

The use of MIP sorbents in capillary electrochromatography (CEC) is attractive in that it combines the selectivity of a molecular recognition process with the enhanced flow dynamic of CEC, which can result in higher efficiency and

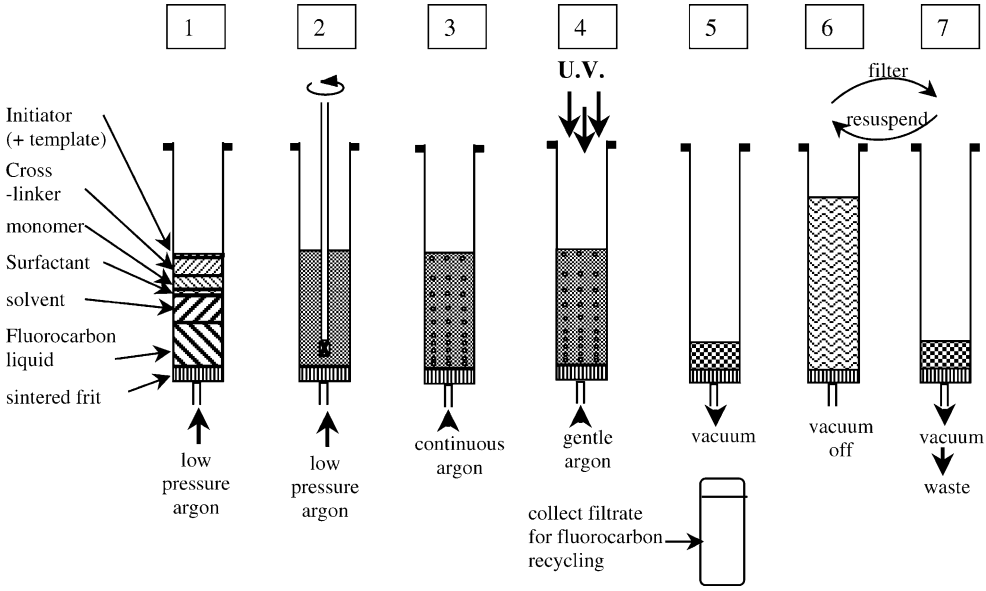


Fig. 10 Preparation of polymers using rapid synthesis in SPE cartridges, showing the seven steps involved [93]

shorter analysis times [96]. References [96, 97] reviewed the MIPs formats and applications for CEC with many examples. Some more recent MIP-CEC research has also been reported [97–101]. Ou et al. [98] prepared tetrahydropalmatine MIPs by in situ thermally initiated copolymerization of methacrylic acid and ethylene dimethacrylate. The capillary was pretreated before the synthesis procedure. First the capillary was derivatized with γ-methacryloxypropyltrimethoxysilane (γ-MAPS) to provide anchoring sites for the polymer. After subsequent flushing with methanol for 10 min, it was dried with a stream of nitrogen gas in an oven. The γ-MAPS was then dissolved in methanol (1:1, v/v) and injected into the capillary with a syringe. It was then kept at 40 °C overnight with both ends sealed with rubber. Finally, the capillary was rinsed with methanol and water successively to flush out the residual reagents and dried again. The capillary was filled with polymerization mixture comprising the template, functional monomer, and cross-linker. The obtained monolithic capillary columns showed good flow-through properties and enantioselectivity. The effects of CEC parameters, such as pH of the buffer, organic solvent, and salt concentration, on electroosmotic flow and recognition selectivity were systematically investigated. During a study into the molecular recognition of MIP in CEC, a monolithic MIP was prepared in a capillary by in situ copolymerization of imprinted molecule [1,1′-binaphthyl-2,2′-diamine (BNA)], methacrylic acid, and ethylene glycol dimethacrylate in porogenic solution, a mixture of toluene and isooctane [99]. The retention and recognition of BNA and its analog, 1,1′-binaphthol (BINOL) were studied with the MIP monolith in CEC. The recognition mechanism of MIP-CEC was complicated since it was a hybrid process, which comprised the features of chromatographic retention, electrophoretic migration, and molecular imprinting. Strong recognition ability and high column performance (theoretically 43,000 plates/m) were achieved in this MIP-CEC mode. To test the function of molecular recognition of the BNA-imprinted monolith, the separations of BNA and BINOL on MIP monolithic column, blank column, and open column were performed (Fig. 11). Since the blank column was synthesized without template, it did not possess recognition sites complementary to the spatial structure of BNA. As a result, BNA and BINOL cannot be separated on the blank column at all. The results showed that the molecular recognition in MIP-CEC mode mainly derived from imprinting cavities on template-imprinted polymer over and above than chromatographic retention and electrophoretic migration. Moreover,

