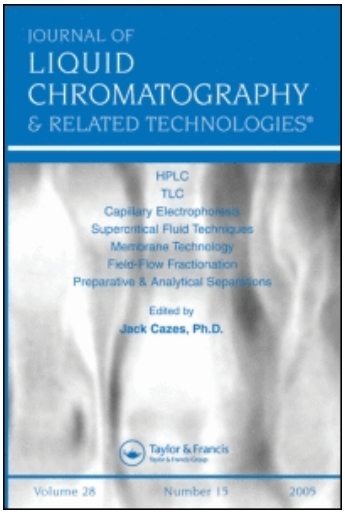

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Affinity Separations on Molecularly Imprinted Polymers with Special Emphasis on Solid-Phase Extraction

Valérie Pichon[a]; Karsten Haupt[b]
[a] Laboratoire Environnement et Chimie Analytique, Ecole Supérieure de Physique et de Chimie Industrielles, Paris, France [b] Université de Technologie de Compiègne, Génie Enzymatique et Cellulaire, Compiègne, France

To cite this Article Pichon, Valérie and Haupt, Karsten(2006) 'Affinity Separations on Molecularly Imprinted Polymers with Special Emphasis on Solid-Phase Extraction', Journal of Liquid Chromatography & Related Technologies, 29: 7, 989 — 1023
To link to this Article: DOI: 10.1080/10826070600574739
URL: http://dx.doi.org/10.1080/10826070600574739

Journal of Liquid Chromatography & Related Technologies®, 29: 989–1023, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600574739

Affinity Separations on Molecularly Imprinted Polymers with Special Emphasis on Solid-Phase Extraction

Valérie Pichon
Laboratoire Environnement et Chimie Analytique, Ecole Supérieure de Physique et de Chimie Industrielles, Paris, France

Karsten Haupt
Université de Technologie de Compiègne, Génie Enzymatique et Cellulaire, Compiègne, France

Abstract: Molecularly imprinted polymers (MIPs) are tailor-made synthetic materials possessing specific cavities designed for a target molecule. They may constitute stationary phases for separation techniques that involve a retention mechanism based on molecular recognition. The physical and chemical properties of MIPs directly result from the procedure used for their synthesis, which is normally adapted to the application in question. This review describes the potential of MIPs in affinity chromatography, mainly for chiral separation using different techniques such as liquid chromatography or capillary electrochromatography. The great potential of MIPs as selective sorbents for solid-phase extraction of target analytes from complex matrices is particularly highlighted.

Keywords: Molecularly imprinted polymers, Affinity separation, Solid-phase extraction, Selectivity

INTRODUCTION

The technique of molecular imprinting allows for the formation of specific recognition sites in synthetic polymers through the use of templates or imprint

Address correspondence to Valérie Pichon, Laboratoire Environnement et Chimie Analytique (UMR CNRS 7121), Ecole Supérieure de Physique et de Chimie Industrielles, 10, rue Vauquelin, 75231 Paris Cedex 05, France. E-mail: valerie.pichon@espci.fr or to Karsten Haupt, Université de Technologie de Compiègne, UMR CNRS 6022, BP 20529, 60205 Compiègne cedex, France. E-mail: karsten.haupt@utc.fr

molecules. These recognition sites mimic the binding sites of biological receptors such as antibodies and enzymes. The result is known as a *molecularly imprinted polymer* (*MIP*). An MIP can potentially be used in any application that requires a specific binding event, such as an affinity separation, biosensors, or in directed organic synthesis and catalysis. For all of these, the proof of principle has already been provided, and the stability, ease of preparation, and low cost of MIPs make them particularly attractive for these applications.

Indeed, industry is currently evaluating the commercial opportunities for MIPs. However, here the proof of principle is not the only criterion for future investment. Companies need to investigate the selectivity of MIPs for their targets in conjunction with their compatibility with the environment in which they are to be used, including biological fluids and tissues. Criteria such as the ready integration of molecular imprinting within existing industrial fabrication processes, yields, cost, and the competitiveness of MIPs with existing affinity materials also need to be examined. To date, the application area of MIPs that appears to be closest to commercialization is solid-phase extraction (in fact, several MIPs are already commercially available for this application). This review focuses on recent developments in the molecular imprinting technique and the use of MIPs in affinity separations and related techniques, with special emphasis on affinity solid-phase extraction as a case study.

General Principle of Molecular Imprinting

The design and synthesis of biomimetic receptors that are capable of binding a target with similar affinity and specificity to antibodies has been a long-term goal of bioorganic chemistry. One technique that has been increasingly adopted for the generation of artificial, macromolecular receptors is the *molecular imprinting* of synthetic polymers.[1,2] Molecular imprinting is a process in which functional and crosslinking monomers are copolymerized in the presence of a target (i.e., the *imprint molecule*), which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, these functional groups are held in position by the highly crosslinked polymeric structure. Subsequent removal of the imprint molecule gives binding sites that are complementary in size and shape to the desired target or analyte. In this way, a molecular memory is introduced into the polymer that is now capable of selectively rebinding this target (see Figure 1).

The complex formed between the monomers and imprint molecule can be produced through reversible covalent bonds or non-covalent interactions, such as hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals forces. A combination of the two approaches can also be used.

Several items have to be considered when comparing the covalent and non-covalent imprinting. The non-covalent approach, which was pioneered

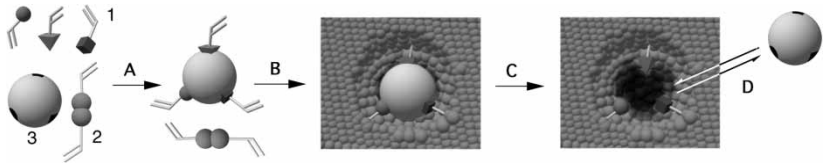

Figure 1. The molecular imprinting principle.

by Mosbach and co-workers,[3] is more flexible with regard to the choice of functional monomers, possible target molecules, and use of the imprinted materials. After polymerization by this method, the imprinted molecule can be removed by simple solvent extraction. However, it should be noted that the pre-polymerization complex in this approach is a reversible system at equilibrium, with a stability that depends on the affinity constants between imprint molecule and functional monomers. This may give rise to some heterogeneity in the imprinted binding sites.

