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Review

Molecularly imprinted polymers and their application in solid phase extraction

Solid phase extraction is routinely used in many different areas of analytical chemistry. Some of the main fields are environmental, biological, and food chemistry, where cleaning and pre-concentration of the sample are important steps in the analytical protocol. Molecularly imprinted polymers (MIPs) have attracted attention because they show promise as compound-selective or group-selective media. The application of these synthetic polymers as sorbents allows not only pre-concentration and cleaning of the sample but also selective extraction of the target analyte, which is important, particularly when the sample is complex and impurities can interfere with quantification. This review surveys the selectivity of MIPs in solid phase extraction of various kinds of analytes.

Keywords: Complex matrices / Molecularly imprinted polymers / Selectivity / Solid phase extraction

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1 Introduction

For the last two or three decades solid phase extraction (SPE) has been the most frequently used pre-concentration technique for trace analysis. Nevertheless, the major disadvantage of conventional SPE sorbents, such as C18, ion-exchange and size-exclusion phases, is the lack of selectivity, leading to co-extraction of matrix interference components with the target analytes. Hence addi-

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Abbreviations: AA, acrylamide; AIBN, azo-N,N'-bis-isobutyronitrile; ALP, alprenolol; BPA, bisphenol A; **BPA-d₁₆, deuterated bisphenol A**; CBZ, carbamazepine; CD, conductometric detection; CIP, ciprofloxacin; CSP, chiral stationary phase; DAD, diode array detection; DAM, diethylamino ethyl methacrylate; DPCSV, differential pulse cathodic stripping voltammetry; DVB, divinylbenzene; ED, electrochemical detection; EDMA, ethylene dimethacrylate; FQ, fluoroquinolone; GCE, glassy carbon electrode; HEMA, 2-hydroxyethyl methacrylate; IC, ion chromatography; IS, immunosorbent; MAA, methacrylic acid; MAE, microwave-assisted extraction; MIP, molecularly imprinted polymer; MMF, mycophenolate mofetil; MPA, mycophenolic acid; NIP, non-imprinted polymer; 4-NP, 4-nitrophenol; OMWW, olive mill waste-water; PETRA, pentaerythriol trimethylacrylate; PRP, propranolol; RAM, restricted access material; SM-MIP, surface modified molecularly imprinted polymer; SW, square wave oxidative voltammetry; TFMAA, trifluoromethacrylic acid; α -TP, α -tocopherol; TRIM, trimethylolpropane trimethacrylate; VP, vinylpyridine

tional clean-up is usually needed before the final chromatographic analysis. However, specific SPE materials can avoid this problem by providing selective extraction.

One class of selective SPE sorbents is immunosorbents (ISs), which rely upon reversible and highly selective analyte–antibody interactions [1–3]. The low stability of ISs and the fact that biological antibodies are both difficult to obtain and expensive have led researchers to synthesize antibody mimics such as molecularly imprinted polymers (MIPs) in order to selectively extract target substances.

Molecularly imprinted polymers are highly cross-linked synthetic polymers that are characterized by molecular recognition properties towards the template molecules, with specificity and binding selectivity similar to naturally occurring binding polymers such as antibodies and enzymes. The polymers are prepared by copolymerization of a functional monomer with a cross-linker in the presence of a template molecule to produce a three-dimensional polymer network. Removal of the template molecule results in a functional polymeric matrix with recognition sites that are complementary in size, shape, and functionality to the template molecule (Fig. 1) [4].

The advantages of MIPs compared to immunosorbents are higher sample load capacity, physical robustness, high strength, resistance to elevated temperature and pressure, inertness to acids, bases, and organic solvents, as well as low cost and ease of preparation, which have enabled them to be used in a large variety of systems. Most MIP-related literature emphasizes stability as the

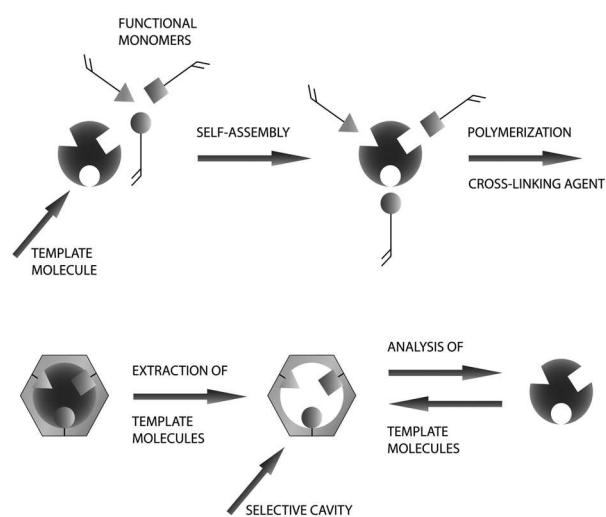


Figure 1. Schematic diagram of the imprinting process.

superior criterion for selecting MIP technology in preference to conventional biologically based recognition schemes, *e.g.* for immunoassays, which are notoriously sensitive to heat and extreme pH. Svenson and Nicholls [5] performed a conclusive study of the theophylline imprinted polymer system, which had been earlier thoroughly researched by Vlatakis *et al.* [6].

Chemical stability studies demonstrated that the polymers kept >95% of their affinity for the imprinted molecule even after 24 hours of exposure to autoclaving treatment, triethylamine, 10 M hydrochloric acid, and 25% NH₃. Heat treatment revealed that the polymers are thermally resilient and retain their chemical affinity, as the MIP did not degrade up to temperatures of 150°C. At higher temperatures decarboxylation is induced, causing loss of MIP specificity.

MIPs can be produced for essentially any type of template molecule; hence a wide range of chemical species have been targeted with the technology and have been reviewed recently [7–16]. The experimental set-up has varied according to the mode of operation (on-line, off-line, and batch mode SPE).

The present review reports the current state of the art with respect to the application of MIPs as selective materials for solid phase extraction. We discuss the use of MIP-SPE in relation to the selective extraction of several compounds from various matrices. To introduce MIPs we also briefly describe various synthetic routes and methods employed for preparation and evaluation of molecularly imprinted polymers.

2 Polymer synthesis and evaluation

Recently the most common procedures for the synthesis of molecular imprints have been reviewed in detail [17].

MIPs can be synthesized according to a number of approaches differing in the way the template is linked to the functional monomer and subsequently to the polymeric binding sites. All strategies aim at the preparation of MIPs able to recognize the imprinted molecule, or structurally related molecules, in a selective way. The binding affinity can be moderate or high, depending on the application.

The right selection of functional monomers is important in molecular imprinting, because the interactions with functional groups affect the affinity of MIPs. The best results have been obtained using templates attached to more than one binding site. The most important types of interactions that can be utilized are: (i) covalent bonding; (ii) π–π interactions; (iii) hydrogen-bonding; (iv) hydrophobic/Van der Waals interactions; and (v) ionic bonding. The different types of interactions involve different levels of specificity.

One of the first approaches to molecular imprinting of organic network polymers introduced by Wulff was based on a covalent attachment strategy [18]. In this case the polymer derivative of the imprint molecule was synthesized. Typically the template is bound to appropriate monomers such as 4-vinylphenylboronic acid and 4-vinylbenzylamine by covalent bonds. The covalent conjugate polymerized under conditions preserving the covalent linkage. After polymerization the covalent linkage is cleaved and the template is removed from the polymer. Upon binding of the guest sample molecule by the imprinted polymers, the same covalent linkage is formed. The advantage of this technique is the controlled stoichiometry between the template and the functional monomer. Owing to the greater stability of covalent bonds, covalent imprinting protocols should yield a more homogeneous population of binding sites. However, this approach requires formation of specific bonds that will tolerate the polymerization conditions. This type of imprinting creates strong interactions as a result of the restoration of the covalent bond between the matrix and the target compound; however, the useful reversible covalent interactions are limited.

Currently the technique most widely applied to generate molecularly imprinted binding sites is represented by the non-covalent route developed by the group of Mosbach [19]. The template is mixed with functional monomers and cross-linkers in an appropriate solvent. In this pre-polymerization mixture, complexes of the template with the monomers are formed as a result of various interactions such as hydrogen bonding, ionic interactions, Van der Waals forces, etc. The strength of these interactions and the resulting complexes are crucial for the templating effect in the final polymer. “Selective” interactions such as spatially oriented hydrogen bonds are preferred in contrast to more generic forces (hydrophobic interactions, Van der Waals forces). The pre-poly-

merization complex is an equilibrium system, the stability of which depends on the affinity constants between the imprint molecules and the functional monomers. Arrangements using non-covalent interactions require the template and the target analyte to form a binding pocket during polymerization.

Aprotic solvents allowing polar interactions between the template and the monomers are typically chosen; however, solubility-miscibility considerations often limit their use. Polar protic solvents suppress the formation of hydrogen bonds, while they promote hydrophobic and ionic interactions between the template and the monomers. The solvent should not only provide the environment for the polymerization reaction, but also produce a porous structure in the resulting polymer. Therefore the choice of polymerization solvent also controls the rigidity and the pore size of the MIP materials. A meso-macroporous structure is necessary to ensure practically useful operating flow and pressure in liquid chromatographic applications. Good accessibility to the imprinted binding sites is essential for selective binding and sufficient sorbent capacity. This is especially important when working with large bulky molecules where the kinetics of the intraporous transfer towards the active sites is restricted due to steric reasons. Since the shape recognition is often best in the solvent used as the porogen in the polymerization of the MIP, this solvent is often also used as the washing solvent, or, in case of selective adsorption, as the extraction solvent [20]. The most widely used organic solvents for the selective washing step are dichloromethane, toluene, and chloroform [21–23]. Protic solvents such as methanol and acetonitrile have found only limited use in specific cases [24–26].

Typical monomers used for non-covalent molecular imprinting include methacrylic acid (MAA), 2- and 4-vinylpyridine (2- and 4-VP), trifluoromethacrylic acid (TFMAA), acrylamide (AA), and 2-hydroxyethyl methacrylate (HEMA). Ethylene dimethacrylate (EDMA) is the most common cross-linker, other cross-linkers often used are divinylbenzene (DVB) and trimethylolpropane trimethacrylate (TRIM).

The cross-linker is added in excess concentrations (the usual molar ratio of cross-linker to functional monomer is 5:1), in order to obtain a rigid, highly dense polymeric network. This enables the imprinted sites to retain their shape and size, and tolerate different environments, “resisting” shrinkage and swelling. The best developed MIPs so far have been based on the above-mentioned monomers or their combinations (cocktail of monomers). In this approach, the formation of the template-monomer complex is expected to occur via selected functionalities of the corresponding molecules. Lately, the development of novel monomers specific for a given template has opened up new possibilities in molecular imprinting. To achieve this aim, conventionally used

monomers are modified, or totally new entities are prepared aiming at a multipoint association with the template molecule. Such complexes are expected to be stronger and more specific for the template molecule, thus the resulting MIP should exhibit higher affinity towards the template.

After the template-monomer complexes have been formed, an azo initiator (usually azo-*N,N'*-bis-isobutyronitrile, AIBN) is added to the polymerization mixture. Free radical polymerization is initiated by heating at 40–60°C or by inducing photochemical homolysis by UV radiation (0–15°C). MIPs prepared at lower temperatures (0°C) by photo-polymerization have been found to exhibit better molecular recognition. This is explained by the formation of more stable complexes of monomers and imprint molecules in the pre-polymerization mixture at low temperatures due to a more favourable entropic contribution, leading to better defined imprints in the resultant polymer [18].

Polymerization is allowed to take place for 10–24 hours. Next, the polymer is further processed to extract back the template molecule. Typical extraction solvents are mixtures of protic solvents with acids (e.g. methanol with 10% acetic acid). Such mixtures should suppress binding interactions and extract the template from the polymer core almost quantitatively. Soxhlet extraction, extensive solvent washing, or alternative acid-base washing are the main experimental protocols used.

Quantitative removal of the template is essential for successful future use of the MIP for two reasons: (i) the more template is removed, the higher is the available number of active sites in the MIP; (ii) it has been repeatedly reported that leakage or so called “bleeding” of the remaining template from the MIP may occur during sample extraction [26–28]. A small amount of template (usually more than 1% of the amount of template in the monomer mixture) remains strongly bound to the polymer, even after careful washing of the MIP. Bleeding is a problem mainly when the MIP has to be applied to extract trace levels of the target analyte and may be reduced by thermal post-polymerization treatment of the materials [29]. A solution to this problem lies in the utilization of an analyte analogue, a “dummy template”, during polymerization [30–32]. Even if leakage of the “dummy template” occurs during sample recovery, this will not interfere with the analysis. Andersson [28] was the first to synthesize an MIP using a template analogue. An MIP selective for sameridine was prepared using a close structural analogue of sameridine as the template. The use of a dummy molecule is particularly advantageous when developing an MIP for class-selective extraction as in the synthesis of MIPs for dopamine [33], atrazine [22], or organophosphorus nerve agents [34]. Other methods, such as thermal decomposition, microwave-

assisted extraction (MAE), and desorption of the template with supercritical fluids have also been employed to remove the template from the MIP [35].

The semi-covalent approach is a hybrid of the two previous methods: Covalent bonds are formed between the template and the functional monomer before polymerization, whereas, once the template has been removed from the polymer matrix, the subsequent binding of the analyte to the MIP exploits non-covalent interactions, as in the non-covalent imprinting protocol [36].

In general, non-covalent imprinting is easier to achieve and applicable to a wider spectrum of templates. Moreover, an excess of the functional monomer relative to the template is usually required to favour template–functional monomer complex formation and to maintain its integrity during polymerization. As a result, a fraction of the functional monomers is randomly incorporated into the polymer matrix to form non-selective binding sites. However, when covalent bonds are established between the template and the functional monomer prior to polymerization, better defined and more homogeneous binding sites form than in the non-covalent approach, since the template–functional monomer interactions are far more stable and defined during the imprinting process [37, 38].

MIPs can be obtained in different formats, depending on the preparation method [39]. Free radical polymerization is the most important synthetic method available today [4]. It is relatively robust and allows polymers to be prepared in high yield using different solvents and different temperatures. In spite of the ease of preparation, bulk polymerization is time consuming, labour intensive, and yields only 30–40% of useable material. Moreover, the non-regular shape of the particles obtained by grinding the MIP prepared by bulk polymerization causes low chromatographic efficiency. This is not a real limitation for off-line SPE applications but can cause broad asymmetric peaks in on-line coupling of SPE with liquid chromatography. Alternative preparation methods such as precipitation polymerization, suspension polymerization, and multi-step swelling polymerization have been shown to produce spherical particles of a desired size; however, their use is still limited [25, 40, 41–47].

The introduction of MIP stationary phases in miniaturized systems can be achieved by packing a capillary column with MIP particles, but it requires frits, which are difficult to fabricate in capillary format [48]. Another approach consists of the *in situ* polymerization procedure that leads to the synthesis of monolithic polymeric MIP materials in the form of rods inside a stainless steel or a PEEK tube [49].

MIP monoliths were primarily developed for capillary electrochromatography (CEC) [50]. However, current research is focused mainly on the use of MIP monolith format as SPE sorbents [51].

Oxelbark and co-workers [52] presented a comprehensive comparison of five chromatographic stationary phases based on molecularly imprinted polymers, in terms of efficiency, imprinting factors, water compatibility, and batch-to-batch reproducibility: a crushed monolith, microspherical particle material, two silica-based composites, and a capillary monolith, all imprinted with the local anaesthetic bupivacaine. Except for microparticles, all formats give satisfactory performance, especially in aqueous mobile phases.

3 MIPs as chromatographic media

The performance of MIP materials is best validated by testing them as chromatographic stationary phases [53, 54]. The MIP material is packed in an HPLC column and retention characteristics of the template and/or analogue molecules are measured in various mobile phases. From the experimental data, useful parameters such as retention factor, imprinting factor, and peak asymmetry are calculated and used to evaluate polymer affinity, cross reactivity, and other features of the MIP. In each polymerization approach, a non-imprinted polymer (NIP) should be synthesized in the same way as the MIP but in the absence of the template molecule in the polymeric mixture, as the reference material to evaluate the imprinting effect and to compare the selectivities of the NIP and MIP materials.

The recognition properties of the MIP materials can be assessed in batch rebinding experiments by measuring the amount of the template taken up by the polymers at equilibrium. First, a predetermined amount of polymer is added to solutions of varied concentrations of the template compound. These mixtures are incubated for a sufficiently long period of time until the binding reaches equilibrium. Then, the polymer is removed by centrifugation or filtration and the concentration of the template in the liquid phase is determined by HPLC, UV, or another analytical technique. The mass of the template adsorbed on the MIP is compared to the amount bound on the NIP, the number of template cavities being correlated with the difference between the amounts adsorbed on both sorbents [55–57]. This approach was recently used to compare the effect of the amount of porogen on the binding properties of MIPs for cholesterol [58]. The binding characteristics can be evaluated from the equilibrium binding experiments by processing with Scatchard equations [59] or by constructing a saturation capacity curve [34].

MIPs can be also evaluated as stationary phases in HPLC by comparing the retention factors (k), separation factors (α), and imprinting factors (I_f) of the template molecule [4, 60], obtained from the retention time t_R of the template on a chromatographic column packed with the

MIP and a second column packed with the NIP materials. The analyte should be retained more strongly on the MIP than on the NIP, because of the selective interactions:

$$k = (t_R - t_0)/t_0, \quad (1)$$

$$I_f = \frac{k_{\text{MIP}}}{k_{\text{NIP}}}, \quad (2)$$

$$\alpha = k_{\text{template}}/k_{\text{comp}}, \quad (3)$$

where t_R is the retention time of the analyte, t_0 is the retention time of the column hold-up volume marker (e.g. acetone), k_{MIP} is the retention factor of the target analyte on the imprinted column, k_{NIP} is the retention factor on the non-imprinted column, k_{template} is the retention factor of template molecule, and k_{comp} is the retention factor of a template analogue or another reference compound. In some studies the selectivity of the MIP was studied using reference compounds structurally related to the template molecule [22, 61]. If the difference between the retention of the reference compounds and the template is low, the MIP shows cross-reactivity [21].

Tamayo *et al.* [30] used silica particles as supports for the preparation of different propazine-imprinted polymer formats. One format involved grafting of thin films of molecularly imprinted polymers using an immobilized iniferter-type initiator (inif-MIP). Another approach employed complete filling of the silica pores with the appropriate polymerization mixture to prepare silica-MIP composite material (c-MIP). Subsequent dissolution of the silica matrix resulted in spherical MIP beads (dis-MIP). These techniques enable fine-tuning of the particle morphology of the resulting MIP particles to enhance molecular recognition selectivity when compared to corresponding control non-imprinted polymer. This study showed that the inif-MIP possess the best efficiency for use as stationary phase in HPLC for the separation of triazine herbicides. Both the retention factor and the imprinting factor were higher than those obtained for c-MIP and dis-MIP, demonstrating clearly that the improved imprinting effect was obtained during the grafting process.

MIPs can be used as Chiral Stationary Phases (CSPs). Molecular imprinting offers a very efficient way of producing receptors for an enantiomer within a stationary phase. By preparing an MIP employing an optically pure R template, chiral separations of remarkable selectivity can be achieved. The R enantiomer preferentially binds to the MIP and thus elutes late, whereas the S enantiomer elutes much earlier. MIP-based chiral separations of various types of drugs, peptides, protected amino acids, alkaloids, and other chiral compounds often exhibited enantioselectivities higher than those obtained with conventional CSPs.

The main drawback of MIP-based CSPs is the heterogeneity of the active binding sites of varying affinity. The

polymerization process activates sites showing non-selective interactions towards sample molecules. This heterogeneity may result in peak asymmetry and low separation efficiency [4]. In this case, non-linear adsorption isotherms are typically observed for the template molecules due to simultaneous action of specific and non-specific interactions. Another limitation of MIPs is the sorption capacity, which is generally lower than with other CSPs. Finally the achievement of molecular recognition in an aqueous environment remains a challenge for MIP technologies.

