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REVIEW

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Molecularly imprinted polymers: new molecular recognition materials for selective solid-phase extraction of organic compounds

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Abstract During the last few years molecularly imprinted polymers have appeared as new selective sorbents for solid-phase extraction of organic compounds in different samples. Molecular imprinting technology involves the preparation of a polymer with specific recognition sites for certain molecules. Once the polymer has been obtained, it can be used in solid-phase extraction protocols, where a careful selection of the most appropriate solvents to be used in the different steps (sample loading, washing and elution) is needed in order to extract the target analyte selectively. This review describes the state of the art of this methodology, including the preparation of imprinted polymers, a process description for molecularly imprinted solid-phase extraction, as well as more recent applications. It is concluded that molecularly imprinted solid-phase extraction is a powerful tool to selectively isolate certain analytes, and future advances are to be expected in order to widen the field of application.

Introduction

In recent decades there has been notable development of different analytical chromatographic techniques for the determination of organic compounds; however, there has been no parallel development in sample preparation to the same extent. In fact, liquid–liquid extraction is still routinely used in many laboratories for the preconcentration and cleanup of drugs, pesticides, polyaromatic hydrocarbons and food additives, among others. In the last 10 years, solid-phase extraction (SPE) has appeared as an alternative to liquid–liquid extraction owing to its simplicity, cost and easy automation, coupled to both liquid and gas chromatography. To date, several sorbents (alkyl-silica, copolymers, graphitized carbon) with different properties are commercially available, and it is possible to find in the literature an adequate SPE procedure to determine any analyte in any kind of sample. However, the sorbents mentioned are not highly selective and therefore the analyte is retained together with other matrix compounds, which hinders its final determination by the current chromatographic techniques. Therefore, the development of complex applications using different washing solvents is necessary, thus reducing the inherent advantages of SPE.

Recently, antibodies immobilized on an adequate support, called immunosorbents, were proposed as selective sorbents for use in SPE applications [1, 2] in order to overcome the aforementioned drawbacks associated with typical nonspecific sorbents. Different immunosorbents have been employed for the determination of pesticides, drugs and polyaromatic hydrocarbons, etc., showing an excellent degree of cleanup owing to the inherent selectivity of the antibodies used. However, the obtainment of antibodies is difficult, time-consuming, expensive and, in addition, it is difficult to guarantee its success. Also, it is important to point out that after the antibodies have been obtained they have to be immobilized on an adequate support, which may result in poor antibody orientation or even complete denaturation. Because of these limitations, an alternative approach to the synthesis of host molecules, which can recognize targeted guest species, has been developed called “molecular imprinting”.

Molecular imprinting, shown schematically in Fig. 1, is based on the preparation of a highly cross-linked polymer around a template (the analyte) in the presence of a suitable monomer. The template and monomer(s) are first mixed in order to form a stable prepolymerization complex in a selected solvent. Subsequently, the polymerization is initiated in the presence of a suitable cross-linker. After polymerization, traditionally bulk polymerization, the polymer is ground and sieved to an appropriate particle size, and the template is removed, leaving cavities complementary in shape, size and functionality. These cavities are able to selectively rebind, in given conditions, the analyte (the template) from a complex mixture. The