Covalent imprinting has been developed primarily by Wulff and coworkers.[4] In this approach, a polymerizable derivative of the imprint molecule is synthesized and, after synthesis, the imprint molecule is removed through chemical cleavage. One problem with this method is that, if the covalent bonds with the target have to be reformed upon use of the polymer, the resulting association kinetics may be slow. On the other hand, the stability of the covalent bonds should yield a more homogeneous population of binding sites in the polymer. Moreover, the yield in binding sites relative to the amount of imprint molecule that is used (referred to as the *imprinting efficiency*) should be higher in this approach than it is for non-covalent protocols.

A simple demonstration of the molecular imprinting effect is shown in Figure 2. In this example, a non-imprinted copolymer of trifluoromethylacrylic acid and divinylbenzene was first synthesized. To the same monomer mixture was also added increasing amounts of a template molecule (theophylline) before polymerization. When the resulting polymers were checked for their ability to bind a labeled analog of the template (i.e., radiolabeled theophylline), even a very small quantity of template (i.e., a 1:5,000 molar ratio versus the amount of functional monomer, trifluormethylacrylic acid) was found to double the binding capacity of the polymer versus the same polymer in a non-imprinted form. Furthermore, it can be seen that, as more template was used during polymerization, a higher capacity was obtained for the desired target in the imprinted polymer. In this particular case, a maximum capacity was reached at a molar ratio of 1:12 for the template versus functional monomer. The figure also shows that cross-linking is important to obtain imprinted binding sites, since the binding capacity of the polymer increases with the degree of cross-linking. In contrast, a non-imprinted control polymer always shows

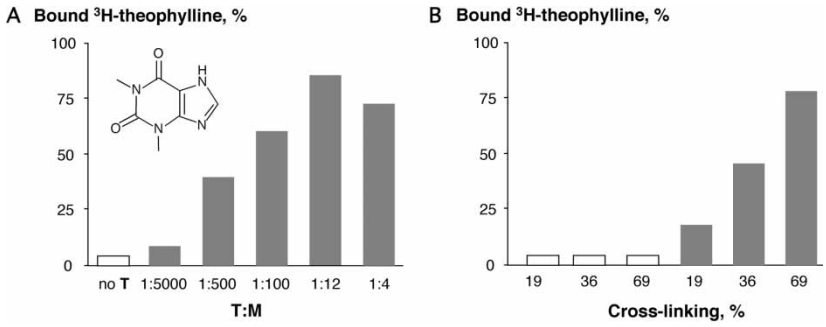

Figure 2. Demonstration of the imprinting effect. (A) Binding capacity for radiolabeled theophylline of a series of polymers that have been synthesized with different amounts of the template. (B) Binding capacity for radiolabeled theophylline of a series of polymers with different degrees of cross-linking. Filled bars: MIPs, empty bars: non-imprinted control polymers. Data from Ref 5.

the same (low) degree of non-specific binding for the three different cross-linking densities used.[5]

The Imprinting Matrix

Various materials can be used as the imprinting matrix for the creation of MIPs. Some examples that will be considered in this section will include acrylic and vinyl polmers, alternative organic polymers, and imprinting matrices such as silica and titanium dioxide.

Acrylic and Vinyl Polymers

Up to present, the majority of reports on molecularly imprinted polymers have described organic polymers synthesized by radical polymerization of functional and crosslinking monomers that have vinyl or acrylic groups, and which use non-covalent interactions between monomers and the template. This can be attributed to the rather straightforward synthesis of these materials and to the large number of available monomers. These monomers can be basic (e.g., vinylpyridine) or acidic (e.g., methacrylic acid). They may also be permanently charged (3-acrylamidopropyl trimethylammonium chloride), hydrogen bonding agents (acrylamide), or hydrophobic substances (styrene).

These rather simple monomers normally have association constants with templates that are too low to form stable complexes. However, in the final polymer, the formation of several simultaneous interactions with the target and a favorable entropy term help to assure tight binding between the imprint and a target molecule. These monomers have to be used in excess

to shift the equilibrium towards complex formation. Association between the polymer and template is governed by an equilibrium that results in the formation of many different complexes. This results in a binding site population that can be rather heterogeneous in terms of the number of functional groups that are incorporated. Some of these functional groups will even be randomly incorporated into the polymer without forming a binding site.

When using a Langmuir model to estimate the affinity constants for these imprinted polymers, models based on two or more sites usually fit the experimental data better than a one-site model.[6] In reality, however, the Langmuir model does not adequately describe most MIPs. To overcome this, it has been suggested to use affinity distribution analysis to better characterise these materials.[7]

As a remedy to this problem of binding site heterogeneity, some have suggested that low-affinity sites be blocked by reacting their functional groups with a chemical reagent (for example, converting carboxyl groups into the methyl ester), while the high-affinity sites are protected by allowing them to be occupied by the template.[8,9] Others have developed monomers that form more stable interactions with the template, or substructures thereof, and which can be used at stoichiometric levels.[10–14] Two examples of monomers recognizing amino and carboxyl groups are depicted in Figure 3. Another possibility for obtaining stronger interactions in the prepolymerization complex, especially in polar solvents like water, is to use coordination bonds with metal chelate monomers.[15–17]

Ultimately, covalent bonds between the template and functional monomers will give the greatest stability for a prepolymerization complex. Whitcombe and coworkers have reported the imprinting of a tripeptide (Lys-Trp-Asp) using a sacrificial spacer (*o*-hydroxybenzamide) between the imprint molecule and monomer. In addition to these covalent bonds,

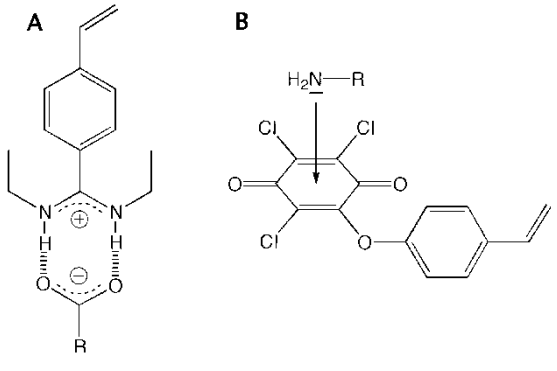

Figure 3. (A) An amidine functional monomer binding to a carboxyl group, and (B) a tetrachloroquinone monomer complexing with an amino group. Structures from Refs. 12 and 14.