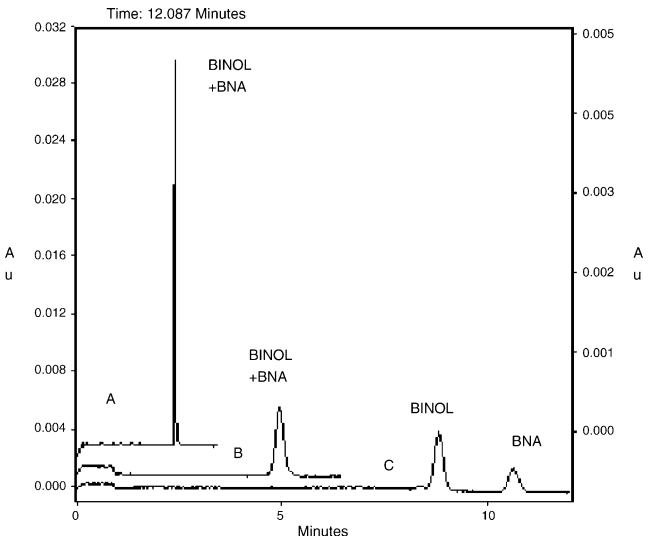


Fig. 11 Chromatograms of the separation of BNA and BINOL on the open column (*A*), blank monolithic column (*B*), and monolithic BNA-imprinted column (*C*) [99]

this research group prepared and investigated MIP-CEC using 4-hydroxybenzoic acid as template [100]. The selectivity of the MIPs was also attractive in the separation and analysis of chiral compounds [101–103].

MIP membrane separation

Recently, MIP membrane was developed for separation and extraction of complex matrices [104–109]. Takeda et al. [104] reported a synthesis of bisphenol A (BPA)-imprinted polymer membrane, which was prepared from BPA, methacrylate, and divinyl benzene. The polymer was hybridized in a porous membrane scaffolding of polystyrene (PS), cellulose acetate (CA), nylon 66 (Ny), and polysulfone (Psf) by a phase inversion process. Figure 12 summarizes the preparation of the hybrid BPA-MIP (HMIP) membranes. Relative to corresponding scaffold polymers in the absence of BPA-imprinted polymer, the hybrid membrane showed excellent BPA recognition. Figure 13 compares HPLC chromatograms of the mixed substrate solution containing BPA, 4,4′-ethylidenebisphenol (BPE), 4,4′-dihydroxydiphenylmetane (BPF), and 2-(4-hydroxyphenyl)ethylalcohol (HPA) for the Psf and Psf-HMIP membranes; each substrate's peak area was observed before and after permeating through the membrane. With Psf membrane (Fig. 13 a and b), four substrate peak areas were not significantly different before and after the permeation. On the other hand, with the Psf-HMIP membrane (Fig. 13 c and d), only the peak intensity of HPA (indicated as peak 1) was almost constant before and after the permeation. The permeation markedly decreased the peak intensities of the bisphenol derivatives; in particular, the decrease of the BPA peak was larger than those of BPE and BPF. The separation factors (α) for BPA (αBPA) were 1.5, 2.9, and 0.4 with CA, Ny, and Psf membranes, respectively. This behavior was confirmed for the non-imprinted membrane without non-selective binding for bisphenols and HPA. In contrast, the HMIP membranes had larger α values for bisphenol derivatives. It means that the HMIP membranes effectively separated the mixture of bisphenols and HPA by permeation. Zhu et al. [105] developed a stir bar method combined with MIP. The stir bars were reproducibly coated with a 180-μm film formed from a formic acid solution of nylon-6 polymer either non-imprinted or imprinted with monocrotophos. The selectivity and extraction capacity were investigated in this study. The extraction showed that the MIP film exhibited high affinity for monocrotophos in dichloromethane and acetonitrile. The extraction and reintroduction could be completed in less than 60 min. The film preparation and extraction performance were demonstrated to be reproducible throughout the study. This MIP film-coated stir bar could