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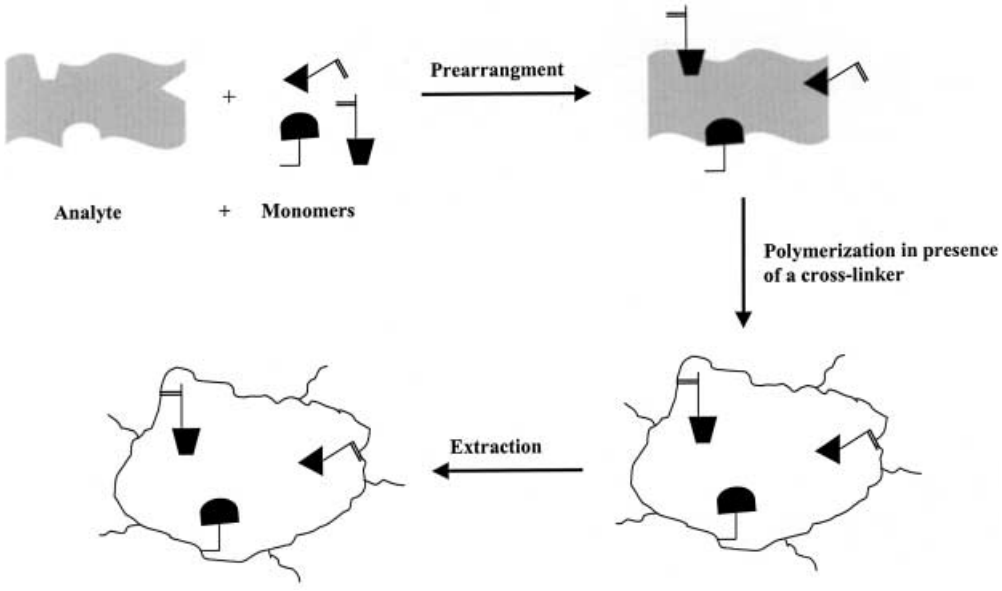


Fig. 1 Preparation of molecularly imprinted polymers

obtainment of molecularly imprinted polymers (MIPs) is easy and inexpensive and they can be easily adapted to different analytical chemistry fields (i.e. chromatographic stationary phases, sensors, catalysis, immunoassays) [3, 4]. In these areas, the use of MIPs as selective sorbents in SPE procedures seems to be extremely promising.

Accordingly, the objective of this article is to present the state of the art of molecular imprinted SPE (MISPE), indicating the factors involved during polymer preparation, current applications and expected future developments in this area.

Preparation of MIPs

As already stated, the first step in the preparation of MIPs involves the prearrangement of the template and monomer(s) used. The template molecule associates with the functional monomer(s) to form a covalent or a non-covalent-bonded complex. The covalent approach is attributed to Wulff and Sarhan [5], who, in 1972, described polymer preparation with chiral cavities for the separation of racemic mixtures. This first example of an imprinted polymer was based on the reversible formation of ester linkages between a sugar and phenylboronic acid, which was derivatized with a vinyl group. It was followed by other polymers based on covalent bonding involving Schiff bases and ketals for amino acid derivatives and ketones, respectively [6]. Imprinting with covalent interactions allows the cavity structures to be probed in detail but this system is not very flexible when choosing the functional monomer(s) and the template species, restricting its range of application to only a few molecules.

A more flexible approach is that known as noncovalent imprinting, introduced by Andersson et al. [7], where the template–monomer interactions involve hydrogen bonding, electrostatic interactions and/or metal ion coordination. This approach can, therefore, cover a wider range of monomers and templates, thus increasing its range of application in chemical analysis. This flexibility has led to a spectacular increase in the number of papers published during the last few years, covering different aspects of molecular imprinting technology [3, 4], and this review will, therefore, focus on the variables affecting the performance of noncovalent imprinted polymers and their applications.

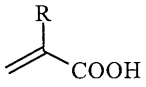
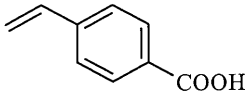
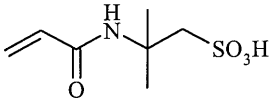
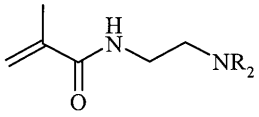
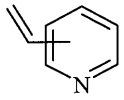
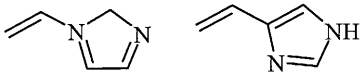
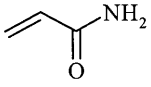
The template, monomer(s), cross-linker and solvent (porogen) used are obviously the key parameters for the obtainment of a successful selective MIP. Since all the parameters mentioned have a strong influence on the overall performance of MIPs in terms of affinity, selectivity, loading capacity, etc., their proper selection will ensure that polymers with the appropriate properties are obtained for a particular application.