Apart from HPLC MIPs have also been used as media for Capillary Electrochromatography (CEC) [39, 62]. In fact, the high efficiency of CEC may potentially overcome the mass transfer and kinetic limitations of MIP-based liquid chromatography by the rectangular plug flow profile in the CEC. Most of work on MIPs as selector phases in CEC reported so far has focussed on chiral separations in which one of the enantiomers serves as the template molecule. MIP-based stationary phases for CEC have been prepared *in situ* as superporous monoliths [50], gel-entrapped particles [63], MIP coatings [64], and nanoparticles [65]. The intrinsic higher efficiency and enhanced flow dynamics of CEC combined with the higher selectivity of MIPs are very attractive for the separation of chiral compounds [50]. MIP monoliths are covalently anchored to the inner wall of fused-silica capillaries so that the use of retaining frits is unnecessary.

4 Use of MIPs in sample pre-treatment

The most important application area of molecular imprinting in the analytical separation field is probably solid phase extraction (SPE) [13]. The technique has been referred to as either MIP-SPE or MISPE and has recently been reviewed [4, 14]. The main benefit of the technique is that the selectivity of an MIP for a target compound or a group of compounds can be pre-determined by the choice of template employed for its preparation. High selectivity of the sorbent may lead to efficient sample clean-up.

SPE is now an established technique for sample preparation due to its simplicity, ease of automation, reduced consumption of organic solvents with respect to liquid phase extraction, and large variety of the chemistries of stationary phases (C8, C18, CN, NH₂, Phenyl, ion exchange, mixed mode, etc.). These generic phases are the first choice for a number of protocols since they offer high capacity, interactions with a wide range of analytes, and good reproducibility.

MIP-SPE has been applied both in on-line and off-line modes. In the first case the MIP sorbent is packed in an HPLC pre-column. Column switching and pulsed elution modes are employed in on-line MIP-SPE. The main

advantage of this design is the possibility of automation in the direct coupling to HPLC or other separation modes. Despite this advantage, the off-line mode is still the most common in practice and has been applied successfully to biological, environmental, and food analysis.

The operational use of SPE cartridges containing molecularly imprinted polymers is very similar to that of other SPE sorbents, consisting in consecutive pre-conditioning, sample loading, washing, and elution steps. The adsorption of the analytes onto an MIP-SPE sorbent may be either due to non-selective interactions, as with conventional reversed phase materials (C8, C18, etc.), or to shape-specific interactions of the template or related analytes with the polymer matrix.

As in traditional SPE, the extraction column can operate in “normal phase” mode or in “reversed phase” mode. In the first approach, a sample should be loaded under conditions enhancing molecular recognition and selective entrapment of the analytes, followed by elution providing enriched sample for further analysis. Usually this selective adsorption/entrapment is employed in the same solvent as is used in the polymerization. Recognition is assumed to be enhanced when the porogen is used as the entrapment solvent because the environment established during the polymerization is reproduced (solvent memory effect) [22].

Another strategy is based on entrapment of the analytes by non-selective sorption on the MIP materials due to hydrophobic interactions. This is usually achieved by loading the sample in an aqueous environment where the target analytes as well as hydrophobic interfering compounds are adsorbed. Next a selective washing step removes interfering matrix compounds from the SPE cartridge, whereas the analyte remains selectively adsorbed on the MIP (selective desorption). In the final elution step, the analyte is recovered in an appropriate solvent. The analyte can be eluted from the column with a pure solvent, solvent mixtures, or with a solvent containing a small amount of acid or basic additive such as acetic acid or pyridine that disrupt the strong and multiple interactions of the analyte with the polymer. This mode employs additional extraction steps, but offers the advantage of straightforward application of aqueous samples (biological fluids or environmental samples).

The washing step has to be optimized in terms of pH, nature, and volume of the washing solvent in order to exploit the polymer's selectivity in recognizing the target molecules. The washing solvent should suppress the non-specific interactions without disrupting the selective interactions between the MIP and the target molecules. For this purpose, low-polarity organic solvents, such as dichloromethane, toluene, or chloroform, are widely used [21, 24]. However, good results have also been obtained with other more polar solvents, such as acetonitrile or methanol [6, 25, 26]. When water samples are per-

colated through MIPs, the clean-up step can be problematic because the washing solvent is usually non-polar, which may give rise to miscibility problems, unless the MIP cartridge is dried by purging with air or nitrogen [24, 66]. Although a clean-up step is often necessary to achieve selective extraction, it can be avoided if the elution step is selective enough.

The first study on MIP-SPE was reported by Sellergren in 1994 [67]. An MIP with recognition sites for pentamidin (a drug used to treat AIDS-related pneumonia) was synthesized and evaluated for use in on-line SPE. The MIP was prepared using methacrylic acid as the monomer and ethylene dimethacrylate as the cross-linker and enabled selective extraction and concentration of pentamidin in biological fluids. The MIP-based SPE yielded a clean extract and enrichment of the sample enabling direct detection.

SPE applications have since been developed for various types of analytes (Table 1), including various modes of on-line SPE, conventional off-line SPE with the MIP packed into columns, or cartridges and batch mode SPE, where the MIP particles are brought into equilibrium with the sample [22, 24, 30, 33, 34, 40, 57, 61, 68–93].

4.1 Off-line protocols

Most of the MIP-SPE applications reported so far were developed in off-line mode, not only because of the relatively simple instrumentation required, but also because elution conditions can be used without having to establish compatibility of the analytical instrumentation with solvent, pH, or acidic or basic additives.

Offline solid phase extraction procedures can handle a wide variety of solvents. In contrast to on-line procedures, miscible and immiscible solvents can be used sequentially when the sorbents are dried in between. A solvent used for elution can be evaporated and the residue containing analyte can theoretically be dissolved in any suitable solvent of choice. In off-line procedures, association and dissociation kinetics is less critical than in chromatographic separations. However, small elution volumes for complete recovery are important, as pre-concentration (enrichment) of a sample is expected in the SPE approach.

The higher the selectivity of the sample pre-treatment procedure, the less selective can be the analytical technique used for the determination of the analyte of interest. If an MIP-SPE method is intended for the extraction of a group of analytes, the imprints need not be selective towards the individual compounds. The MIPs of several individual compounds can be mixed or a mixture of template analytes can be imprinted in one polymerization process.

To perform off-line MIP-SPE, cartridges (usually polyethylene) are packed with the polymer (typically 15–

Table 1. Selected application examples of MIP-SPE technique.

Template	Analyte	Matrix	Polymer system Monomer / Cross-linker	MIP synthesis	Analytical technique	Reference
<i>A: Off-line applications</i>						
Naproxen	Naproxen	Urine	4-VP / EDMA	bulk	HPLC – UV	[24]
Hyoscyamine	Scopolamine	Serum, urine	MAA / EDMA	bulk	HPLC-UV	[77]
Dopamine	Dopamine	Urine	MAA / EDMA	bulk	HPLC-UV	[33]
			MAA / AA / EDMA	bulk		
			AA / Bis / EDMA	bulk		
			MAA / AA / Bis	bulk		
Mycophenolic acid	Mycophenolic acid	Plasma	4-VP / EDMA	bulk	HPLC-UV	[80]
Uric acid	Uric acid	Serum	–	grafted on silica	DPCSV	[81]
Analogues of sildenafil	Sildenafil, Desmethylsildenafil	Plasma	MAA / HEMA / ED-	bulk	HPLC-UV	[84]
Alfuzosin	Alfuzosin	Plasma, soil	MAA / EDMA	bulk	HPLC-DAD	[85]
Enrofloxacin	Enrofloxacin	Urine, tissue sam- ples	MAA / EDMA	bulk	HPLC-UV	[87]
Monocrotophos	Organophosphorus pesticides	Water, soil	MAA / EDMA	bulk	HPLC-DAD	[75]
1-Naphthalene sulfonic acid	Naphthalene sulfonic acids	River water	4-VP / EDMA	bulk	HPLC-UV	[61]
Pinacolyl methyl- phosphonic acids	Alkyl methylphos- phonic acids	Soil	MAA / EDMA	bulk	LC-MS	[34]
Carbamazepine	Carbamazepine	Wastewater, urine	MAA / TRIM	bulk	IC-CD	
			MAA / DVB	bulk	HPLC-UV	[88]
Pinacolyl methyl- phosphonic acid	Alkyl methylphos- phonic acids	Soil	MAA / TRIM	bulk	LC-MS	[82]
Polyphenols	Polyphenols	Olive mill waste water	4-VP / EDMA 4-VP / DVB80 4-VP / PETRA	bulk bulk bulk	IC-CD	
			Allylurea / PETRA	bulk	HPLC-DAD	[91]
			Allylaniline / ED-	bulk		
			MA	bulk		
			MAA / PETRA	bulk		
			MAA / EDMA	bulk		
Metsulfuron	Sulfonylureas	Drinking water	4-VP / EDMA	bulk	HPLC-UV	[76]
Methyl Ametryn,	herbicides		2-VP / EDMA	bulk		
Terbuthylazin	Ametryn,	Juice, soil	MAA / EDMA	bulk	HPLC-DAD	[22]
Pyrimethanil	Terbuthylazin					
	Pyrimidinic fungicides	Wine	MAA / EDMA	grafted beads	HPLC-UV	[83]
Chloramphenicol	Chloramphenicol	Honey	MAA / EDMA	bulk	HPLC-UV	[79]
Quercetin, rutin	Quercetin, rutin	Wine, juice, tea	MAA / EDMA	bulk	HPLC-UV	[90]
			4-VP / EDMA	bulk		
Chloramphenicol	Chloramphenicol	Honey, urine, milk, plasma	DAM / EDMA	bulk	LC-MS/MS	[86]
α -Tocopherol	α -Tocopherol	Bay leaves	MAA / EDMA	bulk	HPLC-UV	[89]
Sudan I	Sudan I	Red chilli powder	MAA / EDMA	bulk	HPLC-UV	[71]
			4-VP / EDMA	bulk		
Ephedrine	Ephedrine	Chinese ephedra	MAA / EDMA	bulk	HPLC-UV	[74]
Esculetin	Esculetin	Ash bark	AA / EDMA	bulk	HPLC-UV	[72]
					LC-MS	
Benzoylecgonine anilide	Cocaine metabolites	Aqueous samples	MAA / EDMA	bulk	HPLC-UV	[70]
Indomethacin	Indomethacin	Tap water, river water	MAA / TRIM	bulk		
			MAA / EDMA	bulk		
			AA / EDMA	suspension polymerization		
<i>B: On-line applications</i>						
Caffeine	Caffeine	Urine, beverages, coffee	MAA / EDMA	bulk	HPLC-UV	[68]
Alfuzosin	Alfuzosin	Plasma	MAA / EDMA	bulk	HPLC-DAD	[92]
Verapamil	Verapamil	Plasma, urine	MAA / EDMA	bulk	LC-MS	[69]

Table 1. Continued.

Template	Analyte	Matrix	Polymer system Monomer / Cross-linker	MIP synthesis	Analytical technique	Reference
Atropine	Atropine, Scopolamine	Gastrointestinal drugs	TFMAA / EDMA	multi-step swelling polymerization	HPLC-UV	[73]
Chlorotriazine herbicides	Chlorotriazine herbicides	River water	TFMAA / EDMA	multi-step swelling polymerization	HPLC-UV	[40]
			MAA / EDMA	multi-step swelling polymerization		
			4-VP / EDMA	multi-step swelling polymerization		
Bisphenol-A <i>p</i> -tert-Butylphenol	Bisphenol-A Bisphenol-A	River water Environmental water	4-VP / EDMA	<i>in-situ</i>	HPLC-UV	[78]
			4-VP / EDMA	two-step swelling polymerization	HPLC-UV	[57]
			MAA / EDMA	grafted on silica	HPLC-ED	
Propazine	Propazine	–	MAA / EDMA	pore filled silica	HPLC-UV	[30]
			MAA / EDMA	spherical beads		

AA – acrylamide, Bis – *N,N'*-methylenebisacrylamide, DAM – diethylamino ethyl methacrylate, DVB – divinylbenzene, EDMA – ethylene glycol dimethacrylate, HEMA – 2-hydroxyethyl methacrylate, MAA – methacrylic acid, PETRA – pentaerythriol trimethylacrylate, TFMAA – trifluoromethacrylic acid, TRIM – trimethylolpropane trimethacrylate, VP – vinylpyridine
HPLC – high performance liquid chromatography, UV – ultra violet detection, ED – electrochemical detection, LC – liquid chromatography, MS – mass spectrometry, DAD – diode array detection, IC – ion chromatography, CD – conductometric detection, DPCSV – differential pulse cathodic stripping voltammetry.

500 mg). After the conditioning, loading, and washing steps, the extract collected from the elution step is introduced into an analytical system, such as a liquid chromatograph [94], a gas chromatograph (GC) [28], or a capillary electrophoresis instrument (CE) [95] (Fig. 2).

Offline MIP-SPE has been applied to the extraction of various compounds from matrices such as environmental samples (river water, groundwater, wastewater from treatment plants, soil extracts) [22, 34, 61, 68, 73, 74, 80, 86, 89, 93, 96, 97], biological matrices such as urine, serum, and plasma [24, 33, 80, 81, 83–85, 87, 88], or food samples [71, 74, 77–79, 83, 85, 89, 90]. Table 1 shows examples of the most relevant studies published so far.

The first off-line application of MIP-SPE for environmental samples was published by Ferrer *et al.* [98]. Two molecularly imprinted polymers were synthesized using terbutylazine as the template and were used as solid phase extraction cartridges for the enrichment of six chlorotriazines in natural water and sediment samples. After a clean-up step with 2 mL of dichloromethane, the matrix components that were non-specifically retained on the MIP were removed. The recoveries were higher than 80% for all the chlorotriazines, except for propazine (53%), after percolating 100 mL of water sample.

Bastide *et al.* [76] prepared an MIP for metsulfuron methyl as the template molecule and vinylpyridine as a functional monomer. The polymer was reported to be

group-selective for sulfonylurea herbicides from aqueous solution.

Cacho *et al.* [96] prepared a semi-covalent imprinted polymer by precipitation polymerization using propazine methacrylate as the template molecule. The binding sites present in the polymeric matrix were characterized by fitting the experimental results of several rebinding studies to the Langmuir–Freundlich isotherm. Analytical methodology based on MIP-SPE was developed for the determination of several triazine herbicides in soil and vegetable samples (corn, potatoes).

Baggiani *et al.* [83] reported MIP-SPE of the fungicide pyrimethanil from wine samples on an MIP prepared from porous chloromethylated polystyrene beads, onto which an initiator-chain transfer agent (iniferter) was coated. Then the beads were dispersed in chloroform and grafted in the presence of methacrylic acid and pyrimethanil as the template molecule. On the MIPs prepared in this way, the optimized extraction protocol was selective for pyrimethanil and the related pyrimidinic fungicides cyprodinil and mepanipyrim, while the non-pyrimidinic fungicides were not extracted. Recoveries from a wine matrix spiked with pyrimethanil were between 80 and 90%.

A number of applications have been directed to the MIP-SPE of drugs from various matrices such as plasma, urine, soil, food samples, etc. Dzygiel *et al.* evaluated

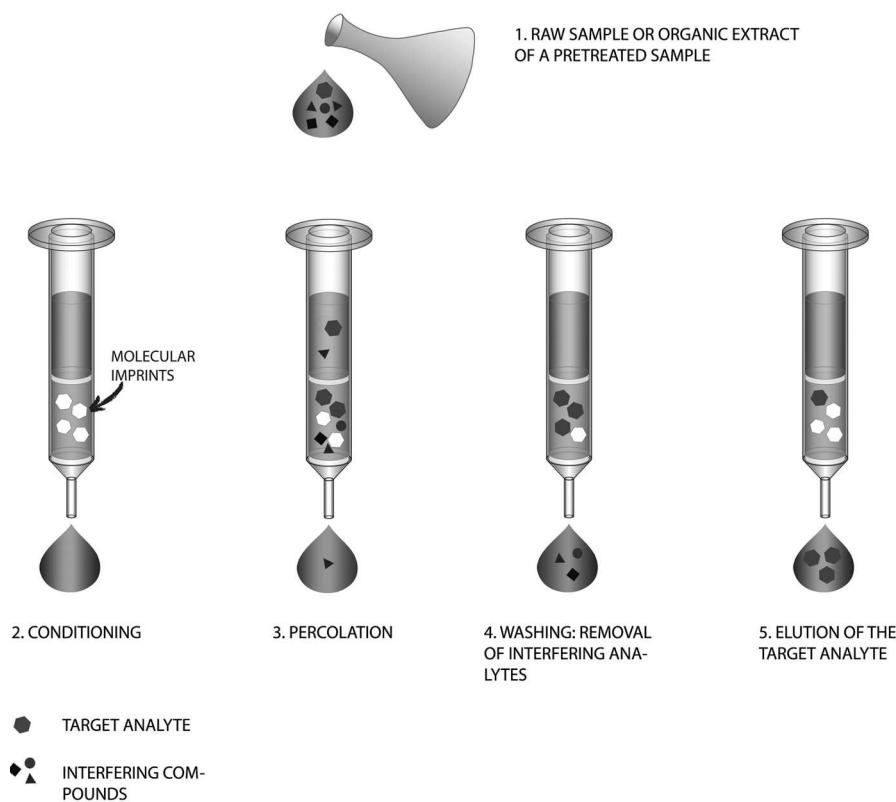


Figure 2. Principle of a solid phase extraction procedure using an MIP sorbent.

molecularly imprinted polymers as selective sorbents for the solid phase extraction of sildenafil used in treatment of male erectile dysfunction and its principal metabolite, desmethylsildenafil [84]. The performance of two MIP sorbents using structural analogues of sildenafil as templates in organic and in aqueous media was studied. The selective extraction of sildenafil gave quantitative compound recovery for aqueous samples in contrast to organic-based samples. The imprinted materials were used as MIP-SPE sorbents for sample pre-treatment for the determination of sildenafil and related compounds in plasma.

Chapuis *et al.* [85] evaluated an imprinted polymer prepared for the alpha-blocker alfuzosin, widely used for the treatment of benign prostatic hyperplasia. The synthesis of the MIP was performed in dichloromethane with methacrylic acid as functional monomer and alfuzosin as template. The presence of selective cavities in the MIP was demonstrated in dichloromethane. The retention mechanism in aqueous media was studied to highlight the effect of pH and of the nature and the amount of cations contained in the biological matrices such as samples of plasma. An optimized procedure was then applied to selectively extract the target drug from human plasma with 60% extraction recovery. Pressurized solvent extraction followed by MIP-assisted clean-up of alfuzosin from soil provided 80%.

Mycophenolic acid (MPA) was selectively trapped and pre-concentrated on an MPA-MIP sorbent using different loading and washing conditions [80]. Good selectivity enabled clean-up of the human plasma samples from interfering compounds with recoveries acceptable for screening MPA in plasma samples (more than 74% for the extraction and more than 87% for the analytical method). A twelve-hour pharmacokinetic profile of MPA was generated for a renal transplant recipient receiving chronic oral dosing of 500 mg mycophenolate mofetil (MMF). The results of this study indicated that this method could be applied for therapeutic drug monitoring of mycophenolic acid in patient plasma.

A method for the determination of the antibiotic chloramphenicol using sample clean-up with a molecularly imprinted polymer was developed using an analogue of the analyte as the template, which avoids residual template leaching or bleeding [86]. The MIP material was used for solid phase extraction of chloramphenicol from various sample matrices such as honey, urine, milk, and plasma. A complete analytical method with LC-MS/MS quantitation was fully validated according to the European Union criteria for the analysis of veterinary drug residues.