non-covalent interactions have also been used. After polymerization, the covalent bonds between the imprint molecule and monomers are hydrolyzed and the spacers are eliminated, giving room and suitable functional groups for non-covalent interactions with the target molecule (Figure 4). In this example, during its rebinding to the polymer, the peptide interacts only through non-covalent interactions, which takes advantage of their faster association/dissociation kinetics than covalent processes.[18]

To obtain an optimized polymer for a given target analyte, combinatorial approaches to MIP synthesis have been used.[5,19,20] In this format, the ingredients of the imprinting recipe, such as the kind and molar ratio of the functional monomers, are varied. This can be performed using automated procedures.[19]

An example of this combinatorial approach is given by an MIP developed for the triazine herbicide terbutylazine. This MIP was optimized and created from a number of different MIPs that were synthesized on small scale (i.e., approximately 55 mg).[20] The functional monomer that was used in the final MIP was selected from a library of six candidates: methacrylic acid, methylmethacrylate, hydroxyethyl methacrylate, trifluoromethyl acrylic acid, 4-vinylpyridine, and *N*-pyrrolidone. Initial screening of these candidates was performed by determine which functional monomer retained the template most strongly. Among the six monomers tested, methyl methacrylate, 4-vinylpyridine, and *N*-pyrrolidone led to polymers that allowed the imprint molecule to be rapidly and quantitatively extracted, while methacrylic acid and trifluoromethylacrylic acid led to polymers that strongly retained the template. Using these last two monomers, a secondary screening for selectivity was performed. For this screening, non-imprinted control polymers were also prepared and analyte binding to the MIPs and control polymers was evaluated in the batch mode. The polymer that gave the highest selectivity for the desired analyte was found to be the one based on methacrylic acid.

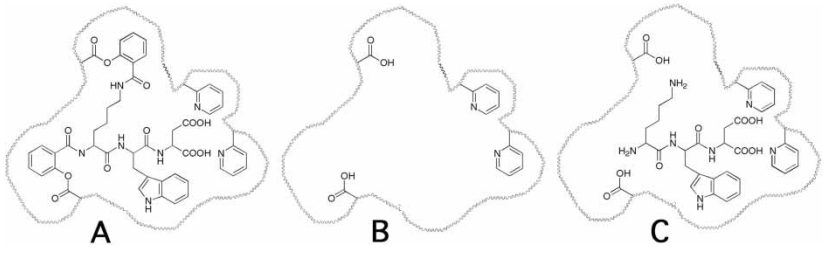

Figure 4. Molecular imprinting of the tripeptide Lys-Trp-Asp using both covalent and non-covalent interactions. The figure in (A) shows a binding site with a covalently bound imprint molecule; (B) illustrates a binding site after chemical cleavage and extraction of the imprint molecule; and (C) shows rebinding of the imprint molecule through only non-covalent interactions. Reproduced with permission from Ref. 18.

Other Organic Polymers

In recent years, other polymers have appeared for MIPs that are either better suited for a specific application or easier to synthesize in the desired form. For example, polymers such as polyphenols,[21] poly(aminophenyl boronate),[22] poly(phenylene diamine),[23] poly(phenylene diamine-co-aniline),[24] polyurethanes,[25] and overoxidized polypyrrole[26] have been used. Compared to polymers based on acrylic and vinyl monomers, the use of these other polymers seems to be somewhat restricted due to their limited choice of available functional monomers.

Other Imprinting Matrices

Sol gels, such as silica and titanium dioxide, are now gaining in importance as imprinting matrices, even though these were introduced many years ago. Silica has been used as an imprinting matrix for inorganic ions[27] and organic molecules.[28–32] With this matrix, either the bulk material can be imprinted by the sol gel method, thus creating microporous materials with specifically arranged functional groups,[27,29,32,33] or an imprinted polysiloxane layer can be deposited at the silica's surface.[28,31,34–36]

Recently, Katz and Davis have reported the molecularly imprinting of bulk amorphous silica with single aromatic molecules by using a covalent monomer template complex, thereby creating shape-selective catalysts.[33] Through the use of physical adsorption experiments, they have been able to directly observe molecular-imprint-generated microporosity, with additional porosity being created in the silica upon template removal.

Another material that has been imprinted using the sol-gel technique is titanium oxide.[37–39] For example, Willner and coworkers have functionalized the SiO_2 gate of an ion-sensitive field-effect transistor (ISFET) with a TiO_2 film that included molecularly imprinted sites for 4-chlorophenoxyacetic acid or 2,4-dichlorophenoxyacetic acid. A titanium(IV) butoxide solution was reacted with the respective carboxylic acid, and the resulting mixture that included the titanium(IV) butoxidecarboxylate complex was deposited onto the ISFET gate. The sol-gel polymerization of the mixture on the SiO_2 gate interface resulted in a TiO_2 film with the embedded carboxylate. Treatment of the film with ammonia solution resulted in the elimination of the carboxylate and the formation of imprinted molecular sites for the respective acid in the TiO_2 film. The functionalized devices that included the imprinted surfaces were used as chemical sensors and revealed selectivity in the sensing of the sodium salts of the imprinted substrates.[37–39] A similar imprinting recipe was used by Kunitake and coworkers, who synthesized thin TiO_2 films imprinted with carbobenzyloxy-L-alanine. The imprinted layers were used as the recognition element in an acoustic sensor, a quartz crystal microbalance, which was able to selectively recognize carbobenzyloxy-L-amino acids.[37–39]

Target Molecules

One of the attractive features of molecular imprinting is that it can be applied to a wide range of targets. The imprinting of small organic compounds, such as pharmaceuticals, pesticides, amino acids, peptides, nucleotide bases, steroids, and sugars is now well established and is considered almost routine. Metal ions and other ions have also been used as templates to induce the specific arrangement of functional groups in an imprinting matrix.[27,40–42]