Template and monomer(s)

The first step in the preparation of MIPs consists of prearranging the template and the monomer(s) in a solvent, the selection of the monomer being dependent upon the template characteristics. The template has to contain in its structure functional chemical groups capable of interacting with the monomer(s) with sufficient strength to form a stable complex. Up to now, since methacrylic acid (MAA) has been the most frequently employed monomer, the templates used have been restricted mainly to those able to interact by hydrogen bonding with MAA. Other monomers used, although to a much smaller extent, are shown in Table 1.

It is important to point out that since the template–monomer interactions are governed by an equilibrium process, a high amount of monomer is used in order to displace the equilibrium to form the template–monomer

Table 1 Monomers typically used in the preparation of imprinted polymers

Functional monomer		Type of interaction
	Acrylic acids (R = H, CH_3, CF_3, CH_2COOH)	Ionic charges, hydrogen bonds
	Vinylbenzoic acids	Ionic charges, hydrogen bonds
	Acrylamidosulfonic acids	Ionic charges
	Aminomethacrylamides (R = H_2, C_2H_5)	Ionic charges
	Vinylpyridines	Ionic charges, hydrogen bonds, charge transfer
	Vinylimidazoles	Ionic charges, hydrogen bonds, metal coordination
	Acrylamides	Hydrogen bonds

complex. In general, a template–monomer molar ratio of 1 : 4 provides enough stability to the complex formed, assuring the obtainment of the desired imprint effect. However, since the excess of free monomers leads to the formation of nonspecific binding sites, the loading, washing and elution conditions to be used in SPE must be correctly selected, as described later.

The solvent used during the prepolymerization step is also of prime importance since it also has a direct influence on the strength of the template–monomer interaction. In general, solvents with a low dielectric constant, such as chloroform and toluene, offer an adequate medium to stabilize hydrogen bonding and/or electrostatic interactions between monomer(s) and templates. Solvents with higher dielectric constants (i.e. acetonitrile) have also been used but the polymers obtained usually show a lower affinity to rebind the template. Protic solvents, such as water and methanol, are not recommended since they not only hinder polymerization but also disrupt the template–monomer hydrogen-bonding interactions.

Finally, the template size and shape has a strong influence on the selectivity of the polymers obtained. In general, slight structural differences near (neighbor carbon) the functional group responsible for the interaction with the monomer lead to the obtainment of highly selective polymers preventing the binding of structurally related compounds [8, 9]. However, in some cases, the absence or presence of groups far from the functional groups has allowed the obtainment of highly selective imprinted polymers. In this respect we have evaluated the recognition of several phenylurea herbicides by two imprinted polymers using fenuron or isoproturon as templates (Fig. 2) [10],

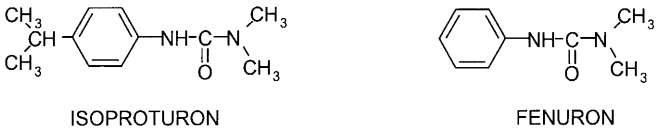


Fig. 2 Chemical structures of fenuron and isoproturon

where the urea moiety is responsible for the interaction with MAA. The isoproturon-imprinted polymer was able to recognize all the herbicides tested since isoproturon, with an isopropyl group in its structure, was the biggest of the compounds tested; however, the fenuron-imprinted polymer was highly selective and recognized only fenuron since it does not possess any substituents in the aromatic ring. This example points to the possibility of tailoring the design of imprinted polymers to suit their subsequent application.

Cross-linker

In order to guarantee the stability of the template–monomer complex during polymerization and to increase polymer porosity, a high degree of cross-linking is necessary. It has been reported [11] that at least 50% of the total monomer in a MAA-ethylene glycol dimethacrylate (EDMA) system has to be EDMA, otherwise no recognition can take place. It is important to stress that the presence of a cross-linker not only preserves the binding sites but also has a direct influence on the physical and chemical properties of the polymeric matrix. From this point of view, EDMA is the cross-linker most often used in methacrylate-based systems, since it provides mechanical and thermal stability, good wettability and rapid mass transfer. Only trimethylolpropane trimethacrylate has shown a similar, or even better, performance than EDMA in the imprinting of peptides [12].