An MIP-SPE method for the extraction of fluoroquinolone antibiotics (FQs) from soil was developed by Turiel *et al.* [54]. The benefits of the use of antibiotics in medicine

are obvious, but widespread abuse in recent years has led to the accumulation of antibiotic residues in various environmental areas, thus increasing the risk of development of antibiotic-resistant bacteria. In the case of fluoroquinolones the use of both sewage sludge and livestock manure as fertilizers in agricultural crops in several countries favours the accumulation of these antimicrobials in soils. Molecularly imprinted polymers for FQs have been synthesized in a single preparative step by precipitation polymerization using ciprofloxacin (CIP), the major metabolite of enrofloxacin, as the template molecule. Combinations of various functional monomers and porogens were tested. No imprinting effect was observed with the vinylpyridine-based polymers, in contrast to the MAA-based polymers. The MIP prepared in methanol porogen solvent using MAA as the functional monomer showed the best performance for the selective recognition of widely used FQs and quinolones. The MIP packed into a stainless steel chromatographic column was applied for screening of FQs in soil samples. Under optimized conditions, soil samples spiked with CIP or with a mixture of fluoroquinolones in concentration of 0.5 µg/g were successfully analyzed.

Another solid phase extraction method for fluorinated quinolones was performed using MIP with enrofloxacin as the template molecule. The imprinted polymer showed a high degree of cross-reactivity for ciprofloxacin and was applied as a selective sorbent in a two-step solid phase extraction method for human urine and tissue samples [87].

A molecularly imprinted polymer was designed for the selective extraction of carbamazepine (CBZ), an anti-convulsant drug widely used in epilepsy treatment, from effluent wastewater and urine samples [88]. The MIP properties were evaluated chromatographically. In solid phase extraction, CBZ-MIP yielded CBZ recoveries of 80% when 100 mL of effluent water spiked with 1 µg/L was percolated through the polymer. For urine samples, 2 mL samples spiked with 2.5 µg/L CBZ were extracted with 65% recovery.

Yang *et al.* developed MIP for the selective extraction of indomethacin (IDM), a non-steroidal anti-inflammatory drug, from tap water and river water samples [93]. Combinations of various functional monomers and polymerization techniques were tested. The MIP of AA-EDMA produced by bulk polymerization showed the highest binding capacity for IDM. Water samples with or without spiking were extracted by the MIP-SPE column and analyzed by HPLC. No detectable IDM was observed in tap water and the content of IDM in river water was found to be 1.8 ng/mL. The extraction recoveries of the MIP-SPE column for IDM in spiked tap and river water were 87.2% and 83.5%.

Puoci *et al.* prepared a sorbent for extraction and purification of α -tocopherol (α -TP) from vegetable samples [89],

which enables selective recognition of α -TP. A non-covalent MIP was prepared using methacrylic acid as the functional monomer and ethylene dimethacrylate as the cross-linker agent. Several MIP sorbents were synthesized at various molar ratios of template, methacrylic acid, and cross-linker. Both thermo-polymerization and photo-polymerization were employed in this study. A good imprinting effect was found in the materials prepared by photo-polymerization with a higher amount of methacrylic acid, whereas no imprinting effect was found in α -TP MIP synthesized by thermo-polymerization. An optimized MIP-SPE protocol was successfully used for the purification of α -TP from bay leaves with 60% recovery.

Theodoridis and co-workers [90] fabricated MIPs for the specific adsorption of rutin and quercetin flavonoids as the template molecules for the preparation of MIP phases in a self-assembly (non-covalent) approach. The imprinting selectivity of the MIPs was evaluated by measuring the retention of several flavonoid compounds on the MIPs, control non-imprinted polymers, and commercial silica-based C18 materials. The MIPs were applied for the selective SPE pre-concentration of the flavonoids from white and red wine, orange juice, and tea samples.

MIPs with caffeic acid and *p*-hydroxybenzoic acid templates were employed for the isolation of polyphenols from olive mill wastewater samples (OMWWs) without previous pre-treatment. Several functional monomers and cross-linkers were tested for both templates. The effect of pH on the binding affinity for the template from aqueous solution was explored. Acidic conditions favour the recognition selectivity for both polymers, which can be used for a quick and efficient SPE isolation of the polyphenol fraction directly from raw OMWW [91].

4.2 On-line MIP SPE-HPLC protocols

On-line MIP-SPE systems have been developed principally by coupling a pre-column packed with a few milligrams of imprinted polymer to an LC analytical column, using the mobile phase as the eluting solvent for the MIP-SPE. The mobile phase desorbs the analytes retained on the MIP and transfers the eluate from the extraction cartridge to the injection loop, the content of which is subsequently analyzed on the HPLC analytical column. This system may be readily built in the laboratory and can be further used as an automated platform for on-line sample preparation (Fig. 3). The number of on-line MIP-SPE publications has increased considerably in recent years. In these studies, the analytes of interest have been extracted from various types of matrices including environmental [57, 78], biological [69, 73, 92], and food samples [68, 99]. Table 1 shows selected relevant studies published so far.

An early application of an on-line coupled-column system combining a molecularly imprinted polymer and a

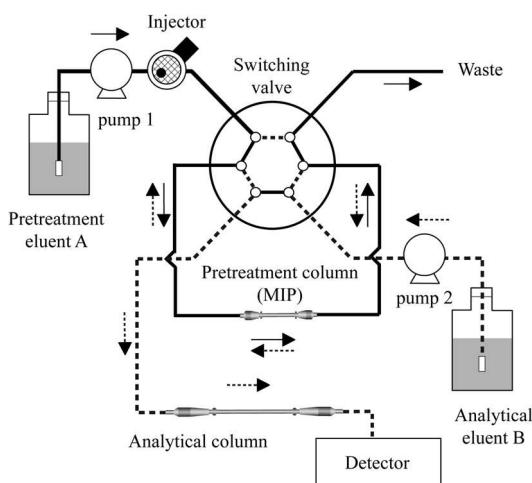


Figure 3. Principle of the on-line coupling to MIP-SPE. Adapted from [73].

C_{18} -silica column was reported for the analysis of triazine herbicides, simazine, atrazine, propazine, and terbutylazine. The MIP showed good performance for selective discrimination of triazines from humic acid in water samples, whereas matrix components in urine and apple extracts were partly retained by the MIP. Triazine-enrichment factors of up to 100 were reported with extraction recoveries of 74–77% [100].

Marcé and co-workers [21] developed an on-line MIP-SPE procedure coupled to HPLC for selective extraction of 4-nitrophenol (4-NP) from water samples. Three MIP polymers have been synthesized using 4-chlorophenol as the template molecule, following different protocols (non-covalent and semi-covalent) and using different functional co-monomers, 4-vinylpyridine (4VP) and methacrylic acid. Only the 4VP non-covalent polymer showed a clear imprint effect. This MIP also showed cross-reactivity for the 4-chloro-substituted phenols (4-chloro-3-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol) and for 4-NP from a mixture containing the 11 priority EPA (Environmental Protection Agency) phenolic environmental pollutants and were applied to selectively extract the 4-chloro-substituted compounds and 4-NP from river water samples.

Surface modified molecularly imprinted polymers (SM-MIPs) have been developed for selective retention of 17β -estradiol (E2) using 6-ketoestradiol as dummy template [101]. MIPs were synthesized using 4-vinylpyridine as the functional monomer. The pre-treatment columns packed with this material were used in on-line configuration coupled to an HPLC-MS system for the pre-concentration and analysis of low levels of 17β -estradiol in river water samples. The pre-concentration of the target compound and the simultaneous elimination of sample

matrix interferences resulted in excellent chromatographic resolution and in a significant increase in sensitivity and reproducibility of the analysis.

A similar on-line configuration coupled with HPLC and electrochemical detection was applied for the determination of bisphenol A (BPA) [57]. This system provided high enrichment factors and prevented possible contamination originating from off-line SPE pre-treatment procedures. Bisphenol A and other phenolic compounds were determined in river water using an on-line procedure with BPA imprinted monolithic pre-columns coupled on-line to HPLC-UV [78]. The monolithic MIP column with good flow-through properties was obtained by changing the molar ratio of the porogens (toluene and dodecanol). If no toluene was added, the polymer had a soft gel-like appearance. The MIP prepared with 20% toluene showed a remarkable imprinting effect. However with 25% toluene in the porogen mixture, the polymer became very dense and almost impermeable. 15% toluene and 85% dodecanol provided the optimal porogen solvent composition. The selectivity and retention properties of the monolith for BPA and other phenolic compounds were evaluated. The results show that both the hydrophobic and hydrogen-bonding interactions play important roles in the recognition process.

An electrochemical method for the determination of sulfamethazine in milk at a low concentration level (25 μ g/L) was reported, which employs sample clean-up and selective pre-concentration of sulfamethazine on a molecularly imprinted polymer and on the surface of a Nafion-coated glassy carbon electrode (GCE). Square wave (SW) oxidative voltammetry of accumulated sulfamethazine was employed for quantification. A mean recovery of $100 \pm 3\%$ was reported for five samples, spiked at the 25 μ g/L level [99].

A molecularly imprinted polymer prepared using caffeine template and methacrylic acid monomer was validated as a selective sorbent for solid phase extraction, implemented in an automated on-line sample preparation method for rapid extraction of caffeine from coffee [68]. The MIP was packed into a polypropylene cartridge, which was coupled to an HPLC instrument. The principle of sequential injection analysis was utilized for a rapid automated and efficient SPE procedure on the MIP. The method was optimized in terms of flow rates, extraction time and volume.

A molecularly imprinted polymer synthesized in dichloromethane was evaluated for the selective extraction of alfuzosin from human plasma and was used for SPE coupled on-line with liquid chromatography. The selective extraction procedure is believed to utilize electrostatic interactions between the target analyte and the selective support in solvents compatible with reversed-phase liquid chromatography. After percolation of plasma sample through the MIP cartridge, washing steps

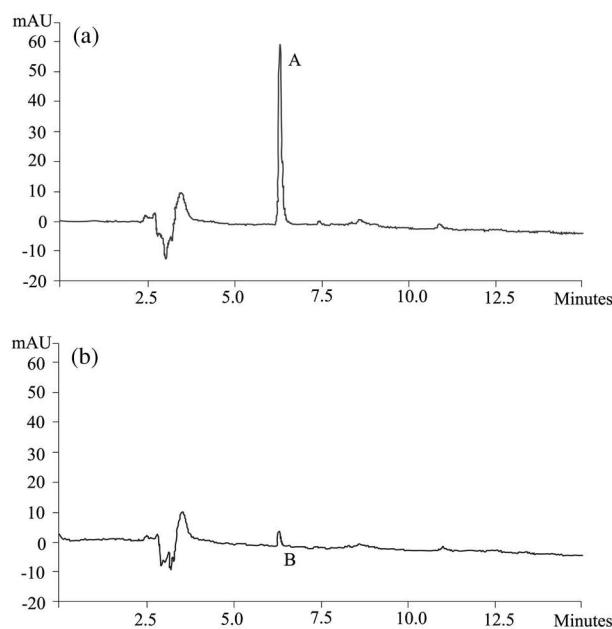


Figure 4. Chromatograms corresponding to the on-line coupling of MIP-SPE to LC–UV for the extraction of 100 μL of treated plasma spiked with 50 ng of alfuzosin on MIP (A) and on NIP (B). W1: 600 μL water/acetonitrile (1:1, v/v), W2: 1200 μL acetonitrile, W3: 150 μL water/acetonitrile (1:1, v/v), E = mobile phase: acetonitrile/2 mM ammonium acetate, 0.2% formic acid. Adapted from [92].

were performed using acetonitrile and a mixture of acetonitrile and water and the target analyte was eluted with non-aqueous HPLC mobile phase (acetonitrile + 2 mM ammonium acetate and 0.2% formic acid). By applying this method to plasma, extraction recoveries close to 100% were obtained for alfuzosin while various pharmaceutical compounds currently found in biological fluids were not retained on the MIP. The high selectivity of the MIP coupled to the chromatographic system permitted easy and fast analysis of the drug with a limit of quantification of 15 $\mu\text{g}/\text{L}$ on UV detection, satisfactory for common therapeutic concentrations of this drug (Fig. 4) [92].

4.2.1 Restricted access materials

Restricted access materials (RAM) possess a hydrophilic surface and a relatively narrow pore size that restricts large molecules from entering the inner pores with less polar stationary phase. These characteristics are responsible for the retention and isolation of low molecular weight compounds, mainly drugs and metabolites [69]. The combination of RAM and MIP allows selective sample preparation to be achieved in the on-line mode [102]. The RAM-MIP media can be employed as pre-columns in column-switching systems for the analysis of biological sam-

ples such as plasma [103] containing some pharmaceuticals such as propranolol [104], atenolol [105], and benzodiazepines [106].

Haginaka *et al.* [107] prepared a RAM-MIP sorbent for direct injection analysis of propranolol (PRP) β -blocker in biological fluids, which showed excellent molecular recognition ability for PRP and good ability for alprenolol (ALP). The MIP was prepared using methacrylic acid as the functional monomer and propranolol as the template molecule by a multi-step swelling polymerization method yielding uniformly sized particles. In the initial step the polystyrene seeds are swollen using an aqueous micro-emulsion of a free radical initiator (2,2'-azobis-2,4-dimethylvaleronitrile) in a water-insoluble solvent. The dispersion of the swollen particles is added to a second aqueous dispersion containing the polymerization mixture. After adsorption of droplets of the polymerization mixture on the swollen particles, polymerization is initiated. The final bead size can be controlled by changing the levels of activating solvent and the volume ratios of the different dispersion phases. Next, a mixture of glycerol monomethacrylate and glycerol dimethacrylate (1:1) was used for hydrophilic surface modification. This RAM-MIP was applied for direct injection analysis of ALP enantiomers in rat plasma using a column-switching HPLC system.

Sambe and co-workers prepared uniformly sized MIPs for atrazine, ametryn, and irgarol by a multi-step swelling polymerization method [40]. The MIP prepared with the atrazine template using methacrylic acid showed good molecular recognition abilities for chlorotriazine herbicides (atrazine, propazine, simazine, and terbutylazine), while the MIPs prepared with ametryn and irgarol templates using trifluoromethacrylic acid showed excellent molecular recognition abilities for methylthiotriazine herbicides (ametryn, prometryn, simetryn, and irgarol). The RAM-MIP for irgarol, having a methylthiotriazine skeleton, was prepared using *in situ* hydrophilic surface modification using glycerol dimethacrylate and glycerol monomethacrylate as hydrophilic monomers during the polymerization process. The RAM-MIP was applied to selective pre-treatment and enrichment of methylthiotriazine herbicides in river water, followed by their separation in a column-switching HPLC system. The recoveries of simetryn, ametryn, and prometryn at 50 pg/mL levels were 101, 95.6, and 95.1%, respectively.

A RAM-MIP material for an isotopologue form of bisphenol A (BPA-d₁₆) prepared by multi-step polymerization [48] showed molecular recognition abilities for BPA, halogenated BPA, deuterated BPA, and other structurally related compounds. This RAM-MIP material was utilized for selective on-line pre-treatment and enrichment of trace amounts of BPA, Cl₄-BPA, and Br₄-BPA in river water, followed by their separation and analysis by LC–MS.

5 Concluding remarks

The use of MIPs in sample preparation has undergone remarkable development during the past few years. The advantages of MIPs as selective sorbents have been demonstrated in many cases. Although some drawbacks such as bleeding of the template molecule or heterogeneity of binding sites have been reported, there are already several ways to circumvent, minimize, or suppress them, making MIP-SPE a powerful analytical tool.

Many successful applications have demonstrated that the use of MIPs for solid phase extraction is a powerful method for the clean-up and the direct selective extraction of trace levels of various compounds from complex matrices. MIPs with specific templates were developed for new selective extraction methods of various molecules of interest such as drugs for the pharmaceutical and clinical analysis domain, organic micropollutants in environmental analysis, or contaminants in food samples. The pronounced chemical robustness of MIPs, permitting their cleaning and reactivation for repeated use in various SPE applications, has recently been proven.

However, ever increasing requirements on analytical information in a variety of fields such as clinical, forensic, food, and environmental analysis connected with the introduction of miniaturized analytical systems to deal with very small sample volumes into laboratory practice necessitate further modification of the format of the imprinted polymers used so far. The use of MIPs connected directly to the detection system for extraction, enrichment, separation, and detection of various target analytes, although still in a rather preliminary stage, will lead to the development of very simple sequential flow analytical methods facilitating their application in routine laboratories.

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A Highly Selective Solid Phase Extraction Sorbent for Pre-Concentration of Sameridine Made by Molecular Imprinting

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Key Words

Gas chromatography
Solid phase extraction
Sample pre-concentration
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Summary

A novel approach to solid phase extraction, based on the use of a highly selective molecularly imprinted polymer, is presented. The versatility of this type of sorbent for solid phase extraction was demonstrated in a model batch-wise pre-concentration of sameridine prior to gas chromatography. Problems associated with leakage of remaining imprint molecules during the desorption phase could be eliminated by the use of a close structural analogue of sameridine as the imprint species. It was found that a major benefit of the imprinted polymer was its specificity, which lead to distinctly cleaner chromatographic traces and ability to improve sensitivity by extracting sameridine from larger sample volumes.

Introduction

Molecular imprinting of ligands in synthetic polymers [1–7] is an emerging technique for the preparation of highly selective sorption materials. The technique entails polymerization of functional monomers in the presence of a templating ligand, or imprint species. The monomers are selected for their ability to interact with the ligand, either by non-covalent interactions (such as hydrogen bonding, ion-pair interactions, etc.), reversible covalent interactions or metal ion mediated interactions. These interactions between imprint species and monomers are preserved during the polymerization of the monomers into a solid, highly cross-linked polymer

network, in which the imprint molecules are embedded. This process gives rise to imprints against the ligand possessing steric and chemical complementarity to the ligand. Subsequent removal of the imprint molecules leaves “memory” sites with affinity for the original imprint species. In some instances binding affinities and selectivities approaching those demonstrated by antigen-antibody systems have been achieved [8, 9]. In most instances, the molecularly imprinted polymers, MIPs, are made as bulk polymers, which are then ground and sieved to particles of suitable size. Such particles may be packed into liquid chromatography (LC) columns and used for the separation of the imprint species from a mixture of structurally similar compounds. A number of MIP studies have focused on chiral separation problems, where the specificity of a given polymer is predetermined by the enantiomer of the ligand that was present during its preparation [10–19]. The technique is now well established and has been recently reviewed [1–7].

Here we present the use of MIPs in a novel approach to highly selective pre-concentration of samples prior to chromatographic analysis. This application relies on the ability of a MIP to selectively bind an analyte, or a group of structurally similar compounds, from a mixture. The target analyte was sameridine, which is a new type of compound with both local anesthetic and analgesic properties [20]. The clinical use of sameridine is to provide anesthesia during surgery and prolonged post-operative analgesia. Two specific problems associated with the use of MIPs in solid phase extractions were addressed: *i*) The potential risk of leakage of imprint molecules during the desorption phase which would give rise to inaccuracy in the concentration determination. *ii*) The difficulty in establishing quantitative and rapid desorption, due to high avidity of the MIP for the analyte. These two problems were solved for the solid phase extraction of sameridine, and a model method for pre-concentration of sameridine was developed and compared with a standard liquid-liquid extraction method presently in use [21].

Experimental

Chemicals

Sameridine (**1**) and compounds **2** and **3** (Figure 1) [20] were from Medicinal Chemistry, Astra Pain Control (Södertälje, Sweden). Ethylene glycol dimethacrylate and methacrylic acid were from Merck-Schuchart (Hohenbrunn, Germany), and 2,2'-azobis(2-isobutyronitrile) from Janssen Chimica (Beerse, Belgium).