Larger organic compounds, such as peptides, can be imprinted through similar approaches, but the imprinting of much larger structures is still a challenge. Specially adapted protocols have been proposed to create imprints of proteins in a thin layer of acrylic polymer on a silica surface.[17] Related work has examined the creation of imprints of cells using a lithographic technique[43] and imprints of the surface structure of mineral crystals.[44]

The group of Ratner has developed an interesting approach for the generation of imprints of proteins on a surface.[45] The protein of interest is first adsorbed onto an atomically flat mica surface. It is then spin-coated with a disaccharide solution which, upon drying, forms a thin layer (1–5 nm) that is attached, through multiple hydrogen bonds, to the protein. This protective disaccharide shell is then covered with a fluoropolymer layer via glow-discharge plasma deposition, which covalently incorporates the sugar molecules. Finally, the polymer layer is attached to a glass substrate using an epoxy glue. After peeling off the mica, the protein is removed by treatment with aqueous NaOH/NaClO. This leaves nanocavities, as revealed by tapping-mode atomic force microscopy. For these surface protein imprints, it has been reported that the resulting cavities are complementary in size and, to some extent, to the functionality of the template protein. For example, it has been shown that a surface imprinted with bovine serum albumin preferentially adsorbs this template protein from a mixture that also contains immunoglobulin G. Moreover, an RNase A imprint preferentially adsorbed RNase A over lysozyme, which is similar to RNase A in its size and isoelectric point, and vice versa.

Physical Forms and Preparation Methods for MIPs

There are various forms in which MIPs may occur, such as bulk polymer monolith or an imprinted particle. This section will examine several of these forms and will discuss procedures used for their synthesis.

Imprinted Particles

Traditionally, MIPs have been prepared as bulk polymer monoliths, which are then treated by mechanical grinding to obtain smaller micron-sized particles. The materials obtained through this somewhat inelegant method seem to be

useful for many applications. However, methods are needed for applications that require MIPs to have a more defined physical form. During the past few years, three main aspects have been addressed during the development of such methods: the synthesis of small, spherical particles with sizes below the micron range, the synthesis of thin layers, and the creation of surface imprints.

MIP nanobeads can be synthesized by methods such as precipitation polymerization and emulsion polymerization. Precipitation polymerization can be performed with similar prepolymerization mixtures to those used for bulk polymers, but with the relative amount of solvent in the mixture now being much higher. When polymerization progresses, imprinted nano- or microspheres precipitate instead of polymerizing together to form a macroporous polymer monolith. One drawback of this method is that, due to the dilution factor, higher amounts of the imprint molecule are needed than in traditional techniques. However, this may be compensated for by this method's typically higher yields. This approach was used by Ye at al. to prepare imprinted particles for binding assays.[46,47] It has been shown in some applications that these particles perform better than those that are obtained through mechanical grinding.[48]

Wulff's group has used an approach that is similar to precipitation polymerization.[49] However, they adjust polymerization conditions so that soluble polymer microgels are produced. These microgels have a molecular weight in the range of 10^6 g/mol, which places them close to proteins with respect to molecular size. Although microgels could be synthesized using a monomer mixture adapted to the imprinting process, this method appears to be less straightforward as a means for obtaining selective imprinted materials and more work in its optimization still needs to be done.

Ishi-i et al. created "imprints" at the surface of fullerenes. This was done by introducing two boronic acid groups into [60]fullerene using saccharides as template molecules. The resulting material gave observable regioselective and stereoselective rebinding of the saccharide.[50,51] Later, the group of Zimmermann published a report on molecular imprinting inside dendrimers.[52] Their method involved the covalent attachment of dendrons to a porphyrin core (the template), cross-linking the end-groups of the dendrons, and removal of the porphyrin template by hydrolysis. This technique appeared to yield homogeneous binding sites, to allow quantitative template removal, and produced only one binding site per polymer molecule. In addition, the materials were soluble in common organic solvents.

Thin Imprinted Polymer Films

When a polymer is needed in the form of a thin film at a surface, one can choose between several standard techniques for polymer synthesis, like spin coating or spray coating. In the MIP field, surface-bound films are often required (e.g., as in the construction of sensors). Several protocols have been used for this, most of which are based on in-situ polymer synthesis.

One elegant way of accomplishing this is to apply the soft lithography technique.[53] This technique can create patterned surfaces with MIPs that are useful, for example, in multianalyte sensors and high-throughput screening systems.[54] Unfortunately, current imprinting recipes are not always compatible with the poly(dimethylsiloxane) stamps used for soft lithography.

MIPs can be synthesized at an electrode surface by electropolymerization,[21,23,24,26] or at a non-conducting surface by chemical grafting.[22,55] Another recent development is the growth of an imprinted polymer from a surface by using polymerization initiators that are chemically bound[56] or physically adsorbed[57] to the surface. This still results in binding sites that are contained in the bulk of the MIP layer, but if a highly porous starting material is used, and if the grafted layer is relatively thin, fast mass transfer will result. For example, Ulbricht and coworkers have photo-grafted an MIP layer with a 10 nm thickness (in the dry state) onto a polypropylene membrane.[55] Sellergren's group has developed a method to synthesize MIP layers at the pore surface of porous silica particles. They were able to control the layer thickness to values between 0.8 and 7 nm.[56]

One of the standard coating techniques used for example in the microelectronics industry is spin-coating. We have recently demonstrated that it is possible to spin-coat the monomer mixture on a flat surface followed by in-situ photopolymerization in the presence of a template, in order to obtain MIP films with thicknesses between 100 nm and several μm. However, with acrylic and vinyl monomers, polymerization of these films is too fast for phase separation to occur, so that non-porous films with very little binding capacity is obtained. However, it is possible to accelerate phase separation by adding a linear polymer such as poly(vinyl acetate) as co-porogen. The porosity of the films can be fine-tuned via the amount and the molecular weight of the poly(vinyl acetate), and even surfaces covered with nanoparticles can be obtained (Figure 5). We found that in contrast to bulk polymers, in these systems phase separation and pore formation is by spinodal decomposition.[58,59]

Imprinting at Surfaces

Imprinted materials with binding sites situated at, or close to, the surface of the imprinting matrix have many advantages. For instance, the imprinted sites in these materials are more accessible and mass transfer is faster. The binding kinetics may also be faster, and target molecules conjugated with bulky labels can still bind. The reason that these materials are not universally used is because their preparation is less straightforward than it is for bulk polymers and requires specially adapted protocols.