Porogen

Apart from its influence on the template–monomer strength interactions mentioned previously, the solvent (porogen) plays an important role in the morphology of the polymer obtained in terms of specific surface area and pore diameter. In general, a low surface area and low macroporosity leads to low template recognition in the subsequent rebinding experiments owing to slow analyte diffusion to sites located in micropores. Consequently, it is possible to obtain a polymer with an inadequate morphology, preventing template recognition even when using a solvent capable of stabilizing the template–monomer complex during the prepolymerization step. Unfortunately, it is quite difficult to predict beforehand the right solvent for the successful production of polymer.

As stated earlier, the polymer obtained, traditionally by bulk polymerization, has to be ground and sieved to the desired particle size (25–50 μm). This process is tedious and time-consuming, and the particles obtained are irregular in size and shape. In addition, only 50% or less of the total amount of polymer is useful for analytical purposes and some binding sites are partially destroyed during grinding, which leads to a considerable loss of loading capacity of the imprinted polymer versus the theoretical loading capacity considering the amount of template used

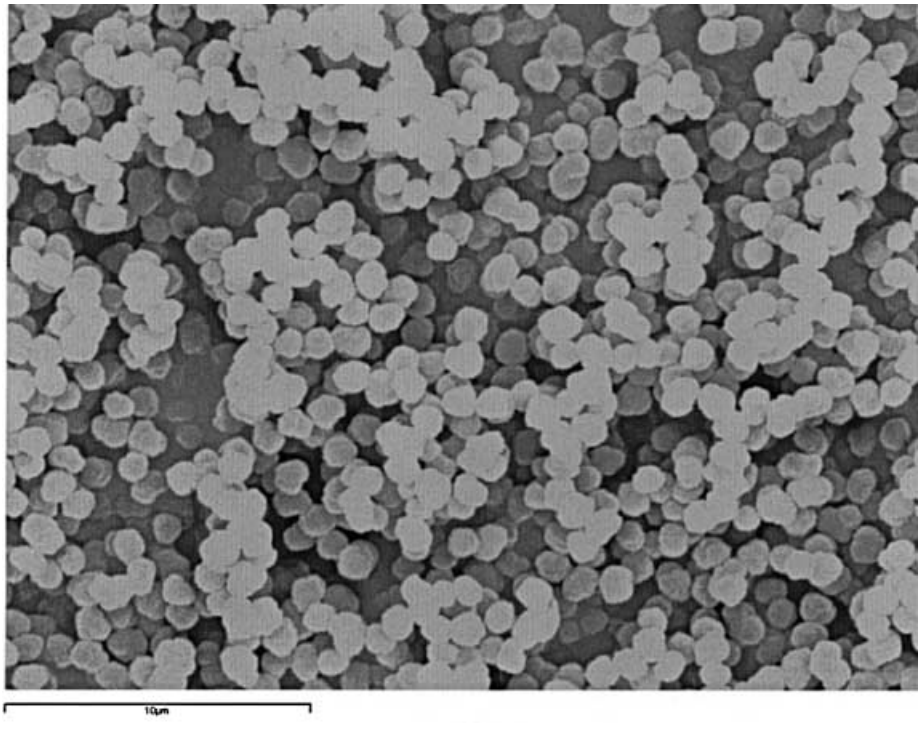


Fig. 3 Scanning electron micrograph of an isoproturon-imprinted polymer prepared by precipitation polymerization

in its preparation. Although the polymers obtained can be useful for most SPE applications, the drawbacks mentioned prevent their industrial production and, therefore, their acceptance in analytical laboratories. Different polymerization strategies have been proposed by several authors for the direct preparation of imprinted polymers, which enables spherical particles to be obtained in the desired particle size. These new methods include imprinting in the pores of preformed beaded silica [13], dispersion polymerization using a polar solvent continuous phase [14], a two-step swelling technique using water as a suspension medium [15], suspension polymerization using a continuous phase composed of liquid perfluorocarbon [16] and precipitation polymerization [17]. As an example, a scanning electron micrograph of an isoproturon-imprinted polymer prepared by precipitation polymerization in our laboratory is shown in Fig. 3 and, as can be observed, uniformly sized microspheres (1 μm) were obtained. Furthermore, imprinted continuous polymers can be obtained in situ in liquid chromatography columns [18].