Polymer Preparation

Azobis-isobutyronitrile (AIBN, initiator, 64 mg, 0.40 mmol) and **3** (190 mg, 0.6 mmol) were weighed into a Kimax test tube and dissolved in 4.68 g of toluene. Methacrylic acid (MAA, 306 µL, 3.6 mmol) and ethylene glycol dimethacrylate (EGDMA, 3.56 g, 18 mmol) were added and the clear solution was cooled on ice and sparged with nitrogen for approximately 5 min. The tube was placed under a UV-source (366 nm) at ambient temperature for 24 h. The test tube was smashed and the hard polymer soaked in methanol in order to remove unreacted monomers. The polymer was ground in a laboratory mortar grinder (Type RM O with mortar cup and pestle of hard porcelain, Retsch, Haan) under wet conditions (water). The particles were sieved through a 25 µm sieve (Retsch, Haan) with water. The particles that passed through the sieve were collected and the retained particles were reground. The fines were removed by repeated sedimentation from ethanol. The sediment was transferred to a filter funnel and washed with 500 mL of 1 M NH₄Ac dissolved in a mixture of 40 % of 2-propanol, 25 % of acetic acid and 35 % of water followed by 300 mL of 25 % acetic acid in ethanol, 300 mL of methanol, 300 mL of 1 N NaOH in 20 % ethanol and 300 mL of methanol. Finally, the particles were dried under vacuum and stored at ambient temperatures until use.

Sample Pre-Concentration

MIP method B: Approximately 600 mg of thawed human plasma, internal standard (**2**, 60 µL, final concentration 50.17 nM) and 10 % Na₂CO₃ (300 µL) were added to glass test tubes. The desired plasma concentration of sameridine for standards were obtained by transferring the appropriate volume of sameridine stock solution to each test tube. 4.8 mL of heptane were added and the tubes were incubated by continuous rotation for 20 minutes. After centrifugation (3000 rpm, 10 min) each organic phase was transferred to a new tube. 440 µL of a suspension consisting of 10 mg/mL MIP in ethanol were added to each test tube and the tubes were incubated for 1 hour. The test tubes were centrifuged (3000 rpm, 15 min) and the supernatants were poured off and discarded. 1 mL of heptane-ethanol (1:1; v/v) was added and the tubes were vortexed, centrifuged and the supernatants discarded. The MIP pellets were treated with a mixture of 1.70 mL of heptane, 130 µL of ethanol and 170 µL of 5N NaOH for 1 hour under continuous rotation.

The tubes were centrifuged (3000 rpm, 15 min), each organic phase was transferred to a new tube and 400 µL of 2.5N NaOH were added. The tubes were vortexed and then centrifuged. Each organic phase was transferred to a borosilicate test tube and evaporated under a stream of nitrogen. The residue was redissolved in 150 µL of n-heptane-ethanol (9:1; v/v) and analysed by GC.

MIP method A was performed as described above except that all volumes except the final 150 µL of heptane-ethanol (9:1; v/v) were exactly one half of the volumes for MIP method B. The standard liquid-liquid extraction method was performed as described [21].

GC-Separation

The GC separations were done essentially as described [21] using a Hewlett Packard 5890, series II chromatograph equipped with a Hewlett Packard 7673 automatic sampler and either a nitrogen sensitive detector (NPD) or a flame ionization detector (FID) (Wilmington, DE). The injection volume was 5 µL and the injection temperature was 260 °C. The capillary was a Hewlett Packard Ultra 2 cross-linked Phenyl methyl silicone (0.17 µm) 25 m × 0.32 mm i.d. The separation started at 120 °C for 1 minute, then the temperature was increased linearly at 40 °C per minute to 280 °C, which was then maintained for 5 minutes. The carrier gas velocity was 1.5 mL min⁻¹.

Results

Polymer Preparation

The preparation of the MIP was done essentially by a well-known procedure described previously for the molecular imprinting of other compounds [9]. Compound **3**, which is a structurally similar analogue of sameridine, **1**, (Figure 1), was used as the imprint species. The resultant MIP was found to bind the imprint species, **3**, sameridine, **1**, and the internal standard, **2**, with approximately equal strength. A ligand displacement experiment was set up in which ¹⁴C-labelled sameridine was used as the tracer. Under the conditions used for the adsorption step the IC₅₀-values were found to be 1.2, 2.4 and 4.4 µM for the imprint species, sameridine and the internal standard, respectively. The IC₅₀-value is the concentration of ligand that displaced 50 % of the binding of the [¹⁴C]sameridine.

The MIP has a macroporous morphology with large surface area which facilitate mass transport in and out of the pores of the polymer. The specific surface area, the pore volume and the average pore diameter were found to be 305 m² g⁻¹, 0.72 mL g⁻¹ and 150 Å, respectively. The pore diameter is the average of all pores, however, pores of approximately 35 and 400 Å were more preva-

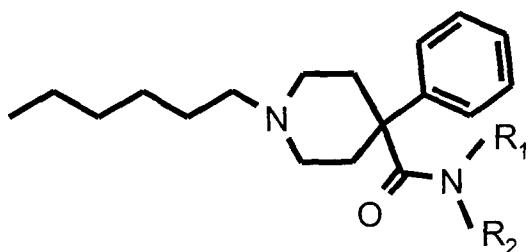


Figure 1

Structures of sameridine (**1**, $R_1 = \text{methyl}$, $R_2 = \text{ethyl}$), internal standard (**2**, $R_1 = R_2 = \text{ethyl}$) and imprint species (**3**, $R_1 = R_2 = \text{methyl}$) [40].

lent than others. Elemental analysis for nitrogen was consistent with removal of 91.5 % of the imprint molecules from the polymer by the washing steps.

Adsorption and Desorption Kinetics

The binding kinetics were studied at three sameridine concentrations, 20, 320 and 3000 nM, using a radioligand binding experiment employing [^{14}C]sameridine. A polymer concentration higher than 1.2 mg mL $^{-1}$ was required to quantitatively bind the analyte from the 3000 nM sample and 0.6 mg mL $^{-1}$ from the 20 and 320 nM samples. In the experiment with the highest sameridine concentration, a high non-specific binding was observed at polymer concentrations above 2.5 mg mL $^{-1}$. The non-specific binding was measured as the binding to a non-imprinted reference polymer. For all three sameridine concentrations the binding interaction completed within 30 minutes.

Radiolabelled sameridine was adsorbed from test samples containing [^{14}C]sameridine at three concentrations: 20, 320 and 3000 nM, and then several means for quantitative desorption of the analyte were investigated. No desorption was obtained with solvents such as acetonitrile, ethanol, methanol, propanol, isobutylmethyl ketone, toluene and mixtures of heptane and ethanol. Partial desorption was achieved with THF (40 %), DMF (60 %) and mixtures of DMF with toluene (50 %) and THF (50 %). Various mixtures of ammonium acetate, ethanol, acetic acid and water did desorb sameridine, however, not quantitatively. Furthermore, such solutions of sameridine are not amenable to direct injection on GC. Eventually, a method for desorption involving treatment with a mixture of ethanol-sodium hydroxide and heptane was found to work. The success of this desorption system is attributed to the fact that the strong base results in a release of the analyte, then the analyte partitions into the organic layer, which drives the desorption reaction to completion. Ethanol was necessary for the aqueous phase to obtain access to the MIP pores, which were filled with heptane after the previous adsorption step. Too much ethanol, however, prevented quantitative extraction of sameridine to the heptane phase. The optimal mixture was found to be heptane-ethanol (99.5 %)-NaOH (5N) in the ratio of 170:13:17

Table I. Experimental protocols for the MIP-based solid phase extraction and standard liquid-liquid extraction only methods.

Standard liquid-liquid extraction	MIP-based solid phase extraction
Extraction of plasma with heptane	Extraction of plasma with heptane Adsorption to MIP Wash MIP
Evaporation of solvent Re-dissolution of analyte GC-determination	Desorption of sameridine Evaporation of solvent Re-dissolution of analyte GC- determination

(v/v). The desorption reaction was completed within 15 minutes.

Analytical Performance of the Complete Method

The complete extraction scheme is described in the Experimental section and is summarized in Table I. Human plasma was extracted with heptane under basic conditions [21], the organic phase was transferred to a fresh tube and the MIP was added as an ethanol suspension. The concentration of MIP during adsorption was set at approximately 0.9 mg mL $^{-1}$ and the adsorption procedure was allowed one hour for completion. The desorption solution was established as a two phase system consisting of ethanolic alkaline and heptane, and the desorption procedure was allowed one hour for completion. A final NaOH wash of the desorbed analytes in heptane was required in order to remove fine MIP particles, which otherwise interfered with the GC-analysis. The heptane was evaporated, the analyte was re-dissolved in a small volume of heptane-ethanol (9:1; v/v) and the concentration of sameridine determined by gas chromatography using conditions described previously [21].

In the first set of experiments, 300 μL of human plasma samples spiked with known amounts of sameridine in the concentration interval from 16 to 120 nmol L $^{-1}$ were subjected to MIP-based pre-concentration (referred to as MIP method A) and the results compared with those obtained using a standard liquid-liquid extraction method (referred to as the standard method) [21]. A second MIP method for analysis was modified so that the volumes used were doubled relative to the first method (MIP method B). In this experiment 600 μL of plasma were analysed and the sameridine concentrations ranged from 8–67 nmol L $^{-1}$. For all sample preparation methods an eight point linear calibration curve which excluded the origin was constructed using internal standard calibration based on peak height. The calibration graphs for MIP method A and the standard method were identical with the equations $y = 0.0113x - 0.0111$ ($R^2 = 0.9934$) and $y = 0.011x - 0.0155$ ($R^2 = 0.9985$), respectively. The slope of the standard curve for MIP method B was the steepest ($y = 0.0239x + 0.0217$;

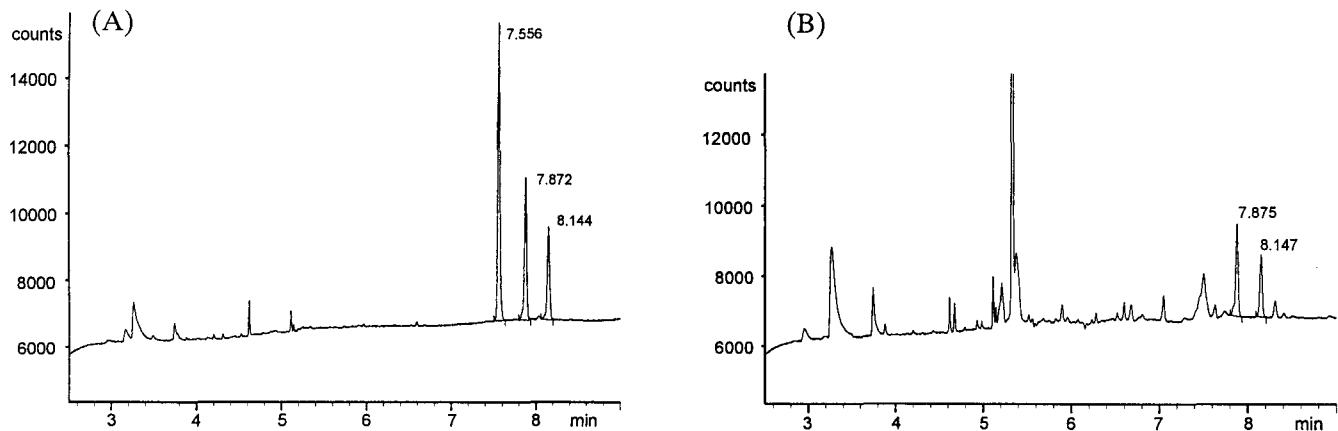


Figure 2

Representative GC traces of spiked human plasma samples. Human plasma was spiked with 66.8 nmol L^{-1} of sameridine and 50.2 nmol L^{-1} of internal standard, and subjected to either (A) MIP-based solid phase extraction or (B) standard liquid-liquid extraction only. The GC-analyses were done as described in the Experimental section. The retention times are: sameridine 7.87 minutes; internal standard 8.14 minutes; and imprint species 7.56 minutes.

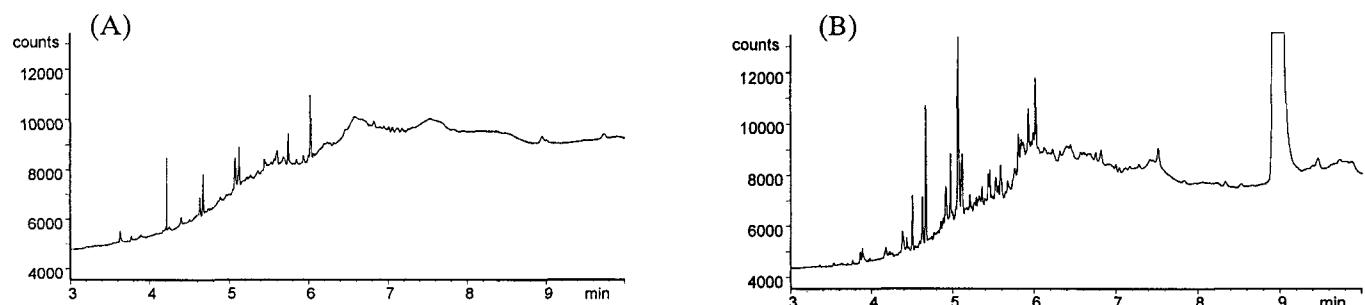


Figure 3

Representative GC traces of blank human plasma samples subjected to either (A) MIP-based solid phase extraction or (B) standard liquid-liquid extraction only. The GC-analyses were done as described in the Experimental section, except that a flame ionization detector (FID) was used.

$R^2 = 0.9977$) because MIP method B was based on pre-concentration from $600 \mu\text{L}$ of plasma into $150 \mu\text{L}$ of heptane-ethanol and was therefore more sensitive than the other methods based on $300 \mu\text{L}$ of plasma.

The chromatograms for samples prepared with the MIP-based method were consistently cleaner than those for the samples prepared without MIP, containing fewer and smaller unidentified peaks (Figure 2). This was even more evident when the GC separations were recorded using a FID, which is a general detector for all organic substances (Figure 3). Due to the low sensitivity of this detector the peaks due to sameridine and internal standard are too small to be detected in these traces. Figure 2 also illustrates the fact that the peak height of sameridine in MIP samples was comparable to the peak height of sameridine in samples prepared by extraction

without MIP. Leakage of imprint species occurred, no matter how carefully the MIP was washed prior to use, and a peak at 7.56 minutes appeared in all GC-traces of samples subjected to MIP extraction (Figure 2A). The imprint species, sameridine and the internal standard are, however, completely separated from each other with retention times of 7.56, 7.87 and 8.14 min, respectively.

The accuracies and intra-assay variations for low, medium and high concentration samples are presented in Table II. The collective data above and in Table II show that the analytical performance, including linearity and slope of the standard graph, accuracy and intra-assay precision, of the MIP-based methods was at least as good as the performance observed for the standard liquid-liquid extraction method. A statistical analysis

Table II. Accuracy and intraassay variation of the MIP based solid phase extraction methods and the standard liquid-liquid extraction method.

Theoretical		MIP Methods			Standard method		
Concentration (nmol L ⁻¹)	Accuracy (%)	RSD (%)	n	Accuracy (%)	RSD (%)	n	
Method for 16–120 nmol L ⁻¹ of sameridine using 300 µL of plasma							
16.00	102.2	8.4	6	107.8	3.9	7	
31.99	94.0	6.3	5	100.0	4.9	7	
119.97	97.3	1.6	6	100.5	2.6	7	
Method for 8–67 nmol L ⁻¹ of sameridine using 600 µL of plasma							
8.01	96.9	4.8	7				
16.02	100.7	3.5	7				
66.76	101.3	4.0	7				

support the qualitative observation that the MIP method was a better predictor of sameridine concentration when the plasma samples contained more unidentified peaks (Figure 2).

Discussion

The results presented here, and in other studies which were published during the preparation of this article [22, 23], demonstrate that MIPs may be used as specific binding matrices for the solid phase extraction and cleanup of biological samples. Of specific interest for the use of MIPs in SPE applications is that remaining traces of the imprint species does not leak from the polymer and contaminate the analyte, which would give rise to an uncertainty in the response. To avoid this risk completely we decided to imprint a close analogue of sameridine, the N,N-dimethyl derivative **3**. Due to their close structural resemblance (Figure 1), it was expected that such imprints would bind sameridine (**1**), the internal standard (**2**) and the imprint species (**3**) with approximately equal strength. Radiolabelled ligand displacement experiments using ¹⁴C-labelled sameridine confirmed that this was indeed the case. This alternate-imprint species approach proved to be crucial to the success of this study since leakage of imprint species did occur, no matter how carefully the MIP was washed prior to use (Figure 2). The imprint species, sameridine and the internal standard were, however, completely resolved from each other. Hence, there is no interference from the imprint species with the quantitation of sameridine using **2** as the internal standard. A general implication of the present work is that neither the analyte(s) nor the internal standard should be used as the imprint species in the preparation of MIPs for sample pre-concentration applications. Instead, a derivative of the analyte, with sufficiently similar structure to give rise to imprints that will exhibit the desired ability to bind the analyte of interest, should be selected. This strategy pre-

vents any analytical errors that may be introduced due to leaking of the imprint species.

The MIP-based pre-concentration of samples showed high selectivity for sameridine and the MIP treatment was shown to eliminate or to greatly reduce the size of the unidentified peaks (Figure 2). The pre-concentration was quantitative and may be used for cleanup of plasma samples prior to determination of the concentration of sameridine using GC [21] or LC [24]. The experiments outlined in this report therefore demonstrate the potential to use MIP in solid phase extraction. The experiments also indicate, however, that the present MIP-based method is time consuming, and suggest that automation of the MIP-based sample preparation method is warranted, if practical use in routine analyses will be feasible. Generally, it is not practical to develop a method for analysis which ensures resolution of every possible contaminant from the analyte of interest. When interfering peaks are detected, normal practice is to modify the chromatography slightly to improve resolution between the interfering peak and the analyte of interest. This practice may, however, lead to time-consuming optimization and validation procedures. A practical application of the present MIP-based sample preparation is that it may be established as a validated and equivalent sample preparation method which is applied only to those samples which require it.

The MIP and standard sample preparation methods for sameridine in human plasma provide equivalent results (Table II). It is interesting to note that the results obtained for ~8–67 nmol L⁻¹ sameridine samples with MIP method B showed less variability than the results obtained for ~16–120 nmol L⁻¹ sameridine samples with the MIP method A which was based on smaller volumes. This result is interesting because it demonstrates the fact that the initial concentration of analyte is not necessarily a sensitivity limiting variable if MIP treatment is incorporated into sample preparation. With the simple extraction method, impurities and unidentified peaks increase linearly with the size of the initial plasma sam-

ple. In contrast, due of the specificity of the MIP for sameridine binding, the MIP sample preparation method have the ability to concentrate sameridine in a selective manner relative to interfering substances. Therefore, with the MIP method it is possible to improve the sensitivity of a given analytical method, and lower the limit of quantitation, by increasing the size of the original sample. Accordingly, MIP treatment may appropriate for samples with extremely low concentrations of sameridine (e.g. < 2 nmol L⁻¹).

Conclusions

We have demonstrated that potential problems associated with leakage of imprint molecules during sample pre-concentration can be prevented by the molecular imprinting of a close structural analogue of the analyte(s) of interest. Provided the imprint species and the analyte(s) can be separated by the subsequent liquid chromatography or gas chromatography, which in most instances can be made a valid assumption, this strategy eliminates any risk of uncertainty in the determination of the concentration of analyte. Further, the results obtained using the MIP based sample pre-treatment are equivalent to those obtained by the present standard method. This finding indicates that sample pre-concentration via MIP is a viable alternative in quantitative analysis. The benefit of the MIP based procedure is its specificity, which leads to a reduction of the number of unidentified peaks and a potential to improve the sensitivity of the method by applying larger sample volumes.