Whitcombe and coworkers have developed a technique for imprinting at surfaces based on emulsion polymerization. In this method, small beads are created in an oil-in-water biphasic system that is stabilized by a surfactant.

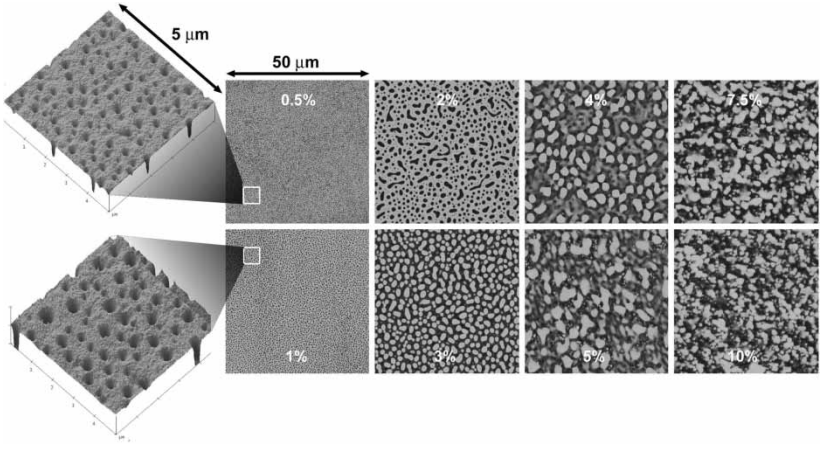

Figure 5. Contact-mode AFM images of spin-coated MIP-film obtained using different concentrations of poly(vinylacetate) as co-porogen 58,59.

The imprint molecule (e.g., cholesterol) is part of the surfactant (i.e., pyridinium 12-(cholesteryloxycarbonyloxy)dodecane sulfate).[60] As a result, all binding sites are situated at the particle surface, as was demonstrated by flocculation experiments using PEG-bis-cholesterol.

Another protocol for the creation of surface binding sites was described by Yilmaz, Haupt, and Mosbach. Here, the imprint molecule is immobilized onto a solid support such as porous silica beads prior to polymerization.[61] The pores are then filled with the monomer mixture, and the polymerization is initiated. The silica is removed by chemical dissolution, which leaves behind a porous polymeric structure that is a negative image of the original bead. The binding sites are now all situated at the surface of the polymer and are uniformly oriented.

APPLICATIONS OF IMPRINTED POLYMERS IN AFFINITY SEPARATIONS

Liquid Chromatography

The first application for MIPs was their use as stationary phases in affinity chromatography. In particular, these were used for the separation of racemic mixtures of chiral compounds. This is possible since the imprinting process introduces enantioselectivity into the polymers that are synthesized (in most cases) from non-chiral monomers.

A unique feature of MIPs versus conventional chiral stationary phases is that they are tailor-made for a specific target molecule, giving them a

predetermined selectivity. For example, when a polymer is imprinted with the L-enantiomer of an amino acid, a column for high performance liquid chromatography (HPLC) that is packed with this polymer will retain the L-enantiomer more than the D-enantiomer. However, a column containing an chemically identical non-imprinted polymer will not be able to separate these enantiomers. Typical values for the enantioseparation factor α of MIPs are between 1.5 and 5, although in some cases much higher values have been obtained.

A pronounced stereoselectivity has been observed with an MIP for the cinchona alkaloids cinchonidine and cinchonine, which resulted in chromatographic α of up to 31.[62] It is even possible to obtain chromatographic supports selective for compounds that contain several chiral centers. For instance, a polymer imprinted with the dipeptide Ac-L-Phe-L-Trp-OMe was able to specifically recognize this imprint isomer over three other stereoisomers, where the LL form was more retained on an HPLC column packed with this MIP than the DD, DL or LD forms (separation factors: $\alpha = 17.8$, 14.2 and 5.21, respectively).[63]

If the molecule of interest contains more than two chiral centers, as is the case with carbohydrates, these properties of molecularly imprinted materials become even more important. As an example, in one study where polymers were imprinted against a glucose derivative, very high selectivities between the various stereoisomers and anomers of glucose were recorded.[64]

These examples are impressive and suggest that good enantioseparations are achievable with MIPs. Unfortunately, in reality the corresponding resolution factors that have been obtained with MIPs are typically rather low. The same is true for the plate numbers of MIP-based columns (i.e., 2,000–5,000 plates/m). This is due to the severe peak broadening and tailing that is often seen for MIP supports, especially for the more retained enantiomer. This, in turn, can be attributed to a heterogeneous population of binding sites in the MIP (with respect to their affinities and accessibilities) and to the low functional capacities of these materials.[65] One has to keep in mind that, for each binding site that is created through imprinting, at least one template molecule has to be present in the polymerization mixture. However, in reality, even a larger amount of the template is needed since the imprinting efficiency (i.e., the number of sites created versus the number of template molecules employed) is much lower than 100%.

Another problem is that part of the template molecules can often not be extracted from the MIP after polymerization. This occurs because some of the template is deeply buried in the crosslinked matrix. Even if extraction is possible, part of the resulting imprint sites have such a low accessibility that they are useless in chromatographic applications.

To obtain a mechanically stable material that is suitable for chromatography, a large percentage of the monomers must be crosslinkers (typically 80–90% for bifunctional crosslinkers). This limits the amount of functional monomers and template molecule that can be added. Moreover, if a non-covalent imprinting protocol is used, the functional monomer has to be

present in excess to shift the equilibrium towards complex formation. This inevitably results in a fraction of the monomers not being situated in binding sites but instead being randomly distributed in the polymer. This creates weak-affinity and nonspecific sites.

Numerous attempts have been made to improve the performance of MIPs and to avoid these problems. The easiest approach is to optimize the conditions for the selected MIP material. This can be done by optimizing the separation protocol that is used with this material, including the separation temperature, mobile phase composition, and use of competing agents or gradient elution protocols[66] to improve peak shapes. It has also possible to chemically block the binding groups in non-specific or low quality sites in MIPs.[8] However, these same sites may be the ones that actually come into play in chromatography because of their faster kinetics than high affinity sites and this approach tends to give only limited improvements in chromatographic behavior. Probably the best approach to overcome these problems is a "preventive" one in which efforts are made to synthesize and select better imprinted materials.