Once the polymer has been prepared it is necessary to remove the template in order to obtain free binding sites. This step is usually carried out by washing the polymer repeatedly with a solvent capable of disrupting the template–monomer interactions or by Soxhlet extraction; however, these procedures cannot remove the template completely and consequently some template leakage was detected during the elution step in the SPE protocols as described later.

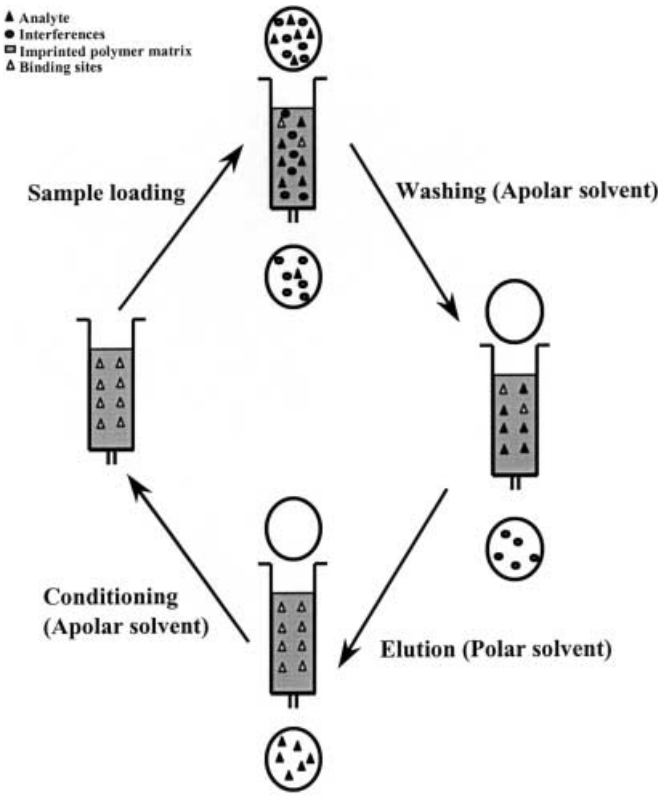


Fig. 4 Molecularly imprinted solid-phase extraction procedure

Molecular imprinted solid-phase extraction

As in other SPE procedures, a small amount of imprinted polymer (typically 50–200 mg) is packed in a cartridge. Subsequently, the common steps of conditioning, sample loading, washing and elution are carried out (Fig. 4). However, in MISPE the selection of solvents is dependent on the kind of template–monomer interactions that took place during polymerization and on the porogen used. As mentioned earlier, to date most of the imprinted polymers are based on template–monomer interaction by hydrogen bonding; therefore, the loading solvent is chosen in order to stabilize this interaction, allowing rebinding of the analyte to specific sites, whereas the elution solvent should be optimized, taking into account its ability to disrupt the hydrogen bonds formed.

Sample loading

Published works have widely demonstrated that the rebinding of templates, in polymers employing hydrogen bonding, takes place using the same solvent as during polymerization and consequently analyte retention decreases when the polarity of the solvent used increases. For instance, it has been reported that polymers prepared in toluene showed better recognition when the loading solvent was toluene than when it was acetonitrile (a more polar solvent). However, it has also been reported that if the polymer was prepared in acetonitrile, lower recoveries were obtained using chloroform (a more apolar solvent) than those obtained using acetonitrile as a loading solvent [19].