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2.17 Molecularly Imprinted Polymers

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2.17.1	Introduction	331
2.17.2	Synthetic Procedures to Produce MIPs	332
2.17.2.1	Factors to Consider When Choosing the Template	333
2.17.2.2	Factors to Consider When Choosing the Functional Monomer	333
2.17.3	MIPs for Sample Preparation	334
2.17.3.1	MISPE	334
2.17.3.1.1	Off-Line Protocols	334
2.17.3.1.2	Online Protocols	335
2.17.3.1.3	In-Line Protocols	336
2.17.3.1.4	Improved Batch Protocols	336
2.17.3.2	Molecularly Imprinted Solid-Phase Microextraction (MI-SPME)	336
2.17.3.2.1	MIP-Coated Fibers	338
2.17.3.2.2	MIP Fibers (Monoliths)	340
2.17.3.3	Other Formats	340
2.17.3.3.1	MSPD	340
2.17.3.3.2	SBSE	341
2.17.3.3.3	Liquid Membranes and MIPs Combination	341
2.17.4	Outlook and Expected Future Trends	342
References		342
Relevant Websites		344

2.17.1 Introduction

Despite the huge development of analytical instrumentation that has occurred since the early 1990s, sample preparation steps must be carried out prior to final determination, which is still considered the bottleneck of the analytical process. Typically, target analytes are solvent-extracted from samples using a wide variety of organic solvents and techniques, and then determined by chromatographic techniques coupled to common detectors or, more recently, to mass spectrometry (MS) or tandem MS. However, even when using the selective detection provided by MS, direct injections of crude extracts are not recommended, since matrix components can inhibit or enhance the analyte ionization, hampering accurate quantification.

The main objectives of sample preparation are the removal of potential interferents, analyte preconcentration, converting (if needed) the analyte into a more suitable form for detection or separation, and providing a robust and reproducible method independent of variations in the sample matrix. More recently, new objectives have been set such as using smaller initial sample sizes, improvement of selectivity in extraction, facilitating the automation, and minimizing the amount of glassware and organic solvents to be used.¹ Keeping these comments in mind, it is evident that the traditional liquid–liquid extraction does not fulfill most of the current requirements, and it has been displaced from laboratories during the last years by new extraction techniques such as solid-phase extraction (SPE), solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), and more recently by matrix solid-phase dispersion (MSPD), micro solid-phase extraction (MSPE), or liquid-phase microextraction (LPME), among others.

Any of the new aforementioned techniques has been demonstrated to be superior to the others but, thanks to the existence of a wide range of suitable phases commercially available, both SPE and SPME are nowadays well established in analytical laboratories. However, the main drawback associated with these methods is the lack of selectivity of the sorbents used, making necessary an extensive optimization of the typical steps involved during the extraction and cleanup of target analytes. Immunosorbents were proposed as an alternative for use in SPE applications in order to overcome the aforementioned drawbacks associated with typical nonspecific sorbents.^{2,3} However, obtaining antibodies is difficult, time consuming, expensive, and, in addition, it is difficult to guarantee its success. It is important that after the antibodies have been obtained they have to be immobilized on an adequate support, which may result in poor antibody orientation, limiting molecular recognition. Besides, the use of organic solvents is not recommended since it might lead to antibody denaturation. One of the most powerful and reproducible ways of providing selectivity for the whole analytical process is by incorporating molecularly imprinted polymers (MIPs) into the sample preparation steps.^{4–10}

Molecular imprinting allows the molding of complementary binding sites, usually for small lipophilic molecules, into synthetic polymers. The technique, as outlined in Figure 1, entails copolymerization of mono- and polyfunctional monomers in the presence

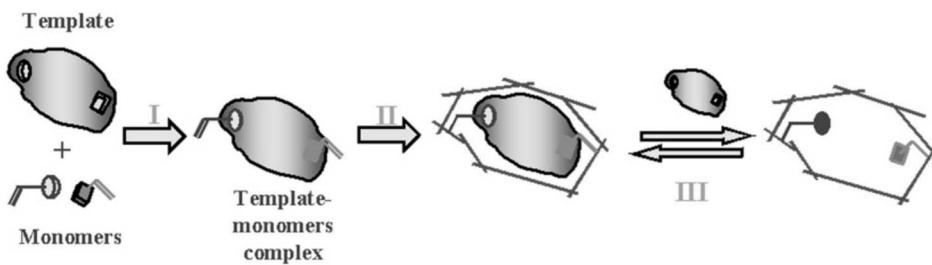


Figure 1 Principle of molecular imprinting relying on self-assembly of functional monomers and a template (I), polymerization (II), and (III) removal of the template providing empty cavities.

of a template, which is thereafter removed to leave sites that can be reoccupied by the template or closely related compounds. This leaves a highly cross-linked and durable MIP containing templated sites or imprints of the template added in the polymerization step.¹¹ Therefore, the behavior of MIPs emulates the interactions established by natural receptors to selectively retain a target molecule (i.e., antibody–antigen) but without the associated stability limitations, which has led to their assessment in a range of molecular recognition-based applications targeting small molecules. MIPs have therefore gained ground as a robust selector phase in SPE. Numerous examples now demonstrate their superior performance over more generic phases in delivering clean extracts and reproducible recoveries of mainly low molecular weight analytes.¹² The analyst may gain by using these phases in obtaining methods that are faster, more sensitive, and more selective compared with those relying on generic sample pretreatment methodologies.

2.17.2 Synthetic Procedures to Produce MIPs

The most versatile approach to the synthesis of molecularly imprinted sorbents is based on self-assembly of the template and a complementary functional monomer prior to polymerization (Figure 1). Thus the template remains associated with the growing polymer during synthesis and, by adding a large portion of a cross-linking monomer, sites complementary to the template are formed that remain stable after template removal.

Although the preparation of a MIP by this method is technically simple, it relies on the success of the stabilization of the relatively weak interactions between the template and the functional monomers. The use of solvents of low polarity and the addition of an excess of functional monomer are the common means of ensuring that the template molecule is complexed to a maximal degree. However, a large proportion of the functional monomer is not involved in complexation of the template and is instead distributed randomly throughout the polymer matrix during the polymerization.¹³ This is a major cause for the binding-site heterogeneity observed in these materials. For instance, using 9-ethylidene as template and methacrylic acid (MAA) as functional monomer, MIPs were obtained that displayed a heterogeneous binding-site population for the template in chloroform with one class of high-energy binding sites ($K = 77\,000\text{ M}^{-1}$, $Q = 20\text{ }\mu\text{mol g}^{-1}$) and one class of lower energy sites ($K = 2400\text{ M}^{-1}$, $Q = 86\text{ }\mu\text{mol g}^{-1}$).¹⁴ It could be shown that the low-energy site class represented nontemplated sites contributing to the nonspecific binding of the MIP.

The polymerization is performed in the presence of a pore-forming solvent called a porogen. To stabilize the electrostatic interactions between the functional monomers and the template, the porogen is often chosen to be aprotic and of low to moderate polarity. Obviously, it also must solubilize all components in the prepolymerization mixture. This restricts the choice of porogen and limits the ability to fully control the morphology of the materials. One difficulty in the process is thus to find a compromise between the two aspects of materials morphology and molecular recognition. Since water disrupts the weak electrostatic interactions present during imprinting, the use of suspension and emulsion polymerization procedures are usually not possible, and polymerization is most commonly carried out in one phase leading to polymer monoliths that need to be crushed and sieved in order to provide particles suitable as packing material.¹⁵ Alternatively, polymer beads may in some cases be produced by various bead polymerization techniques,^{15,16} or preformed support beads may be used to produce imprinted composites.¹⁷ After washing and conditioning the thus-obtained sorbent is then ready for testing or direct use in SPE.

MAA is the most widely used functional monomer in imprinting, resulting in MIPs showing high selectivity for a large number of target molecules. However, it does not provide a universal solution to imprinting problems, and for several targets it fails to produce sufficient binding affinity and selectivity. In this case other monomer or combinations of monomers need to be found. Another fundamental problem in imprinting concerns the selection or design of a template that is likely to produce a sufficient cross-reactivity for the desired target.¹⁸ Apart from functional and shape complementarity with the target, the template needs to be stable and soluble in the prepolymerization mixture and during polymerization. Usually the polymer matrix of the MIP needs to be optimized in order to minimize nonspecific binding of the target or matrix components in the sample matrix for which they are intended. This requires fine tuning of the type and amount of cross-linking monomer as well as the porogenic solvent.

2.17.2.1 Factors to Consider When Choosing the Template

The majority of analytes targeted so far exhibit moderate to high solubility in the corresponding polymerization medium, and can therefore be used as template in the conventional procedure. However, one problem in molecular imprinting concerns the small amount of template that remains strongly bound to the polymer after extraction. This may not constitute a problem in preparative separations, but when the materials are used for sample preparation prior to analytical quantification, bleeding of this fraction will cause false results.¹⁹ Therefore a close target analog is often chosen as template, leading to binding sites that also can capture the target analyte. In addition to being a closely related analog to the target, the template should possess functionalities that interact with the functional monomer, it should be stable and inert during the polymerization, and it should not cause interference during the subsequent quantification of the analyte.^{20–26}

Moreover, the design of a MIP capable of not only recognizing its template but also another target compound or a group of structurally related compounds is a key activity when developing a new MIP. Although MIPs typically exhibit a very high selectivity and affinity for their template, they can also cross-react with structurally similar compounds.

For instance, the propazine-imprinted polymer prepared as shown in Figure 2 does not totally exclude other triazinic herbicides, but instead exhibits enhanced retention for all compounds containing a triazinic group.²⁷ This type of cross-reactivity can be easily understood, since all retained analogs do contain the triazinic substructure, and the principle has been exploited in the development of broad-spectrum molecularly imprinted solid-phase extraction (MISPE) applications for classes of related compounds (e.g., nitrophenols, nitroimidazoles, benzodiazepines, amino alcohols (e.g., β -receptor agonists or antagonists), β -lactam antibiotics, and fluoroquinolones).

2.17.2.2 Factors to Consider When Choosing the Functional Monomer

The guiding principle in the choice of functional monomer is functional group complementarity.²⁸ Thus, for templates containing acidic groups, Brönsted basic functional monomers (e.g., 2- or 4-vinylpyridine, diethylaminoethylmethacrylate) are preferably chosen whereas acidic functional monomers (e.g., MAA, trifluoromethylacrylic acid, itaconic acid) are used to target Brönsted bases. For carboxylic acids and amides high selectivities have also been observed with primary amide-containing monomers (e.g., methacrylamide). Other neutral solvating monomers that commonly enhance the imprinting effect are *N*-vinylpyrrolidone and hydroxyethylmethacrylate.

In the case of poorly polar to apolar templates, with few polar interaction sites it may instead be beneficial to use amphiphilic monomers, stabilizing the monomer-template assemblies by hydrophobic and Van der Waals forces or, in the case of extended π -systems, through charge transfer stabilization. Similarly to biological systems, a large number of complementary interactions is expected to increase the strength and fidelity of recognition. Thus, the use of comonomers that may orthogonally target different subunits of a complex template is often a successful strategy.^{29,30} This leads to terpolymers or higher polymers, and the screening

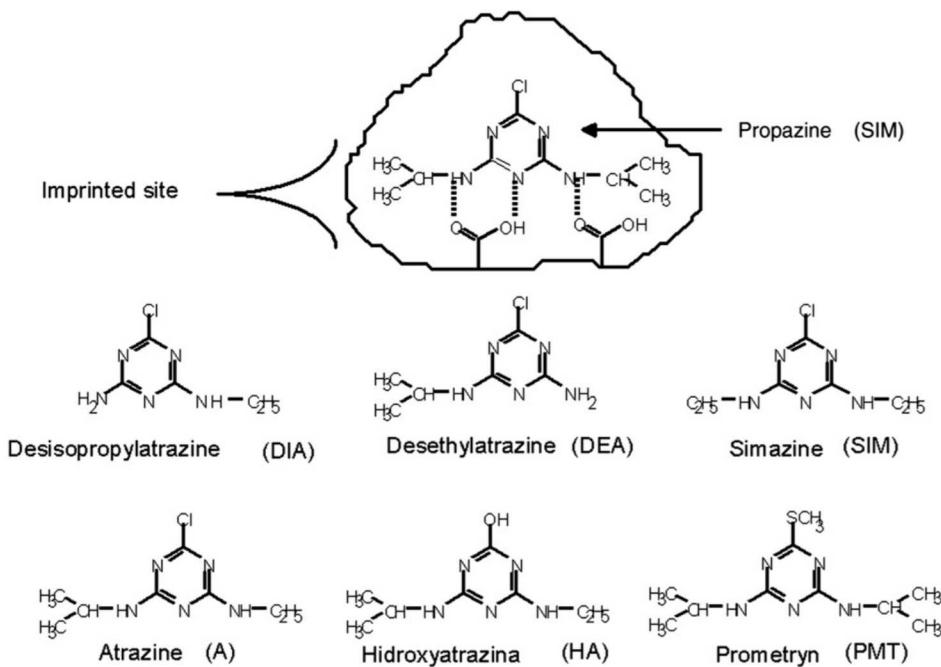


Figure 2 Expected interactions of propazine-methacrylic acid by hydrogen bonding inside binding sites and chemical structures of related triazines.

and optimization task is here considerably more complex and is best carried out using high-throughput polymer synthesis techniques, experimental design, and parallel techniques to analyze binding data.¹⁸ As discussed below, comonomers can also be chosen in order to impart compatibility of the material with a certain solvent, i.e., to reduce nonspecific binding of target analogs or matrix components.^{26,31}

The selection of suitable monomers may also be aided by molecular modeling and computational methods.³² A virtual library of functional monomers is created and screened for all possible interactions the individual monomers may engage in with the template. Monomers with the highest binding scores are subsequently selected to produce full-scale MIPs with hopefully superior recognition properties.

Finally, the search for the optimal structural motif, which can complement the template functionality, may also be guided by results from the areas of host–guest chemistry. Polymerizable host monomers designed to strongly complex a given guest in solution have been used to successfully imprint similar guests.^{33,34} In some cases, this has resulted in a quantitative yield of high-energy binding sites (stoichiometric imprinting), which can recognize the guest also under aqueous conditions.

2.17.3 MIPs for Sample Preparation

2.17.3.1 MISPE

Different modes of MISPE have been assayed, including batch SPE where the MIP is incubated with the sample, conventional off-line SPE where the MIP is packed into cartridges, and several modes of online SPE. The former has been displaced by the conventional off-line SPE format, but it is important to highlight the work by Andersson et al.²² for the determination of *sameridine* in human plasma samples, since this was one of the first examples demonstrating the great potential of MIPs for the selective extraction of target analytes. In this seminal work, an imprinted polymer prepared using a *sameridine* analog as template was incubated for 1 h with spiked human plasma. After the corresponding washing step, *sameridine* was eluted and further determined by gas chromatography, leading to clean chromatographic traces thanks to the high selectivity provided by the MIP. In addition, it was demonstrated for the first time that the use of a close structural analog of the analyte as imprint species prevents the problems associated with template bleeding, one of the typical drawbacks associated with the use of MIPs.

2.17.3.1.1 Off-Line Protocols

Off-line MISPE protocols do not differ from other SPE procedures. Typically, a small amount (15–500 mg) of imprinted polymer is packed into polyethylene cartridges. Then, after the conditioning, loading, and washing steps, analytes are eluted, ideally free of coextractives, and the elution extract is further analyzed by liquid chromatography, gas chromatography, or capillary electrophoresis.

Recent years have seen a huge development of off-line MISPE methods for the determination of a great variety of analytes in environmental samples (river water, groundwater, wastewater, seawater, and soil extracts), biofluids (urine, plasma, serum, and blood), and food samples. In general, the sample is loaded onto the MIP cartridge in a low-polarity solvent, since in such media specific interactions are maximized, and after a washing step for the removal of compounds nonspecifically bound to the polymeric matrix, analytes are eluted with a solvent able to disrupt the typical noncovalent interactions between the analyte and the imprinted polymer. As an illustrative example, Figure 3 shows the chromatograms obtained in the analysis of parabens in soil samples after MISPE on a benzylparaben-imprinted polymer using MAA and divinyl benzene as monomer and cross-linker respectively, prepared by precipitation polymerization.³⁵ From such a figure, it is evident that the high degree of cleanup provided by MISPE allows the determination of target analytes at trace concentration level by high-performance liquid chromatography (HPLC)/ultraviolet (UV), reaching detection limits comparable with those obtained by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Similarly, Mohamed et al.³⁶ convincingly demonstrated that the use of MISPE may result in considerable savings in time and cost of the analysis. In their work, the advantages of MISPE, using a commercial MIP, were assessed by comparing the data generated from a reference method relying on standard generic SPE for the determination of chloramphenicol in milk (raw milk, skimmed milk, and milk powder). The MIP-based procedure only required a previous centrifugation step whereas the traditional approach involved a protein precipitation step, an SPE cleanup, and three liquid–liquid extraction steps. Apart from the expected cleaner chromatograms obtained following the MISPE procedure, a considerable increase in sample throughput was achieved (18 samples processed within 3 h compared to 8 h with the classical approach), demonstrating convincingly the suitability of MISPE in food analysis. Another aspect of the improved sample cleanup refers to the required instrumentation. Whereas the reference method relying on generic SPE requires LC electrospray ionization–MS/MS to achieve the necessary sensitivity and selectivity, acceptable limits of detection using the MISPE procedure may be achieved using more available LC-UV or fluorescence detectors.