During the last few years, work with MIPs in chromatography has focused on two key aspects. The first of these is the synthesis of uniformly shaped and sized particles with narrow pore-size distributions and improved mass transfer properties. The second is the development of MIPs with better quality binding sites, ideally using stoichiometric ratios of the template and functional monomer.

Uniformly sized spherical MIP particles for chromatography can be synthesized in a variety of ways. These include organic-in-water suspension polymerization, suspension polymerization using perfluorocarbon liquids as the dispersing phase[67] and multistep swelling procedures.[68] These materials should have better chromatographic behavior than the more common ground bulk polymers. For example, it has been shown that a 25 cm $\times$ 4 mm I.D. column filled with MIP beads prepared through suspension polymerization in perfluorocarbon could resolve 1 mg of Boc-DL-Phe at flow rates up to 5 mL min^{-1}, a result which is not easily obtained when using a ground bulk polymer as a column packing.[67]

Capillary Electrochromatography

Capillary electrochromatography (CEC) might be one of the more promising chromatographic techniques to be used in combination with MIPs, in particular for chiral separations.[69,70] *MIP-capillary electrochromatography* (or *MIP-CEC*) profits from the inherent separation power of this method. Compared to MIP-based HPLC, better resolutions (due to efficiencies in CEC of more than 100,000 plates/m)[69] and larger separation factors can be achieved.

In one study, a chiral separation of the beta-blockers propranolol and metoprolol was achieved with MIP-CEC. The polymer for this separation

was cast in situ in the capillary in the form of a macroporous monolith that was attached to the inner wall. This capillary was prepared and conditioned within a few hours.[71] When this capillary was used in CEC, the components of racemic propranolol were resolved within only 120 s (Figure 6), and when samples were injected that contained mainly the *R*-enantiomer of propanolol, small amounts (1%) of the *S*-enantiomer could also be distinguished by this approach. Other possible uses for MIPs in combination with CEC or capillary electrophoresis include MIPs that are in the form of continuous polymer rods,[72] particles included in a gel matrix,[73] or small particles that are suspended in the carrier electrolyte.[74]

Thin Layer Chromatography

MIPs have also been used as stationary phase in thin layer chromatography (TLC), although the number of publications in these areas remain limited.

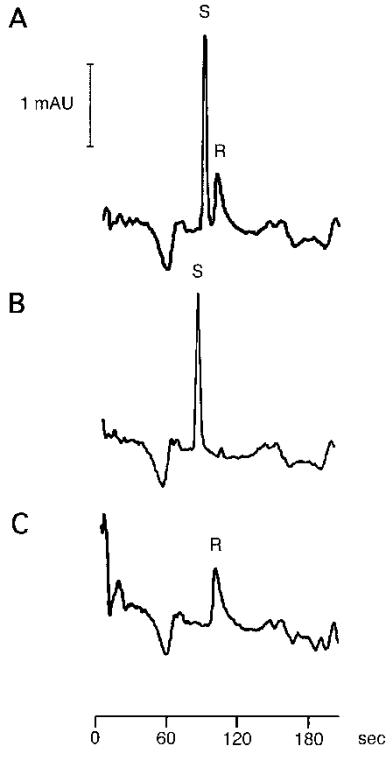

Figure 6. Capillary electrochromatographic separations for (a) racemic propranolol, (b) *S*-propranolol, and (c) *R*-propranolol on an *R*-propranolol-imprinted polymer. Adapted with permission from Ref. 71.

A first report was published by the group of Mosbach who used finely ground imprinted polymer coated onto an inert support for chiral TLC.[75] They where able to show that the racemates of a number of amino acids could be resolved, but problems were encountered due to band broadening, which led to the formation of zones rather than small spots or thin bands. This in turn led to band overlap and poor resolution, and measurements of R_f values were made more difficult. Optimization of particle shape, size and porosity would probably result in a considerably improved shape of the bands. This method may be attractive for the determination of the enantiomeric purity of compounds such as chiral drugs, owing to its simplicity, its speed and the possibility of running multiple parallel samples. More recently, Suedee et al. reported similar work where they used imprinted polymer particles of below-micrometer size for TLC.[76,77] The polymer was mixed with $CaSO_4$ and wet-coated onto microscope slides. For example, the separation of enantiomers (such as ephedrine) and diastereomers (such as quinine/quinidine) was possible on a polymer imprinted with quinine, with separation factors typically below 1.5.[76] However, they also reported that some of the quinine imprint molecule remained in the MIP after processing of the particles. It is therefore not sure whether the separation power of the material can be solely attributed to the imprinted sites or if the chiral ligands remaining in the polymer matrix also contributed.

Membrane-Based Separations

Chromatographic separation techniques are well established and widely used, however they do have some limitations, especially in the scale-up of separation processes. For larger-scale separations, they are therefore often replaced by membrane-based techniques, since membranes can often be used in continuous mode, as compared to the batch-wise operation in chromatography.

Imprinted membranes have been prepared in different ways; they can be cast directly as a thin layer on a flat surface[78] or between two surfaces.[79] A different way to obtain MIP-membranes has been proposed by Ulbricht and coworkers; they have photo-grafted a MIP-layer of 10 nm thickness (in the dry state) onto a polypropylene membrane by physically adsorbing the photoinitiator onto the membrane.[55] Alternatively, MIP-membranes can be prepared by a phase inversion precipitation technique.[80] Imprinted membranes have great potential for applications in separation, especially chiral separation, but they can also be used as recognition elements in biomimetic sensors.[81,82] Depending on the structure of the membrane, the target molecule can be selectively adsorbed (retained) by the membrane, which can thus be used for adsorptive separation.[83] If pore flux is limited, selectively transport through the membrane may take place. For example, a free-standing membrane imprinted with 9-ethyladenine showed faster transport of adenosine than of guanosine.[78] A theophylline-imprinted membrane

cast on the surface of a porous aluminum membrane transported theophylline faster than the structurally related caffeine, and vice versa.[84] Such membranes have the potential to be used in continuous separation processes.