Although these comments can be considered as a general rule, the adequate loading solvent has to be optimized in each application in order to prevent nonspecific interactions. For instance, the retention of clenbuterol in a blank polymer (prepared without a template) and in an imprinted polymer, both prepared in acetonitrile, was complete when acetonitrile was used as a loading solvent, owing to nonspecific interactions between the clenbuterol and the polymeric matrix. However, on adding 1% acetic acid to the acetonitrile (mixture capable of disrupting hydrogen-bonding interactions) the binding decreased to 33% in the blank polymer, whereas it remained complete in the imprinted polymer [20].

As already mentioned, the polymers are prepared mainly in aprotic solvents and this does not allow the direct loading of water-rich samples and so prevents the MISPE of analytes in environmental water and biological fluids; however, in some cases it is possible if the polymers interact with the analytes by nonspecific hydrophobic forces. In this respect, Matsui et al. [21] directly loaded 500 mL water samples spiked with several herbicides (simazine, asulam, thiram, propyzamide and iprodine) on an atrazine-imprinted polymer. All the herbicides tested (except asulam) were quantitatively retained in the

polymeric matrix by hydrophobic interactions. Subsequently, the polymer was washed with dichloromethane, achieving selective binding of simazine to the sorbent, whereas the remaining herbicides were eluted from the cartridge. Similar polymer behavior was reported by other authors in the MISPE of clenbuterol from calf urine [20], and triazines [22] and 4-nitrophenol [23] from environmental water although with less success, and thus further research should be done in this interesting field.

Washing step

This step is carried out in order to maximize the specific interactions between the analytes and the imprinted polymer with the simultaneous elution of interfering compounds nonspecifically retained in the polymeric matrix. Usually, the solvents used during this step are the same solvents used in the sample loading step; however, when the nonspecific interactions are important, it is necessary to increase the polarity of the solvents used in order to make a clear distinction between specific binding of the analytes in the preformed cavities and nonspecific interactions with the polymeric matrix [24, 25].

Elution

The analytes are usually eluted with polar (acetonitrile) and protic (methanol) solvents and mixtures of the two, including in many cases traces of weak acids (acetic acid) and bases (triethylamine). In this way, the template–monomer interactions based on hydrogen bonding are disrupted and the analytes are released from the polymer. It has been reported that imprinted polymers can undergo large volume changes owing to switching of solvents during an experiment [26]. This may cause swelling or shrinkage of the polymers, leading to changes in site accessibility. In this respect, Zander et al. [27] reported a decrease in the recovery from 100% to less than 50% in an MISPE of nicotine from a nicotine-imprinted polymer when the amount of water present in the elution solvent was increased from 2.5 to 30%. This may indicate, according to these authors, that the analyte is trapped in the smaller pores of the polymer. Thus, as a general rule, quantitative recoveries can be obtained using an elution solvent based on acetonitrile or methanol with a slight amount (1–10%) of acetic acid, trifluoroacetic acid, triethylamine or similar compounds, allowing the disruption of hydrogen bonding without any major impact on the polymer morphology.

Another problem associated with the elution step is related to the remaining template not removed from the polymeric matrix. Until now, it has been difficult to remove all the template and some template leakage is detected during the elution step, which is obviously a clear error source in trace analysis. As has been clearly explained by Rashid et al. [24], if an assay is capable of detecting down to 50 $ngmL^{-1}$, even a template molecule leakage of 0.00001% would produce significant interference, which is a very realistic approximation, taking into account the high amount of template used during polymerization. Several approaches have been proposed to overcome this drawback, including harsh washing conditions prior to use, which may affect the polymer morphology, polymer heating [27], parallel extraction of blank solutions [20], assuming leakage reproducibility between cartridges, and using a structural analogue to the analyte as a template molecule [28]. The last of these approaches is most successful as the analyte is not used as a template. In this respect, Matsui et al. [29] prepared a dibutylmelamine-imprinted polymer as a triazine herbicide-selective sorbent. Since dibutylmelamine is not used for agricultural purposes, even if it remains in the polymer there is no possibility of disturbance in triazine analyses.