In general, MISPE provides a high degree of selectivity but, in some cases, the complexity of the samples under study leads to the coextraction of some matrix components. This problem has been circumvented by introducing an initial cleanup step using a nonimprinted polymer (NIP).³⁷ In this approach, the sample is first loaded onto the corresponding NIP and thus analytes and matrix components are nonspecifically retained. Then, target analytes (and a small amount of interfering compounds) are eluted during the washing steps whereas the majority of matrix compounds remains bound to the NIP, thus achieving a first cleanup of the sample extracts. Subsequently, the obtained washing solution is loaded onto the corresponding MIP for further cleanup. Similar approaches have been followed for the determination of phenylurea herbicides.³⁸

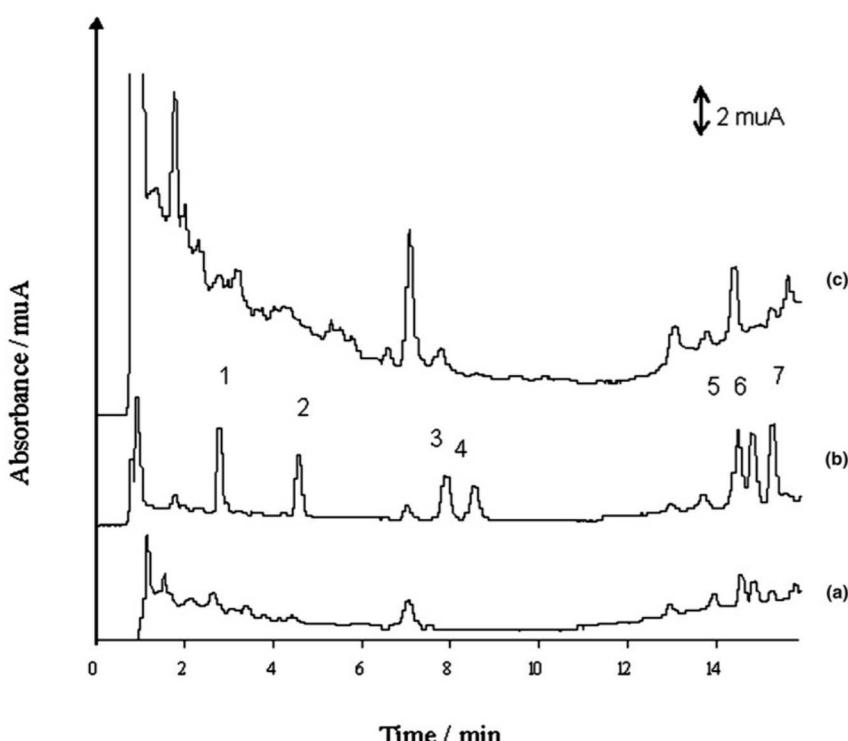


Figure 3 Chromatograms obtained in the analysis of soil samples. (a) Molecularly imprinted solid-phase extraction (MISPE) of a nonspiked sample; (b) MISPE of a sample spiked with 5 ng g^{-1} of each compound; (c) Direct injection of nonspiked sample extract. Peak identification: (1) Methylparaben, (2) Ethylparaben, (3) iso-Propylparaben, (4) Propylparaben, (5) iso-Butylparaben, (6) Butylparaben, and (7) Benzylparaben. *Reproduced from Nuñez, L.; Turiel, E.; Martín-Estebar, A.; Tadeo, J.L. Talanta 2010, 80, 1782, with permission from Elsevier.*

Aqueous samples can also be directly loaded onto MIP cartridges. However, in this case MIPs behave like a reverse-phase sorbent and thus both target analytes and matrix components are retained through nonspecific interactions. Then, a washing solvent able to remove matrix components and to redistribute nonspecifically bound analytes to the selective imprints is introduced. However, the success of such a procedure is not always achieved and thus efforts have been directed toward the synthesis of water-compatible MIPs by incorporating hydrophilic surface properties to the polymer in order to reduce nonspecific hydrophobic interactions. This goal can be mainly achieved by using polar porogens,^{39–41} hydrophilic comonomers (e.g., 2-hydroxyethyl methacrylate, acrylamide) or cross-linkers (e.g., pentaerythritoltriacylate, methylenebis(acrylamide)),^{31,42} and/or designed monomers capable of stoichiometrically interacting with the template functionalities.^{43,44} Such approaches have provided recognition of target analytes by MIPs in aqueous media to a certain extent. The contributions from the group of Moreno-Bondi are particularly noteworthy.^{43–45} Stoichiometrically imprinted polymers showing good target binding in aqueous media were prepared using penicillin G (PenG) procaine salt or enrofloxacin (ENR) as template molecules and a stoichiometric quantity of a recently developed urea-based functional monomer to target the single oxyanionic species in the template molecule. The optimized MISPE-HPLC procedures allowed direct extraction of β -lactam antibiotics on the PenG MIP and fluoroquinolones on the ENR MIP from river water samples with minor use of organic solvents. For instance, in the latter case the selective washing was achieved using acetonitrile/HEPES buffer, pH 7.5 (10:90, v/v) and elution with 2% trifluoroacetic acid in methanol. Good recoveries and precision were obtained for river water samples of all the antibiotics tested, resulting in limits of detection ranging from 0.01 to $0.30 \mu\text{g l}^{-1}$ when 100-ml water samples were processed.

2.17.3.1.2 Online Protocols

In this format, a small precolumn packed with the imprinted polymer (typically about 50 mg) is placed in the loop of a six-port injection valve. After loading the sample and washing out interfering compounds, the analytes are eluted by the mobile phase and then separated in the analytical column. Masqué et al.⁴⁶ described the first application of an online MISPE procedure coupled to HPLC for the selective extraction of 4-nitrophenol from a mixture of phenolic compounds in river water samples.

Despite the clear advantages of online protocols, fewer than 25 papers have been published on online MISPE studies. This low activity might be attributed to the usual lack of compatibility of the mobile phase required to perform the separation on the analytical column and the elution solvent necessary to desorb analytes from the MIP precolumn. This problem can be circumvented by directing the eluent from the MIP precolumn to a second injection loop and subsequently injected on the chromatographic system,⁴⁷ or by mixing the organic elution solvent with an aqueous-rich solvent before reaching the analytical column.⁴⁸

In addition, and in order to prevent a large amount of matrix compounds reaching the MIP precolumn, online two-step MISPE procedures have been proposed. In this regard, Mullett et al.⁴⁹ developed an online two-step SPE-LC-MS procedure for the determination of verapamil and its metabolites in urine, plasma, and cell cultures using a restricted access material (RAM) cartridge and a verapamil-imprinted polymer. In brief, the sample was loaded onto the RAM cartridge in which analytes were retained by the alkyl hydrophobic bonded phase, while hydrophilic electroneutral diol groups bound to the external surface of the spherical particles protected the sorbent from contamination by proteins. Then after this first cleanup, analytes were desorbed from the RAM precolumn using a mobile phase of pure acetonitrile, and transferred to the MIP precolumn. Finally, the analytes retained by the MIP were desorbed and transferred to the analytical column for chromatographic separation. Using this approach, verapamil and its metabolites were easily quantified by LC-MS in less than 35 min.

2.17.3.1.3 In-Line Protocols

Extraction, enrichment, separation, and determination of target analytes can be achieved in one single step by the direct coupling of a MIP column in line with the detection system, thanks to the high selectivity provided by MIPs. MISPE with direct in-line UV-detection was first described by Sellergren⁵⁰ for the determination of pentamidine in urine. After loading 100 ml of diluted urine sample, the column was washed with 100 ml of a buffer solution (pH 9) and pentamidine eluted in ~1.5 ml of buffer at pH 3. The selective elution performed allowed the successful detection of pentamidine free of coextractives by UV at 270 nm. However, since a large volume of aqueous samples was loaded, a high amount of matrix compounds were retained on MIP columns by hydrophobic interactions. Thus, it was necessary to use a large volume of washing solutions, leading to a quite long analysis time.

As mentioned above, in general the recognition capabilities of MIPs are enhanced in organic media. In this regard, Turiel et al.⁵¹ developed an analytical method for the determination of the fungicide thiabendazol (TBZ) in fruit sample extracts in acetonitrile. Organic sample extracts (50 µl) were injected onto a TBZ-imprinted polymer column using a 100% MeOH mobile phase. Then after 2.4 min, the mobile phase was switched to methanol/acetic acid (80:20, v/v) in 0.1 min, keeping these conditions constant for 5 min before returning to the initial conditions. Following this methodology, the high selectivity of the MIP column permitted the target analyte to be retained on the column while the interferences were rapidly eluted, and thus TBZ was unambiguously detected and quantified in less than 15 min.

MISPE with pulsed elution (MISPE-PE) represents an alternative in which a small volume of elution solvent, instead of a steep solvent-switch, is used.⁵² In this first work, theophylline in chloroform-diluted serum samples was injected onto a theophylline-imprinted polymer packed into a stainless-steel column using chloroform as mobile phase. Subsequently, after interfering compounds were washed out from the column, 20 µl methanol was injected, being theophylline eluted free of coextractives and determined directly spectrophotometrically at 270 nm. MISPE-PE has been subsequently improved by the application of successive 20-µl pulses of different solvents, a procedure known as MISPE with differential pulsed elution (MISPE-DPE),⁵³ in order to wash out remaining interfering compounds and retain the analyte fraction nonspecifically. Both MISPE-PE and MISPE-DPE allow analyte enrichment through injection of large volumes of sample and provide a high analytical sensitivity, thanks to the narrow band obtained by pulsed elution.

2.17.3.1.4 Improved Batch Protocols

As mentioned above, batch MISPE has been displaced by other SPE formats. However, batch MISPE has reemerged using magnetic MIP particles^{54–56} which, after extraction, can be separated from the media by a simple magnet, as shown in Figure 4, avoiding in this manner the tedious filtration and/or centrifugation steps.

The synthesis of magnetic MIPs, although involving several steps, is rather simple. First, after obtaining Fe₃O₄ particles they are treated with surface modifiers (ethylene glycol, oleic acid, and poly(vinyl alcohol)). Then the polymerization solution (template, monomers, initiator, and porogen), the modified Fe₃O₄ particles, and a dispersing medium (i.e., water) are adequately mixed and dispersed. Finally, the suspension is heated allowing polymerization taking place onto the surface of modified Fe₃O₄ particles. Once the magnetic MIP beads are obtained they can be used in batch procedures for the extraction and cleanup of target analytes from complex samples. Such a procedure has been successfully used for the extraction of triazines from soil, soybean, lettuce, and millet,⁵⁴ tetracycline antibiotics from egg and tissue,⁵⁵ and bisphenol A in environmental water and milk.⁵⁶

A different approach, so-called molecularly imprinted micro-SPE (MIMSPE), was recently proposed by Feng et al.⁵⁷ The MIMSPE device consists of the MIP particles enclosed within a polypropylene flat-sheet membrane envelope, and its preparation is schematically depicted in Figure 5. The membrane presents a porous structure with a pore size of 0.22 µm, making it ideal to hold submicrosphere MIP particles not suitable for MISPE since they cannot be held back by the typical frits used in SPE cartridges. Once an MIMSPE device is obtained, it is conditioned and placed in the sample. The device tumbles freely within the sample solution during extraction for a certain period of time under stirring. After extraction, the device is manually removed with the help of tweezers and placed in a vial for elution of analytes. This approach has been successfully applied to the selective extraction of phenolic compounds from tap, river, and sewage waters, with recoveries higher than 80.0% and relative standard deviations lower than 10% at 0.10 mg l⁻¹ concentration level.⁵⁷

2.17.3.2 Molecularly Imprinted Solid-Phase Microextraction (MI-SPME)

Solid-phase microextraction (SPME) was introduced by Arthur and Pawliszyn⁵⁸ in the early 1990s and is based on the partitioning of target analytes between the sample and the stationary phase, which is typically coating the surface of a fused silica fiber. Originally

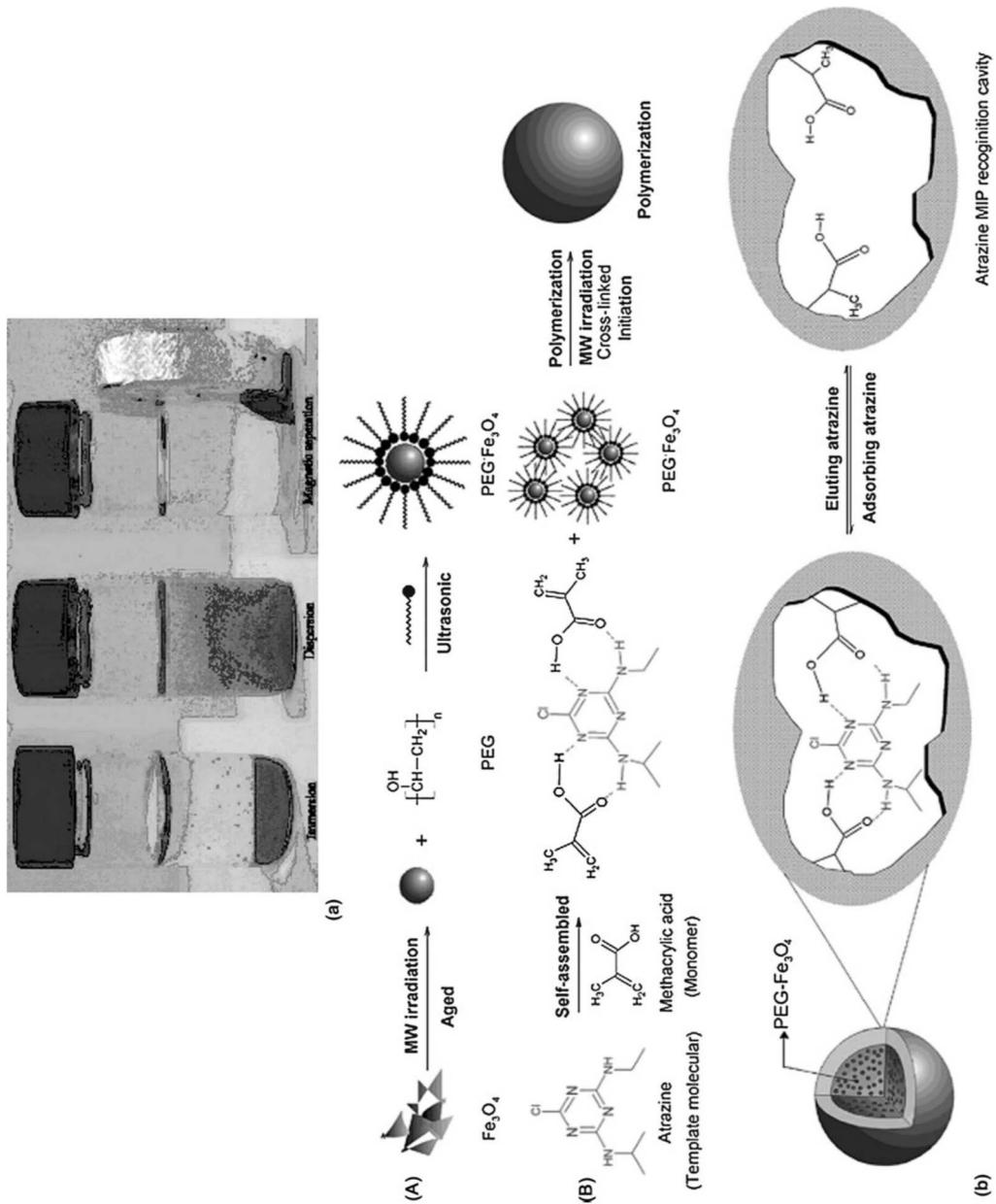


Figure 4 (a) Photographs of immersion, dispersion, and magnetic separation of magnetic molecularly imprinted polymer (MIP) beads. (b) Schematic representation of polyethylene glycol modification of iron oxide particles (A) and atrazine mag-MIP beads preparation (B). MW, microwave; PEG, polyethylene glycol; mag-MIP, magnetic MIP. Reproduced from Zhang, Y.; Liu, R.; Hu, Y.; Li, G. Anal. Chem. 2009, 81, 967, with permission from the American Chemical Society.

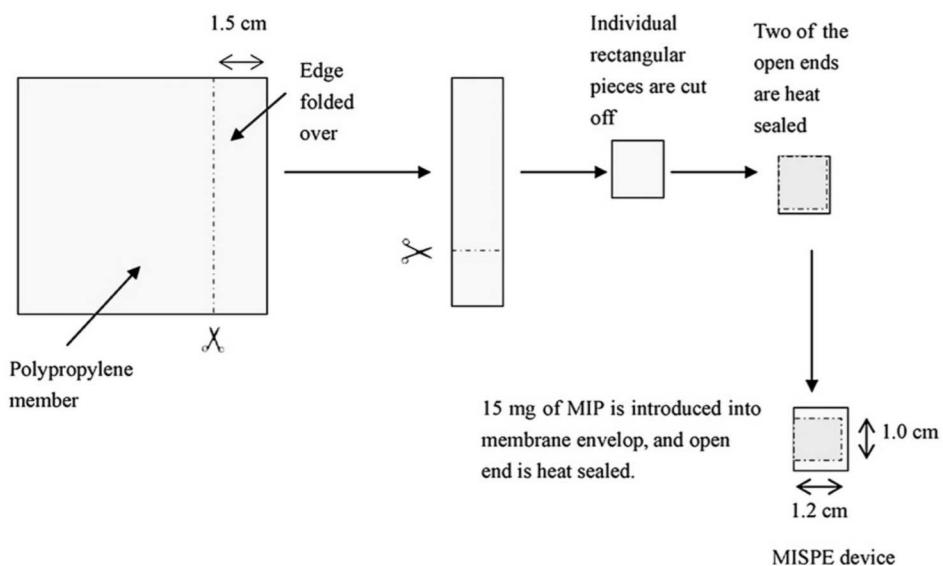


Figure 5 Preparation of a molecularly imprinted micro solid-phase extraction (MISPE) device. Reproduced from Feng, Q.; Zhao, L.; Lin, J.-M. *Anal. Chim. Acta* **2009**, 650, 70, with permission from Elsevier.

analytes were thermally desorbed directly onto the injection port of a gas chromatograph (nowadays it is still the typical procedure), although they can also be eluted using suitable solvents to be further analyzed by chromatographic techniques.⁵⁹ Its simplicity of operation, its solventless nature, the availability of commercial fibers, and the developments toward the automation of the whole process have led to SPME becoming a tool routinely used for certain applications, and its use in analytical laboratories is expected to continue growing in the future.⁶⁰

However, SPME has, in common with SPE, the lack of selectivity of the extraction process, since the commercially available fibers are based in nonselective sorbents to cover the scale of polarity. Thus, recent years have seen an increasing interest in the preparation of tailor-made fibers with the aim of providing certain selectivity to the extraction process. In this sense the combination of molecular imprinting and SPME would ideally provide a powerful analytical tool with the characteristics of both technologies (simplicity, flexibility, and selectivity).

The simplest way for combining both technologies, proposed by Mullet et al. in 2001, consisted in packing a capillary with the MIP particles for in-tube SPME; this format was successfully used for the selective determination of propranolol in serum samples.⁶¹ However, this methodology requires using some extra instrumentation (pump, multiport valves), and has other important drawbacks such as the lack of compatibility between the solvent needed to desorb analytes from the MIP and the mobile phase used (a typical drawback of online MISPE protocols). Thus, the preparation of silica fibers coated with a MIP to perform SPME could be considered a better alternative.

2.17.3.2.1 MIP-Coated Fibers

The suitability of this approach was nicely demonstrated by Koster et al. in 2001, reporting the preparation of the first MIP-coated silica fiber for the SPME of brombuterol from human urine.⁶² In this work, silica fibers were activated by silylation and subsequently immersed in a polymerization solution composed of clenbuterol, MAA, ethylene glycol dimethacrylate, and azobisisobutyronitrile in acetonitrile to carry out polymerization for 12 h at 4 °C under irradiation at 350 nm. According to the authors, fibers with a polymeric film thickness of ~75 µm were obtained in a reproducible manner and were successfully used for the selective extraction of brombuterol from urine samples. However, this format presented serious difficulties in controlling polymeric film thickness, since the desired graft polymerization is invariably accompanied by polymerization in solution when azo-initiators are used. Thus, the obtained fiber (with MIP coating on the surface) has to be pulled cautiously out of the solid polymer.