Solid Phase Extraction

Solid-Phase Extraction (SPE) is routinely used for the extraction of compounds at the trace level from liquid samples or for the purification of extracts resulting from the treatment of complex matrices. Despite their attractive features, the classical SPE sorbents retain analytes by non selective hydrophobic (with alkyl-bonded silicas, polymers, carbonaceous sorbents,...) or polar interactions (with silica, amino-bonded silica, alumina,...) that lead to a partial co-extraction of interfering substances. In order to enhance the selectivity of the extraction, immunosorbents (ISs) based on the high affinity and the selectivity of antigen-antibody interactions allowing a selective extraction of the target analyte and of compounds having a similar structure were developed. Several reviews have been published in recent years demonstrating the interest of immunoextraction as a selective sample pretreatment method.[85–87] Nevertheless, the development of an IS is time consuming and relatively expensive. These drawbacks have led to the recent application of the molecularly imprinted polymers (MIPs) to this field. So, MIPs are often called synthetic antibodies in comparison with the ISs. They offer some advantages including easy, cheap and rapid preparation and high thermal and chemical stability.[88]

The first application of molecularly imprinted polymers in Solid-Phase Extraction (MISPE) was carried out by the group of Sellergren in 1994 for the extraction of pentamidine present at low concentration in urine.[89] Since this work, MIPs have been largely applied to the selective extraction or to the clean-up of target analytes from various complex matrices. Figure 7 illustrates the increase of the number of publications that concern the development of MISPE methods applied to real samples. Most of these applications of MIP are listed in Table 1.[89–151] It highlights the development of MIPs for a large variety of compounds extracted from biological fluids, environmental matrices and food.

Principle of SPE on MIPs

The principle of selective extraction on MIPs is the same as on an immunosorbent. In the most common approach, the MIP particles are packed into a disposable cartridge or a small-size column between two frits. After a conditioning step, the sample is percolated through the MIP and a washing step allows the removal of the interfering compounds retained by non specific interactions. This step must be optimized in order to keep the target analytes strongly retained inside the specific cavities of the MIP. The

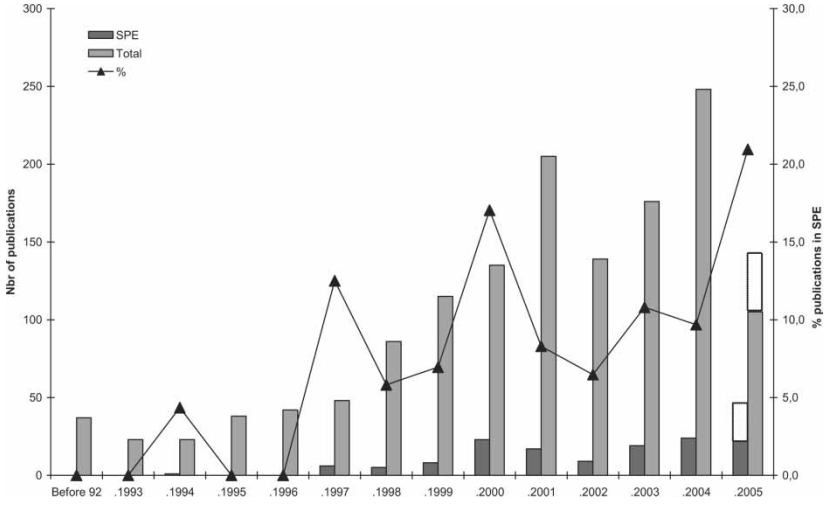

Figure 7. Evolution of the number of manuscripts dedicated to molecular imprinted polymers in general and applied to SPE specifically. Main source: *http://www.smi.tu-berlin.de/*

desorption of analytes is achieved by percolating a solvent able to disrupt the interactions between the monomer residues and the analytes in order to recover them. However, the nature of the different solvents involved in a MISPE procedure can be very different from those used in immunoextraction. Due to the use of biological reagents, immunosorbents are particularly well adapted to the direct percolation of aqueous samples, the washing and the desorption steps mainly consisting in the use of hydro-organic mixtures. In return, due to the synthesis of the polymers in organic media, the MIP-based extraction procedures mainly consist in the use of organic solvents. This point will be largely developed below.

Synthesis of the MIP

Most molecularly imprinted polymers produced for SPE purposes were prepared in bulk. The polymer rod is then crushed, ground and sieved to obtain particles mainly in the 25–50 μm size range. The non regular shape of the particles does not constitute a real limitation for SPE applications. However, other methods of polymerisation such as precipitation[126] and suspension polymerisation,[94,149] were recently used for SPE materials. Haginaka's group proposed the use of a multi-step swelling approach combining restricted access media to exclude macromolecules and a MIP to selectively retain target analytes from biological fluids.[96,129] In this approach, the difficulty is to develop a hydrophilic surface that does not modify the selectivity of the MIP.

Table 1. MISPE of compounds from real matrices coupled off-line or on-line with analytical methods (mainly liquid chromatography)

Target analytes	Template	Matrices	Monomer/solvent	Sample pretreatment	Coupling	Date	Ref
Alkyl-phosphonates	Pynacolil-methylphosphonate	Human serum	MAA/MeCN	Centrifugation and liquid-liquid extraction with MeCN	off	2001	[90]
		Soil	MAA/MeCN	Extraction with pressurized hot water	off	2005	[91]
Alfusozine	Alfusozine	Plasma/soil	MAA/CH_2Cl_2	Plasma:dilution with MeCN, filtration—Soil: solvent extraction	off	2005	[92]
Atropine/ scopolamine	Atropine	Tablets	TFMAA/multistep swelling	Extraction with HCl	on	2005	[93]
Benzo(a)pyrene	Benzo(a)pyrene	Waters, instant coffee	2-VP/CH_2Cl_2, bulk, microsphere	Dilution with MeCN	off	2004	[94]
Bisphenol A	Terbutylphenol	Surface water	4-VP/emulsion synthesis	No	off	2003	[95]
	Bisphenol A d16	Serum	4-VP/toluene/ multistep sweeling + RAM	No	on	2005	[96]
Bupivacaine	Bupivacaine	Plasma	MMA/toluene	Dilution in citrate buffer	off	2003	[97]
Bupi-, ropi-, mepivacaine	Pentycaine	Plasma	MAA/toluene	Dilution in citrate buffer	off	2004	[98]
Caffeine	Caffeine	Urine, coffee, drinks	MAA/MeCN	Dilution in water	on	2004	[99]
Catechol	Cathecol	Aqueous effluent	4-VP/MeCN	No	off	2005	[100]
Cephalexin	Cephalexin	Plasma, serum	TFMAA/MeCN	SPE (C_{18})	on	2003	[101]
Cephalexin and analogs	Cephalexin	Serum	TFMAA/MeCN	SPE (C_{18})	on	2004	[102]