Applications

In the last 5 years there has been a considerable increase in the number of papers published on the optimization of polymer preparation and SPE procedures. Although few of the procedures developed have been applied to the extraction of target analytes from real samples, it is possible to differentiate three types on the basis of the format employed:

- Off-line protocols.
- MISPE with pulsed elution.
- On-line MISPE coupled to liquid chromatography.

Off-line protocols

Since off-line MISPE does not differ from a typical SPE procedure (Fig. 4), it involves loading the sample on the sorbent, usually placed in a cartridge, washing out possible interfering compounds, eluting the analytes and final determination by, mainly, chromatographic techniques. This approach has been used for the extraction of sameridine in human plasma using a methacrylic-based polymer prepared using a sameridine structural analogue as a template molecule [28]. The performance of the method developed was assessed by comparing it with the classical method based on liquid–liquid extraction, demonstrating clearly that cleaner extracts were obtained using the imprinted polymer. In a similar way, other authors have demonstrated the potential of MIPs for SPE of tamoxifen from plasma and urine [24], atrazine from beef liver extracts [30], 7-hydroxycoumarin in plasma [31], cholesterol from gastrointestinal fluids [32], clenbuterol from calf urine [20] and bupivacaine from human plasma [25].

MIPs have been compared to polyclonal antibodies owing to the fact that they have different binding sites. Thus, this “cross-reactivity” can be exploited in order to preconcentrate as many analytes as possible within a group of related compounds. This approach has allowed the simultaneous extraction of atrazine and simazine in a model water sample spiked with several herbicides using an atrazine-imprinted polymer prepared by suspension

polymerisation [21], phenylurea herbicides from ground water and soil sample extracts using an isoproturon-imprinted polymer [10] and nicotine and its oxidation products from nicotine chewing gum extracts using a nicotine-imprinted polymer [27].

Recently, Ferrer et al. [22] reported the use of a terbuthylazine-imprinted polymer for the extraction of several triazine herbicides in ground water and sediment samples. It is important to stress that 100 mL ground water samples were directly preconcentrated on the polymer, confirming the potential of imprinted polymers for nonspecific retention of analytes on the basis of hydrophobic interactions which turn on affinity interactions using an apolar solvent.

MISPE with pulsed elution

This method was introduced by Mullett and Lai in 1998 [33] and, although it may be considered as an on-line protocol, its particular characteristics require it to be discussed separately. MISPE with pulsed elution is based on the use of a small solvent volume (20 μl) to elute the analytes (pulsed elution) retained on an imprinted polymer packed into a column directly connected to the detection system. In this first work, theophylline in chloroform-diluted serum samples (20 μl) was extracted on-line on a theophylline-imprinted polymer packed into a stainless steel column (8 cm × 0.4 cm inner diameter) using chloroform as the mobile phase. Subsequently, after any potential interfering compounds had passed through the column (about 2 min), 20 μL methanol was injected, and theophylline was eluted free of coextractives and was determined directly spectrophotometrically at 270 nm.

MISPE with pulsed elution derives from a previous work by Sellergren [34] where the direct determination of pentamidine in urine samples using a pentamidine-imprinted polymer is described. In this case, the analyte was eluted in continuous mode by changing the mobile-phase composition, but it was not pulse-eluted in a small solvent volume, which prevented the obtainment of very low detection limits.

MISPE with pulsed elution has been improved by the application of successive 20-μl pulses of different solvents, giving rise to the procedure known as MISPE with differential pulsed elution [35], which more efficiently removes any remaining interfering compounds bound to the imprinted polymer as well as the analyte fraction retained nonspecifically. In addition, the columns used were reduced in size, providing a better desorption of the bound analyte. This approach has been successfully applied to the determination of nicotine in tobacco [36] and 4-aminopyridine in serum [37], allowing the determination of these analytes in less than 6 min with high reproducibility (2%) and low detection limits (0.5–1 $\mu g mL^{-1}$) using 20 μl sample.