In this regard, Li's group further detailed the key factors influencing both the morphology and the width of the obtained MIP in a series of papers dealing with the preparation of different MIP-coated silica fibers.^{63–66} In all works, silica fibers were activated by silylation by immersing them into a solution of 3-(methacryloxy)propyltrimethoxysilane in acetone prior to being immersed in polymerization solution (polymerization took place at 60 °C). Although the procedure and further pulling out of the fiber are tricky, the obtained coatings resulted in being homogeneous and dense, and good reproducibility could be achieved by strict control of polymerization conditions. The polymerization time is a key aspect of this procedure, since it affects both coating thickness and fiber preparation reproducibility. Besides, it is crucial for successfully pulling of the fragile fiber out of the semisolid polymer. In general, a polymerization time of 6 h has been reported as optimum. Shorter time leads to bad uniformity and thin coating, and

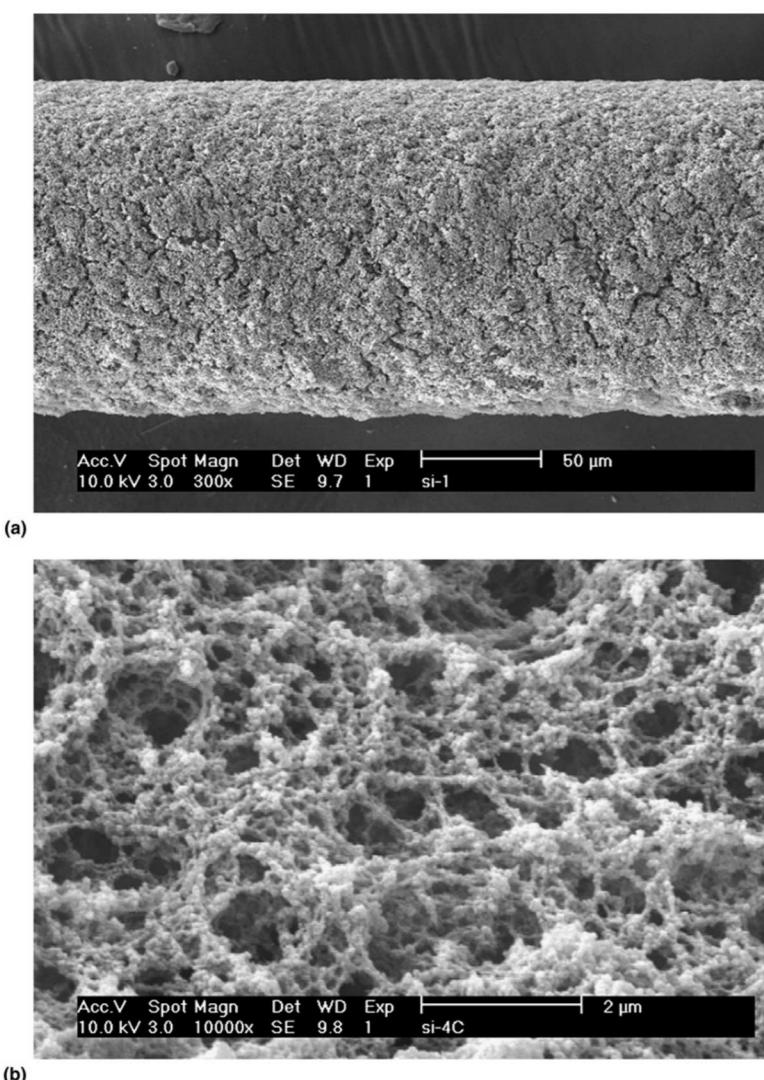


Figure 6 Scanning electron micrographs of tetracycline MIP-coated fiber at $\times 300$ (a) and $\times 10\,000$ (b) magnification. Preparation conditions of MIP-coated fiber: solvent: acetone; monomer: acrylamide; cross-linker: trimethylolpropane trimethacrylate; initiator: azo(bis)-isobutyronitrile; polymerization time: 6 h; coating times: 10. *Reprinted from Hu, X.; Pan, J.; Hu, Y.; Huo, Y.; Li, G.J. J. Chromatogr. A 2008, 1188, 97, with permission from Elsevier.*

longer time produces a too solid polymer, preventing the safe pulling out of the fiber. Moreover, a proper selection of the porogen is crucial, and it has been demonstrated that apolar solvents (i.e., toluene) lead to a homogeneous MIP coating with thickness of 25.0 μm (relative standard deviation (RSD) 2.9%),⁶⁴ whereas a thickness of 1.5 μm was achieved using acetone.⁶⁵ This problem can be circumvented by immersing the obtained fibers again in fresh polymerization solutions, resulting in fibers repeatedly being coated by identical polymerization procedures until the desired thickness is reached. In this manner, after ten polymerization cycles, the coating thickness reached a value of 19.5 μm with an RSD of 6.7%. The scanning electron micrographics of this tetracycline MIP-coated fiber are shown in Figure 6. It is evident that not only a homogeneous and dense morphological structure was achieved (Figure 6(a)) but also a highly cross-linked and porous structure was obtained (Figure 6(b)), which is vital to guarantee sufficient extraction-desorption kinetics in the MIP fiber.⁶⁵

Alternatively, organic-inorganic hybrid sol-gel imprinted coated fibers have also been proposed in the literature for the SPME of ascorbic acid⁶⁷ and polybrominated diphenyl esters (PDEs).⁶⁸ Theoretically these devices should provide improved adsorption-desorption kinetics (although it has not been fully demonstrated) and a much better thermal stability, which is quite important when analytes are directly desorbed at a gas chromatography injection port. In this regard, PDEs extracted by hybrid inorganic-organic sol-gel MIP-coated fibers were thermally desorbed at 270 °C without observing losses in fiber performance after repeated extraction-desorption cycles.

2.17.3.2.2 MIP Fibers (Monoliths)

A completely different and much simpler approach has been proposed independently by the groups of Martín-Esteban⁶⁹ and Djordan⁷⁰ for the preparation of MIP fibers inside fused silica capillaries. In this method, capillaries are cut to approximately 30-cm long pieces and four windows of about 1.0 cm are prepared by burning the protecting polymer layer. Then the capillary is filled with the polymerization mixture and both capillary ends are closed with two small pieces of rubber. The filled capillaries are put into an oven and polymerization takes place typically at temperatures higher than 60 °C for a certain period of time. Finally, capillaries are cut and immersed in an aqueous solution of NH₄HF₂ under agitation, with silica walls being etched away. In this manner, MIP monoliths of 1 cm length are obtained, where its thickness is dependent on the inner diameter of the silica capillary used. Moreover, as can be also observed in Figure 7(a), the obtained fibers were flexible and it was possible to bend them to a certain extent, preventing the easy breakage traditionally associated with the coated fused silica fibers. Similarly to MIP-coated fibers, solvent, cross-linker, and polymerization time have a direct influence on the porosity, and thus in the final analytical performance, of the obtained fibers.

The obtained fibers have been successfully applied to the SPME of target analytes in different kinds of samples and show a good thermal stability, making them suitable for SPME–gas chromatography. In this regard, Djordan's group have reported detailed studies on thermal stability of monolithic fibers, concluding that they are stable up to 280–300 °C, keeping its performance unaltered.^{70–72}

2.17.3.3 Other Formats

2.17.3.3.1 MSPD

MSPD, introduced by Barker et al. in 1989,⁷³ is based on the complete disruption of the sample (liquid, viscous, semisolid, or solid), allowing sample components to disperse into the solid sorbent. The sample is placed in a glass mortar and blended with the sorbent until a complete disruption and dispersion of the sample on the sorbent is obtained. Then the mixture is directly packed into an empty SPE cartridge. Finally, analytes are eluted after a proper washing step to remove interfering compounds. The main

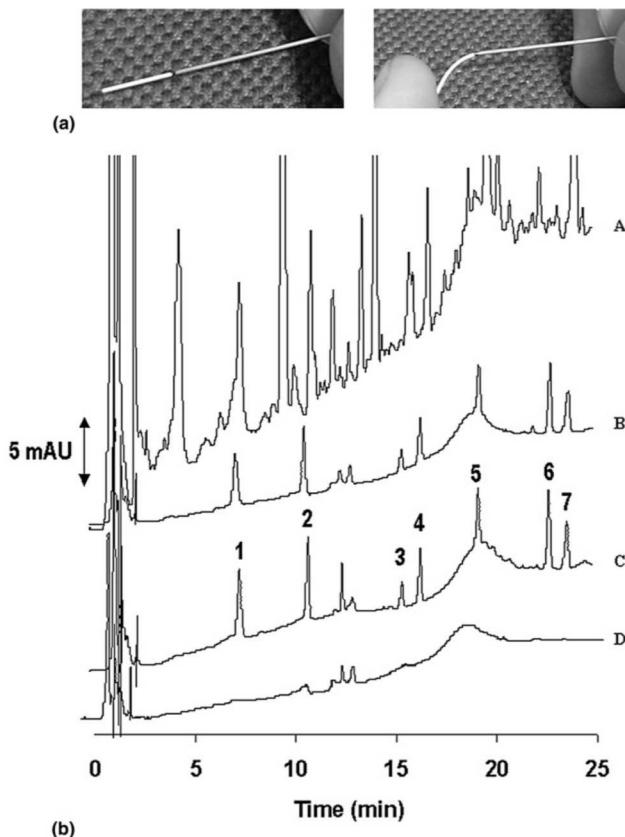


Figure 7 (a) Flexible MIP fiber (monolith); (b) liquid chromatography ultraviolet chromatograms obtained at 220 nm for (A) a soil sample extract directly injected without any previous cleanup, (B) a soil sample extract enriched with triazines at 0.1 mg L⁻¹ concentration level after molecularly imprinted solid-phase microextraction (MI-SPME), (C) a 0.1 mg L⁻¹ standard solution of triazines after MI-SPME, and (D) a nonspiked soil sample extract after MI-SPME. Peak numbers: (1) desisopropylatrazine; (2) desethylatrazine; (3) simazine; (4) cyanazine; (5) atrazine; (6) propazine; (7) terbutylazine. *Reprinted from Turiel, E.; Tadeo, J.L.; Martín-Esteban, A. Anal. Chem. 2007, 79, 3099, with permission from the American Chemical Society.*

difference between MSPD and SPE is that the sample is dispersed through the column instead of only onto the first layers of sorbent, which typically allows for clean final extracts and avoids the necessity of performing a further cleanup.

As mentioned above, MISPE has been successfully applied to the cleanup of complex biological samples such as hair and tissue. However, due to the complexity of these samples, a previous cleanup step using a typical nonselective sorbent might be required. To simplify the whole procedure, Crescenzi et al.⁷⁴ employed MSPD for the extraction of clenbuterol from bovine liver, and further isolation and cleanup using a bromoclenbuterol-imprinted polymer cartridge. First, liver sample is mixed and homogenized with a C₁₈ sorbent, and the mixture packed onto an empty cartridge and placed on top of the MIP cartridge. Subsequently, clenbuterol is eluted from the MSPD cartridge onto the MIP cartridge using acetonitrile containing 1% acetic acid. Under these conditions MIP is able to retain target analyte and, after a washing step with the same solvent, clenbuterol is eluted with acidified methanol for further LC analysis. The complete extraction procedure is rapid and simple, and provided quantitative recoveries allowing a detection limit below 0.1 µg kg⁻¹ to be reached.

The direct use of MIPs as dispersant sorbents is not straightforward, due to the high water content of the samples typically extracted by MSPD. As mentioned above, selective recognition by MIPs is not favored in aqueous samples. However, the use of water-compatible MIPs in MSPD has been demonstrated for the selective extraction and cleanup of fluoroquinolones from eggs and tissue,⁴⁰ and serum samples.⁴¹ The average recoveries obtained ranged from 72.2 to 114.1%, depending upon the analyte and the sample, with RSDs of less than 7.0%, demonstrating clearly the superior performance of MIPs compared with that achieved using conventional sorbents (C₁₈ and florisil), where recoveries ranging from 24.9 to 84.6% were obtained.

2.17.3.3.2 SBSE

SBSE is based on the partitioning of target analytes between a liquid sample and a stationary phase-coated stir bar.⁷⁵ Until now only polydimethylsiloxane (PDMS)-coated stir bars have been commercially available, restricting the range of applications to the extraction of hydrophobic compounds (organochlorine and organophosphorus pesticides) due to the apolar character of PDMS. In general, the yield of the extraction process is much greater when using a stir bar rather than an SPME fiber, both coated with PDMS, due to the larger volume of the extraction phase used. However, the greater coating area of magnetic stir bars is simultaneously its main drawback, since the extraction kinetics are slower than for SPME fibers and a high amount of interfering matrix compounds are coextracted with target analytes.

Keeping these comments in mind, it is obvious that the preparation and use of MIP-coated stir bars would extend the applicability of SBSE in sample preparation. In this regard, Zhu et al. have developed stir bars coated with a MIP consisting of a film formed from a formic acid solution of nylon-6 polymer, imprinted with monocrotophos to extract successfully and selectively the organophosphorus pesticides mevinphos, omamatoate, monocrotophos, and phosphamidon from dichloromethane solution for the analysis of environmental soil samples,⁷⁶ and with L-glutamine to extract this amino acid.⁷⁷ Moreover, the MIP-coated stir bars showed not only the expected high selectivity but also rapid equilibrium adsorption, thanks to the porous nature of the imprinted polymer obtained combined with a proper thickness of the coated polymer film (~160–180 µm). More recently, Li's group described the preparation and use of MIP-coated stir bars for the determination of β₂-agonists⁷⁸ and triazines,⁷⁹ following a similar procedure to that described above for the preparation of MIP-coated fibers for SPME, which seems to be a more generally applicable procedure. Further research is expected in this area in the near future.

2.17.3.3.3 Liquid Membranes and MIPs Combination

As mentioned above, although in general MIPs provide a high degree of selectivity, in some cases the complexity of the samples under study leads to the coextraction and coelution of some matrix components. Moreover, it has been reported that some matrix compounds are in certain cases strongly bound to the polymeric matrix, resulting in a loss of the recognition capabilities of imprinted polymers, thus leading to a decrease in the recoveries after few runs.³⁸ The reported use recently of supported liquid membranes might circumvent such drawbacks.

The combination of liquid membranes and MIPs was reported for the first time by Chimuka's group for the extraction of 17β-estradiol from aqueous samples⁸⁰ and triazines from lettuce and apple extracts.⁸¹ In both works, membrane liquid–liquid extraction (MLLE) combined with MISPE is carried out in a specially designed stainless-steel extraction device. The extraction unit is composed of two compartments separated by a membrane. Membranes are firstly equilibrated with a water-immiscible organic solvent, which is also the acceptor solution. Preparation of the unit involves initial loading of the aqueous sample in the lower compartment, with the membrane subsequently positioned at the compartment interface and sealed in place by the upper compartment. Finally, the upper compartment is filled with the organic acceptor phase, followed by a small amount of MIP particles (30–50 mg), after which the unit is closed. Target analytes are extracted from the aqueous donor phase into the organic acceptor phase. In the organic phase, analytes are bound to an imprinted solid support held in the phase by the membrane. After a fixed extraction time (60–90 min), the MIP particles are separated from the organic phase by filtration through a syringe filter. Nonspecifically bound analytes are removed by washing with additional acceptor phase, and specifically bound compounds released from the particles by passing a suitable elution solvent. Following this approach, enrichment factors ranging from 6 to 40 are achieved, depending on the analyte and the sample analyzed, with an impressive degree of selectivity even with complex samples such as wastewater or urine. It is expected that there will be further developments in this area in the near future, particularly toward the reduction of the long extraction times.

Recently, a novel liquid–liquid–solid microextraction (LLSME) technique based on porous membrane-protected MIP-coated silica fiber has been developed.⁸² In this seminal work, a terbutylazine-imprinted polymer-coated silica fiber⁶⁴ was protected with

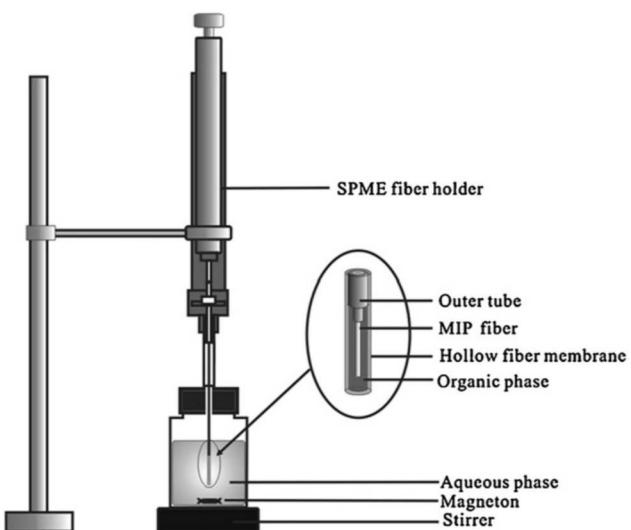


Figure 8 Schematic representation of the liquid–liquid–solid microextraction device. SPME, solid-phase microextraction. *Reprinted from Hu, Y.; Wang, Y.; Hu, Y.; Li, G. J. Chromatogr. A, 2009, 1216, 8304, with permission from Elsevier.*

a length of porous polypropylene hollow fiber membrane, which was filled with water-immiscible organic phase. Subsequently the whole device, schematically depicted in Figure 8, was immersed into aqueous sample for extraction of triazines as model compounds. The target analytes were firstly extracted from the aqueous sample through a few microliters of organic phase residing in the pores and lumen of the membrane, and were finally extracted onto the MIP fiber. This procedure was applied in the analysis of triazines in sludge water, watermelon, milk, and urine samples, reaching low detection limits ($0.006\text{--}0.02 \mu\text{g l}^{-1}$), satisfactory recoveries (71.1–108.7%), and good repeatability (RSD 1.2–9.6%, $n = 5$). LLSME technique performance was compared to that of molecularly imprinted polymer based solid-phase microextraction (MIP-SPME) and hollow fiber membrane-based liquid-phase microextraction (HF-LPME), respectively. From this comparison, it was concluded that the LLSME integrates the advantages of high selectivity of MIP-SPME and enrichment and sample cleanup capability of the HF-LPME into a single device, making it a promising sample preparation method for complex samples.

2.17.4 Outlook and Expected Future Trends

MIPs programmed to recognize a specific analyte or a group of analytes are highly compatible with current sample pretreatment procedures. In contrast to generic SPE sorbents, these dedicated sorbents are capable of cleaning up complex samples to unprecedented levels from small sample volumes. Overall this translates into fast and robust analytical methods with unprecedently low detection limits, satisfying regulatory prescriptions. Although today's advanced high-resolution MS detectors can sometimes provide sufficient sensitivities with minimal sample pretreatment, the robustness gained with MISPE and the clean extracts obviate the need for these still expensive detectors. Moreover, the recent developments achieved in the synthesis of imprinted fibers for SPME as well as the incorporation of MIPs into other extraction techniques such as MSPD or SBSE also highlight the adaptability of MIPs to almost any extraction technique. In this regard, a refinement of its use in such techniques, its combination with others (i.e., liquid membranes), and the development of micro-MISPE devices will bring new selective and simple analytical methods in the near future. Allowing interdisciplinary research to further refine these molecular recognition elements may pave the way for MISPE as one of the breakthrough technologies in twenty-first century separation science.

See also: Principles and Practice of Solid-Phase Extraction; Microwave Extraction; Solid-Phase Microextraction; Recent Advances in Solid-Phase Microextraction for Environmental Applications; Recent Advances in Solid-Phase Microextraction for Forensic Applications; Matrix Solid-Phase Dispersion

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Relevant Websites

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Direct Drug Determination by Selective Sample Enrichment on an Imprinted Polymer

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A new approach to sample enrichment and analyte determination is reported. An imprinted dispersion polymer capable of molecular recognition of pentamidine (PAM), a drug used for the treatment of AIDS-related pneumonia, was used in solid-phase extraction in order to selectively retain PAM from a dilute solution. At a physiological concentration (30 nM) this gave an enrichment factor of 54 using a PAM-selective polymer whereas the enrichment factor on a benzamidine- (BAM-) imprinted reference polymer was only 14. The high selectivity of the polymer allowed the drug to be detected directly in the desorption step, thus eliminating the need for a successive chromatographic analysis. In this way, PAM could be enriched and directly analyzed when present in low concentration in a urine sample.

In the medical and environmental fields, the analysis of target molecules in complex mixtures often requires pre-treatment steps.¹ First, if the analyte is present in low concentration, it needs to be concentrated in order to be detected by standard analytical techniques. Second, if it is present in a complex mixture of similar compounds, a cleanup step is required. Solid-phase extraction (SPE) where the analyte is sorbed onto a solid phase has become an important sample preparation technique.² Compared to liquid/liquid extraction (LLE), it offers convenience with respect to automation and field sampling as well as safety due to the low solvent consumption. In the most common technique, uncharged analytes are adsorbed on a hydrophobic sorbent. High enrichments as well as efficient sample cleanup can thus be obtained in one step. Enrichment and cleanup of hydrophilic analytes is usually more difficult to obtain, leading to disturbances in the subsequent chromatographic analysis. In affinity chromatography, immobilized biomolecules exhibiting high affinity and specificity toward their substrates have been used for this purpose. However, these phases are often associated with a complicated preparation scheme, poor stability, and poor reproducibility. Sorbents that can be tailor-made to bind an analyte would in these instances find potential applications.