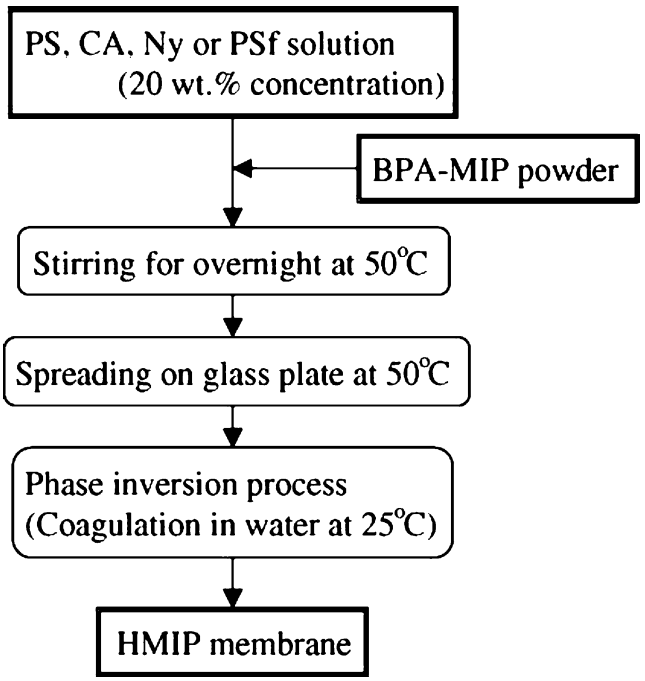


Fig. 12 Preparation of HMIP membrane [104]

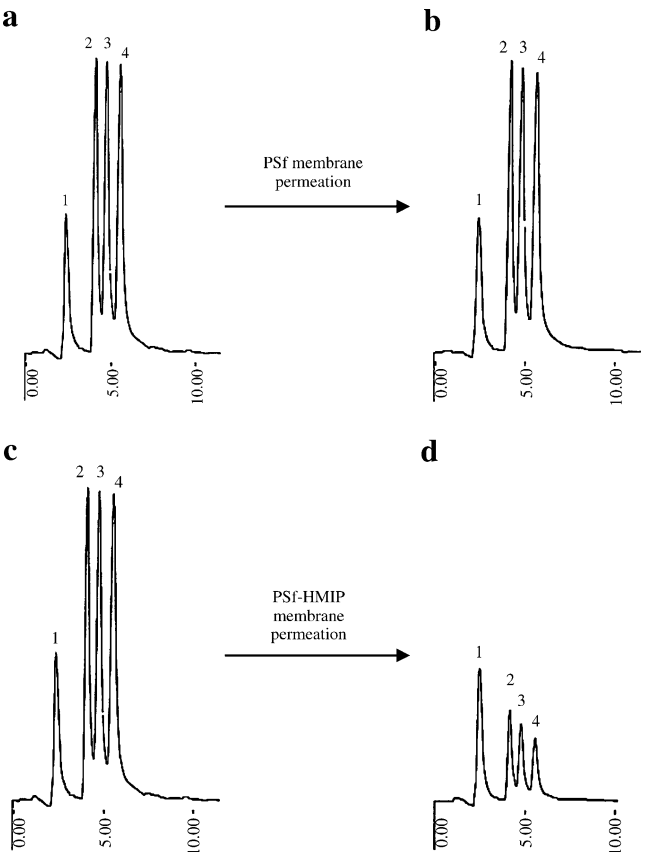


Fig. 13 HPLC chromatograms of bisphenols and HPA mixture for separation experiment before and after permeation through PSf and PSf-HMIP membranes; 20 μM each of HPA (*1*), BPF (*2*), BPE (*3*), and BPA (*4*) was mixed in the solution before (**a** and **c**) and after (**b** and **d**) permeation through the PSf and PSf-HMIP membranes [104]

Table 3 Comparison of the proposed sensor with other methods for the determination of caffeine [111]

Method	Calibration range	Recovery (%)	Detection Limit	Ref.
Voltammetry	5–200 μM	98–104	2 μM	[37]
Flow injection	10–80 μM	98–103	0.20 μM	[38]
SFC-FTIR	600 pg–90 ng	–	60 pg	[39]
MIP-PMAA/PVC sensor	1×10^{-9}–1×10^{-3} mg mL^{-1}	52–122	3.76×10^{-11} mg mL^{-1}	This work
UV-VIS (AOAC 12.028)	0.001–2.0 mg mL^{-1}	98–101	4×10^{-5} mg mL^{-1}	This work

be applied successfully for selective extraction of monocrotophos and its close structural analogy from soil sample.