On-line MISPE coupled to liquid chromatography

In this format, a small precolumn (typically 1 cm × 4.6 mm inner diameter) packed with the imprinted polymer is placed in the loop of an injection valve. After preconcentrating the sample and washing out interfering compounds, the analytes are eluted by the mobile phase and are separated in the analytical column. This approach is especially appropriate for multianalyte determinations using imprinted polymers capable of recognizing several structurally related compounds.

This mode was first used in the molecular imprinting field by Bjarnason et al. [38] for the determination of triazine herbicides in complex aqueous samples, urine and

Table 2 Molecularly imprinted solid-phase extraction methods reported in the literature for the analysis of organic compounds

Analyte	Template	Matrix	Reference
Sameridine	Sameridine analogue	Human plasma	28
Tamoxifen	Tamoxifen	Plasma and urine	24
Atrazine	Atrazine	Beef liver extracts	30
7-Hydroxycoumarin	7-Hydroxycoumarin	Plasma	31
Cholesterol	Cholesterol	Gastrointestinal fluids	32
Clenbuterol	Clenbuterol	Calf urine	20
Bupivacaine	Pentycaine	Human plasma	25
Atrazine and simazine	Atrazine	Water spiked with a mixture of herbicides	21
Phenylurea herbicides	Isoproturon	Ground water and soil	10
Nicotine and its oxidation products	Nicotine	Chewing gum extracts	27
Triazine herbicides	Terbuthylazine	Ground water and sediment	22
Theophylline	Theophylline	Serum	33
Pentamidine	Pentamidine	Urine	34
Nicotine	Nicotine	Tobacco	36
4-Aminopyridine	2-Aminopyridine	Serum	37
Triazine herbicides	Simazine	Water, urine and apple extracts	38
Triazine herbicides	Ametryn	Tap water	39
4-Nitrophenol	4-Nitrophenol	River water	23

apple extracts, although the setup used differed slightly from that mentioned previously. In this case, the samples were first enriched on a precolumm filled with octadecyl-silica and subsequently the analytes (and interfering compounds) were eluted on-line and repreconcentrated on an imprinted precolumn. Finally, after the usual washing step, the analytes were eluted using the mobile phase. Thus, the on-line combination C_{18}-SPE column using an imprinted column offers both the high extraction efficiency of SPE in aqueous samples and the high selectivity of MIPs.

A similar approach was evaluated by Ferrer and Barceló [39] using an ametryn-imprinted polymer precolumn for the extraction of ametryn and other related triazines from tap water, and they obtained cleaner chromatograms than those obtained using a C_{18}-silica precolumn alone.

To date, to the author's knowledge, only one paper has been published on the direct coupling of MISPE to liquid chromatography [23]. In this work, although the recoveries obtained were rather low (36%) using only 10 mL sample, the potential of an imprinted polymer for 4-nitrophenol was demonstrated by the extraction of this analyte from river water completely free of coextractives. It is important to stress that this imprinted precolumn was used to preconcentrate at least 70 water samples with no noticeable deterioration in performance, clearly demonstrating the promising future of MISPE.

The methods that have been reported to date for the analysis of organic compounds using MISPE are shown in Table 2.

Conclusions and outlook

MISPE is a powerful analytical tool which can solve many problems occurring in the determination of organic compounds in complex samples thanks to the high selectivity of imprinted polymers. This inherent selectivity allows the extraction of target analytes and their subsequent elution free of coextractives. Up to now, MAA has been the most employed monomer, which restricts MISPE application to analytes able to interact by hydrogen bonding with MAA. Thus, the use of other monomers with different chemical functionalities will increase the number of compounds to be used as templates and, consequently, the MISPE application field.

Although at present most of the methods developed are based on polymers prepared by bulk polymerization, which prevents their scale-up and commercialization, new polymerization strategies are expected to be developed for the obtainment of imprinted particles of the desired size and shape. In addition, the preparation of polymers capable of recognizing analytes in aqueous samples has to be improved.

Finally, the near future will very likely see further development of methods based on on-line coupling of MISPE to liquid and gas chromatography, as well as the preparation of imprinted fibers for use in the solid-phase microextraction process.

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