Molecular imprinting⁴⁻⁸ has been used to prepare materials exhibiting antibody-like affinity and selectivity toward an

analyte.^{7,8} The technique consists of three key steps: (1) copolymerization of functional monomers, preorganized around a template molecule, with a cross-linking monomer; (2) displacement of the template from the resulting network polymer, leaving behind binding sites complementary to the template; (3) investigation of the materials in a batch or chromatographic mode for molecular recognition of the template.

Materials have thus been prepared showing pronounced affinity for enantiomers of basic compounds,^{5,6} for nucleotide bases,⁷ and for commercial drugs.^{5e,8} Recently, we reported on imprinted dispersion polymers as new easily accessible affinity stationary phases for chromatography.⁹ Of particular interest was the material with affinity for the antiprotozoal drug pentamidine (PAM), which has recently been found effective towards a number of AIDS-related disorders.¹⁰ Due to the adverse side effects, there is a need for reliable methods for quantitation of the drug in biofluids.

In this report a PAM-selective imprinted dispersion polymer is used for selective enrichment and analysis of PAM present in low concentrations.^{9a,11} It was anticipated that the high selectivity of the polymer would allow the analyte to be quantified directly in the desorption step, making subsequent analytical procedures unnecessary.

EXPERIMENTAL SECTION

Preparation of Imprinted Sorbent (Scheme 1). The polymers were prepared by following a modified version of an earlier described procedure.⁶ Pentamidine (PAM; 0.125 mmol) or benzamidine (BAM; 0.25 mmol, Aldrich) in the

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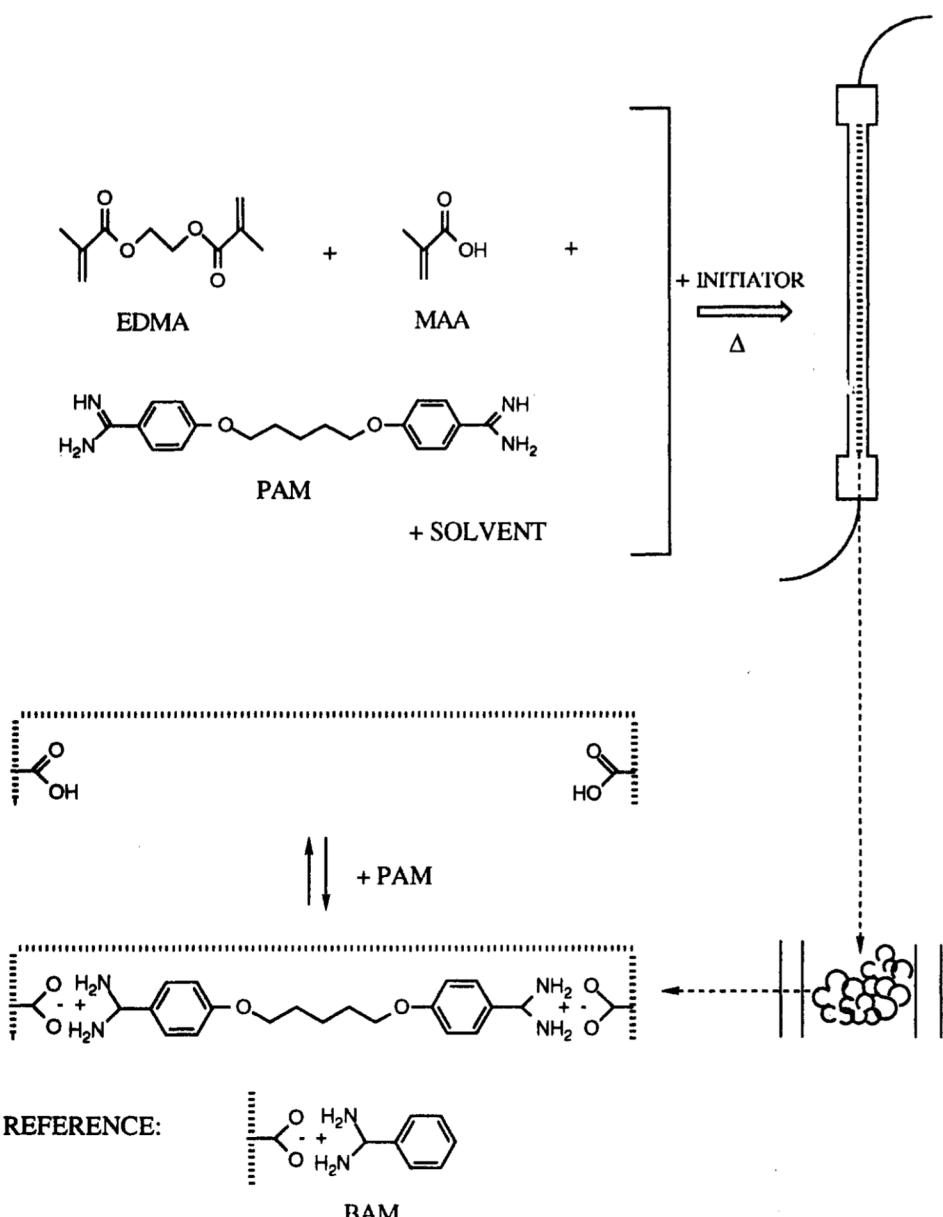
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Scheme 1



free base form¹² was dissolved in 2-propanol (2.8 mL) and ethylene glycol dimethacrylate (EDMA; 12 mmol). Addition of methacrylic acid (MAA; 0.5 mmol) caused formation of a precipitate which dissolved upon addition of water (1.3 mL). The initiator azobis(isobutyronitrile) (12 mg) in 2-propanol (0.5 mL) was added and the solution purged with nitrogen and heated to 40 °C for homogenization. The solution was then transferred under nitrogen to glass tubes (length 150 mm, o.d. = 5 mm, i.d. = 3 mm); the tubes were sealed and left in an oven at 60 °C for 24 h, giving the PAM-imprinted (PPAM) and BAM-imprinted (PBAM) polymers.

Chromatographic Evaluation of Imprinted Sorbents. The glass tubes were cut and equipped with column end fittings.

(12) Pentamidine isethionate (Rhône Poulenc Pharma, Helsingborg, Sweden) was converted to the free base by basifying an aqueous solution (K_2CO_3) of the drug and collecting the formed precipitate. Purification was done by redissolving the precipitate in EtOH, filtration of the EtOH solution, and finally evaporation giving PAM as a white solid.

They were then connected to a simple HPLC equipment and 50 mL of MeCN/potassium phosphate (KP) buffer (0.05 M, pH 2, 7/3 v/v) was passed at 5 mL/min. Due to some compression of the column bed, the inlet end fitting was removed and the tube cut to a length of 100 mm. After reconnecting the column, MeCN/KP buffer (0.05 M, pH 5, 7/3 v/v) (pH 5 mobile phase) was passed at a flow rate of 0.3 mL/min until a stable base line was attained. PAM or BAM (2 nmol) in 20 μ L of mobile phase was injected and the retention time (t) measured to the peak maxima of the UV absorption at 270 and 240 nm, respectively. The time for the void volume (t_0) to pass was measured by injecting 20 μ L of MeCN/H₂O (7/3 v/v). The capacity factors (k') were calculated as $k' = (t - t_0) / t_0$.

Enrichment and Analysis of PAM. (a) **Sample Sorption.** The columns containing PPAM and PBAM were equilibrated in pH 5 or pH 7 mobile phase at a flow rate of 2 mL/min for 30 min. Then a solution (100 mL) of PAM or BAM (10, 30,

or 60 nM) in the mobile phase was passed through at a flow rate of 2 mL/min.

(b) Sample Desorption. A pH 2 mobile phase was used as eluent. This entered at the column inlet at a flow rate of 0.1 mL/min, monitoring UV absorption at 270 (PAM) or 240 nm (BAM). Fractions (1 mL) were collected at the detector outlet and the UV spectra recorded. The amount of PAM or BAM in the eluate was determined from the peak area by reading off the corresponding concentration from a standard curve of injected standards (1–10 nmol).

Urine Sample Determinations. In the urine sample determination, fresh urine (30 mL) was diluted with KP buffer (0.1 M, 30 mL) after which the pH was set to 5. Half of this solution (30 mL) was spiked with PAM. The spiked and the blank solutions were then diluted with 70 mL of MeCN. The final concentration of PAM in the spiked solution was 60 nM.

(a) Sample Sorption. After equilibration at pH 5 of the column packed with PPAM, 100 mL of the spiked or the blank solution was passed at a flow rate of 2 mL/min. The column was then washed by passing 100 mL of a pH 9 mobile phase at a flow rate of 2 mL/min followed by 2.5 mL of a pH 5 mobile phase at 1 mL/min.

(b) Sample Desorption. Desorption was then carried out by passing a pH 3 mobile phase at 0.1 mL/min and the amount of PAM determined as described above after subtraction of the blank absorption.

RESULTS AND DISCUSSION

In the chromatographic mode, the retention of PAM on the sorbents varied strongly with the mobile-phase pH.⁹ While no retention was observed at pH 2, PAM was 7 times more retained on PPAM ($k' = 16$) than on PBAM ($k' = 2.3$) at pH 5. At pH 7, however, PAM was strongly retained on both columns and no peaks were detected. The retention of BAM depended less on pH and was similar on both columns. Thus, k' increased from 0 at pH 2 to 0.3 at pH 5 and 2.9 at pH 7. As observed and theoretically analyzed in the resolution of D,L-phenylalanine anilide on a L-phenylalanine anilide imprinted material,¹³ a cation exchange retention mechanism is operating. Both retention and selectivity can thus be controlled in a predictable manner by adjusting the pH in the mobile phase. The pH control is ideally suited for use in sample enrichment.

A dilute solution of PAM at pH 5 was passed through the columns containing the sorbents PPAM and PBAM. Desorbing with a pH 2 mobile phase gave the elution curves seen in Figure 1. The observed peak was assigned to PAM after a comparison of the UV spectrum of the collected fractions with that of PAM. In a control experiment, a solution lacking PAM was passed through the column. Upon desorption, no peak was produced, which shows that the eluted PAM is not the result of leaking residual template. In a second control experiment, no enrichment was observed when a dilute solution of the reference BAM was passed.

As seen in Figure 1, the eluted peak from PPAM is clearly larger than that from PBAM, showing that selective enrichment of PAM can be performed using the PPAM column. The amount of eluted PAM, obtained from a standard curve, was divided by the volume within which the peak eluted. This gave the concentration of desorbed PAM ($[PAM]_{out}$), the

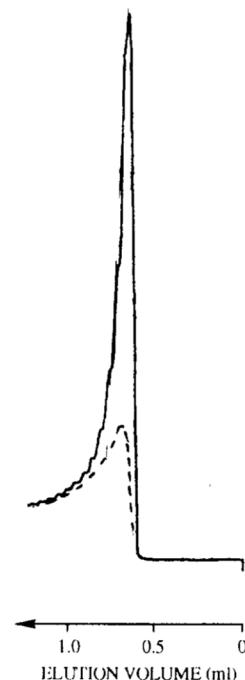


Figure 1. Desorption of PAM with a pH 2 mobile phase from PPAM (solid line) and PBAM (dashed line) after passage of 100 mL of a 30 nM PAM solution at pH 5. Flow rate 0.1 mL/min. UV detection at 270 nm. Mobile phase MeCN/KP buffer (0.05 M, pH X, 7/3 (v/v)).

Table 1. Sample Enrichment of PAM on a PAM- (PPAM-) and a BAM- (PBAM-) Imprinted Dispersion Polymer Used as Sorbent in Solid-Phase Extraction

sorbent ^a	pH _{in} ^b	[PAM] _{out} ^c (nM)	EF ^d	rec ^e (%)	SF ^f
PPAM	5	1615	54	10.7	4.0
		410	14	2.7	
PBAM	7	13350	445	89	1.5
		9050	300	60	

^a Glass columns (length 100 mm, o.d. = 5 mm, i.d. = 3 mm) containing *in situ* prepared imprinted dispersion polymers PPAM and PBAM as sorbents. ^b pH in the sample solution. A 100-mL aliquot of a dilute solution of PAM ([PAM]_{in} = 30 nM) in MeCN/KP buffer (0.05 M, pH 5 or 7, 7/3 v/v) was passed at a flow rate of 2 mL/min. ^c Concentration of desorbed PAM in the minimum volume containing the peak (0.2 mL). Desorption was done by passing a pH 2 mobile phase at 0.1 mL/min with UV detection at 270 nm. The amount of desorbed PAM was then read off from a standard curve. The results are averages of two independent experiments with errors of less than 6%. ^d Enrichment factor, [PAM]_{out}/[PAM]_{in}. ^e Recovery, [amount of desorbed PAM]/[amount of PAM in sample]. ^f Selectivity factor, [EF on PPAM]/[EF on PBAM].

associated enrichment factor (EF), and the selectivity factor (SF) (Table 1). Both the enrichment and the selectivity varied with the sample pH. At pH 5, the enrichment factor of PAM on PPAM was at least 4 times higher than on PBAM although the recovery of PAM was rather poor. At pH 7 on the other hand, large enrichment factors were obtained on both columns, resulting in a lower selectivity while the recovery was nearly quantitative. These results reflect the chromatographic behavior of the phases. The selectivity decreases with increasing pH in the mobile phase. An explanation was proposed suggesting that the selective sites, are more acidic than the existing excess of nonselective sites, leading to an increase in the nonselective binding with increasing pH.¹³ Apparently when one is choosing the sample pH a compromise

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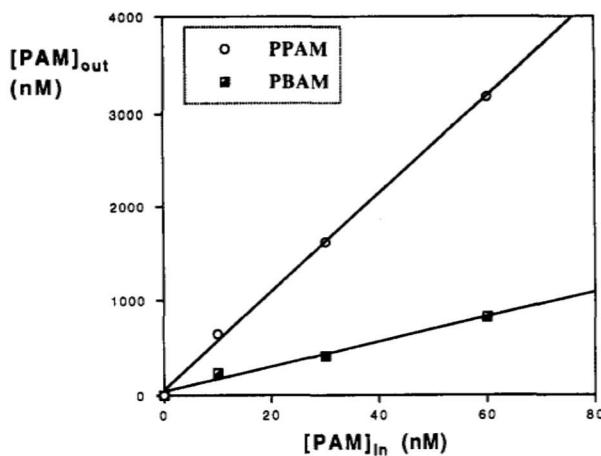


Figure 2. Concentration of desorbed PAM ($[PAM]_{out}$) versus concentration of PAM in the sample solution ($[PAM]_{in}$) passed through the columns containing PPAM or PBAM as sorbent. Conditions otherwise as described in Figure 1 and Table 1.

has to be made between enrichment and recovery on one hand and selectivity on the other.

A plausible explanation for the poor recovery at pH 5 is that the breakthrough volume for the analyte has been exceeded. This is not due to sample overload since the number of sites (maximum value 50 $\mu\text{mol/g}$) by far exceeds the maximum amount of sample passed through the columns (maximum value 6 nmol). Rather it is the limited retention of PAM at pH 5 that causes the breakthrough volume to be low. The entire sorbent is thus in equilibrium with the sample solution. In such cases the enrichment factor can be expressed as¹⁴

$$EF = (1 + k')V_0/V_{out} \quad (1)$$

where V_0 is the void volume of the sorbent and V_{out} is the desorption volume of the analyte. With a $V_0 = 0.54$ mL and a $V_{out} = 0.2$ mL, the enrichment factor of PAM on PPAM at pH 5 can thus be estimated as $EF = (1 + 16)0.54/0.2 = 46$. This value agrees well with the experimentally obtained enrichment in Table 1, which indicates that the chromatographic data can be used for predictions of sample enrichment factors. Moreover this suggests that the amount of sorbent as well as the volume of sample solution can be reduced while the same level of enrichment is maintained. Note that a lower sample volume is likely to result in an increase in the recovery.² The whole system therefore seems suited for miniaturization.

For routine use in drug analysis it is important that the enrichment factor is constant over a range of sample concentrations close to those found in biological samples. As seen in Figure 2 the concentration of PAM in the eluate increased linearly with the sample concentration, indicating that the adsorption isotherm is linear in the corresponding concentration interval. In addition to the high selectivity observed, this suggests that a simple analytical procedure can be followed that obviates the need for subsequent chromatographic analysis. This was tested by passing a spiked or a blank urine sample through the PPAM column at pH 5 followed by recording of the UV elution profile upon desorption. After passage of the sample solution, the column

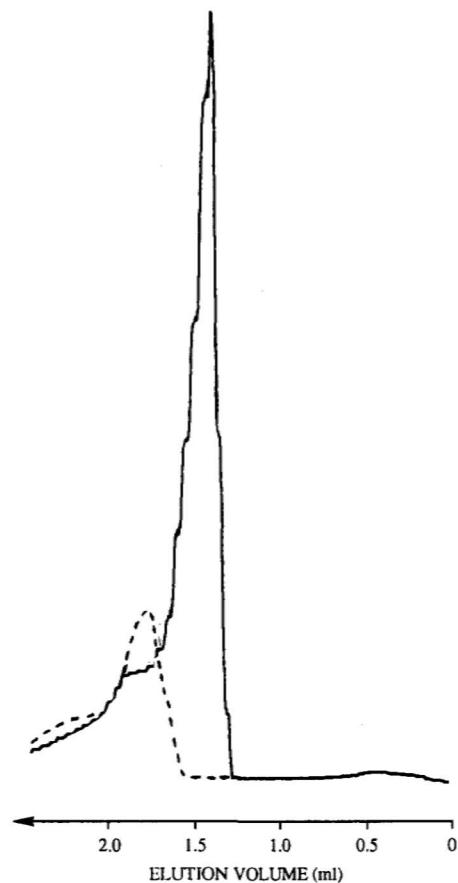


Figure 3. Desorption of PAM from PPAM with a pH 3 mobile phase after having passed 100 mL of a spiked ($[PAM]_{in} = 60$ nM, solid line) or a blank (dashed line) urine sample solution at 2 mL/min. The urine solution contained 30 mL of a urine/KP buffer solution (1/1 v/v) adjusted to pH 5 and mixed with 70 mL of MeCN. Desorption flow rate 0.1 mL/min. UV detection at 270 nm. $[PAM]_{out} = 1722 (\pm 30)$ nM. EF = 29 (± 0.6). Recovery, 10.2% (± 0.2 %). The results are averages of two independent experiments.

was extensively washed at pH 9 in order to remove sorbed basic compounds with pK_a values below 9. Desorption was then carried out at pH 3 instead of pH 2 in order to resolve the drug from sorbed impurities. When PAM was present in the urine sample solution, a large peak appeared in the elution profile (Figure 3). The peak identity was confirmed as described above, and the calculated enrichment and recovery agreed roughly with the results reported in Table 1. Although the sample concentration is higher than the normal physiological concentrations, it is apparent from Figure 3 that lower concentrations can be determined with this technique. MS or diode array detectors would simplify the verification of peak identity.

The presented analytical scheme can thus in favorable cases enrich and cleanup a sample to a level that allows direct analyte determination upon desorption. Solid-phase extraction on imprinted materials may therefore make final chromatographic or immunological steps unnecessary. In the determination of PAM this appears to be an attractive alternative to the LLE techniques presently employed.¹⁵ Finally, in view of the increasing number of highly selective imprinted phases,^{7,8}

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the technique may offer a solution to analytical problems dealing with the determination of adducts or metabolites of close structural similarity.

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