Sensor

Because of their superior stability, MIPs are attractive recognition elements in sensors, and many different transducers have been used in combination with MIPs [110]. Example applications of MIPs in sensors include electrochemical sensors, fluorescence probes, and bionics sensors [111–119].

References [13, 120–122] reviewed the development of sensor technology. Over the last ten years, great efforts have been made to combine MIP technology with electrochemical sensors. References [120] and [121] reviewed the development of electrochemical sensors in detail. The majority of the sensor systems explored to date have used radically initiated polymerization with acrylic or vinylic polymers as recognition elements, but other phases (e.g., electrogenerated polymers, monolayers, and sol–gel systems) have also been tested [120].

Benilda et al. prepared a piezoelectric quartz sensor coated with MIP for caffeine. The MIP was prepared by copolymerizing methacrylic acid and ethylene glycol dimethacrylate in the presence of azobis as initiator, caffeine as template molecule, and chloroform as solvent [111]. The sensor exhibited a linear relationship between the frequency shift and caffeine concentration in the range from 1×10^{-7} to 1×10^{-3} mg mL^{-1} with a correlation coefficient (r) of 0.9935 and a sensitivity of about 24 Hz ln^{-1} (concentration, mg mL^{-1}). A steady-state response was achieved in less than 10 min. The performance of the sensor shows a promising and inexpensive alternative method of detecting caffeine. The proposed sensor was compared with other methods for the determination of caffeine (Table 3). The sensor exhibited very good linearity, high sensitivity, and high selectivity compared to the other methods.

Fluorescence-based measurement is an attractive determination method [40, 45, 123]. Fluorescence probes combined with MIP offer high selectivity and sensitivity. Graham et al. [40] prepared an environmentally sensitive fluorescent probe, in which 7-nitrobenz-2-oxa-1,3-diazole (NBD) was located adjacent to the analyte DDT binding site to transduce the binding of the analyte. The probe could detect DDT quantitatively in water with a detection limit of 50 ppt in a response time of <60 s. However, the sensing film design was limited by the relatively minor changes in fluorescence intensity upon binding DDT. Shughart et al. [45] used NBD-Cl as the fluorescent label to prepare a fluorescence probe sensor to analyze 9-anthrol. Figure 14 shows the development of fluorescence from the original synthesis (step a) to the formation of MIP monolith (step e). Kubo et al. [123] prepared a cyclobarbital-selective molecularly imprinted polymer using a fluorescent functional monomer, 2-acrylamidoquinoline. The resultant imprinted polymers exhibited enhancement of the fluorescence intensity when cyclobarbital was bound. This result showed that this fluorescent responsive imprinting method could be

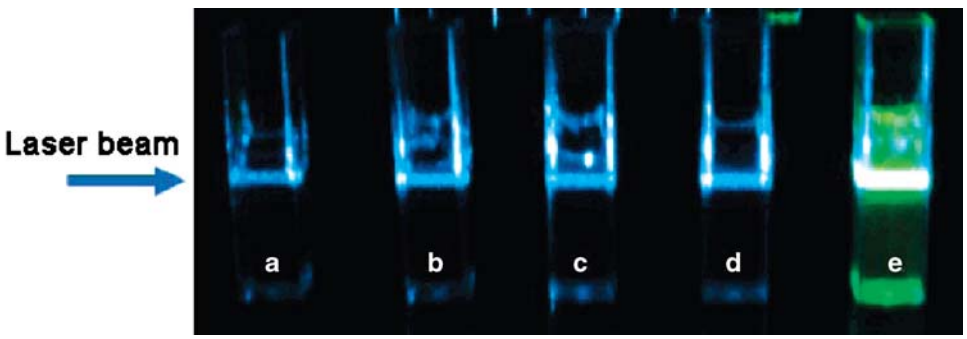


Fig. 14 Appearance of fluorescence from the original synthesis (**a**) to the formation of fluorescent MIP monolith (in step **e**) [45]

useful in the development of sensors for quantification of non-fluorescent compounds.

Outlook

Selectivity is the most powerful attribute of MIPs in the separation and purification of complex samples such as environmental and biological samples. The number of relevant papers published each year has increased, indicating the growing interest in molecular imprinting technology. MISPE, MIP-HPLC columns, and MIP sensors, for example, have made certain analytical work simpler and more efficient. Some new preparation methods should be developed to reduce the issue of remnant template molecule. And the regularity of the material should be improved to enhance the affinity, selectivity, capacity, and efficiency of MIP-HPLC columns. MIPs should have prospects in probe and sensor applications.

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