Ceramide	Ceramide	Yeast	Monomers mixture/toluene-heptane, in-situ	Extraction in $CHCl_3$/MeOH	on	2003	[103]
Chloramphenicol	Chloramphenicol	Ophtalmic solution, milk	DEAEM/THF	Sol: dilution in phophate buffer. Milk: precipitation with acid, centrifugation	on	2003	[104]
Chloro-, nitro-phenols	Chlorophenol	River water	4-VP/MeCN	Acidification	on	2003	[105]
Chlorophenoxy acetic acids	Trichlorophenoxy acetic acid	River water	4-VP/MeOH-H_2O	Acidification	off	2001	[106]
Clenbuterol	Clenbuterol	Liver, urine, milk	MAA/MeCN	LLE on column (hexane/CH_2Cl_2)	off	2001	[107]
	Clenbuterol	Calf urine	MAA/MeCN	No	off	2000	[108]
Darifacin	Darifacin	Plasma	MAA/THF, ethyl acetate	Precipitation with MeCN, centrifugation	off	1999	[109]
Diphenylphosphate	Ditolylphosphate	Urine	2-VP/$CHCl_3$	Dilution in citrate buffer	off	2004	[110]
(−)-ephedrine	(−)-ephedrine	Herbal ephedra	MAA/MeCN	Extraction with NaOH/$CHCl_3$	off	2005	[111]
Esculetin	Esculetin	Ash bark	Acrylamide/EtOH	Extraction with water	off	2005	[112]
Harmine, Harmaline	Harman	Seeds	MAA/toluene-MeCN, MeCN, THF	Soxhlet extraction (EtOH)	on	2002	[113]
Ibuprofen	Naproxen	Rat plasma	4-VP/emulsion synthesis	No	on	2000	[114]

(*continued*)

Table 1. Continued

Target analytes	Template	Matrices	Monomer/solvent	Sample pretreatment	Coupling	Date	Ref
Metformin	Metfromin	Plasma	TFMAA/MeCN	Precipitation with MeCN, centrifugation	on	2004	[115]
Microcystin-LR	Microcystin-LR	Drinking water	AMPSA/UAEE/DMSO	Addition of buffer (pH 4)	off	2003	[116]
Naphtaene sulfonates	1-naphtalene sulfonate	River water	4-VP/MeOH, water	No	off	2004	[117]
Naproxen and analogs	Naproxen	Urine	4-VP/toluene	Acidification pH 3	off	2004	[118]
Nicotine	Nicotine	Nicotine chewing gum	MAA/$CHCl_3$	Extraction in ethyl acetate/NH_3	off	1998	[119]
Nitrophenol	Nitrophenol	River water	4-VP or MAA/MeCN	Acidification	on	2000	[120]
Ochratoxin A	Ochratoxin A	Wine	Py/MeCN	No	on	2005	[121]
Pentamidine	Pentamidine	Urine	MAA/propanol	Dilution in buffer, MeCN	off	1994	[89]
Phenorbital	Anorbital	Urine, medicines	MAA/$CHCl_3$ (suspension)	Dilution in water	off	2003	[122]
Phenytoin	Phenytoin	Plasma	MAAM/MeCN-THF	No	off	2001	[123]
Phenylureas	Isoproturon	Surface water	MAA/toluene	SPE (PS-DVB)	off	2001	[124]
	Isoproturon/linuron	Corn waters	MAA or TFMAA/toluene	Extraction in MeCN, centrifugation	off	2005	[125]
			MAA or TFMAA/toluene	SPE (VP-DVB)	off	2005	[126]

	Fenuron	Plant samples	MAA/toluene	Extraction in MeCN, centrifugation	on	2003	[127]
Pirimicarb	Pirimicarb	Water surface	MAA/$CHCl_3$	No	on	2002	[128]
Propanolol and analogues	Propanolol	Plasma	MAA/multistep sweeling + RAM	No	on	2003	[129]
			MAA/toluene	Precipitation of protein with ACN	off	2004	[130]
Quercetin	Quercetin	Red wine	4-VP/MeCN	?	off	2002	[131]
	Quercetin	Plasma	AA/THF	Dilution in acetic acid	off	2003	[132]
	Piceatannol	Medicinal herb	4-VP/MeCN-THF	Extraction by EtOH, liquid-liquid extraction ($CHCl_3$, EtOAc)	on	2003	[133]
Sameridine	Sameridine	Plasma	MAA/toluene	Liquid-liquid extraction (heptane)	off	1997	[134]
Scopolamine	Hyoscyamine	Urine, serum	MAA/toluene	Serum: precipitation with MeCN, centrifugation Urine: acidification	off	2003	[135]
Sudan I	Sudan I	Chili powder	4-VP/$CHCl_3$	Extraction with $CHCl_3$	off	2005	[136]
Sulfamethazine	Sulfamethazine	Milk	MAA/MeCN	No	off	2005	[137]
Sulfonylureas	Metsulforon-methyl	Water and soil	TFMAA/CH_2Cl_2	Addition of EDTA	off	2002	[138]
	Monosulfuron	Soil	MAA/DMF	Extraction by water-MeOH	off	2004	[139]
Theophylline	Theophylline	Serum	MAA/CH_2Cl_2	Liquid-liquid extraction ($CHCl_3$)	on	1998	[140]
Triazines	Atrazine	Beef liver	MAA/$CHCl_3$	Extraction in $CHCl_3$	off	1997	[141]

(*continued*)

Table 1. Continued

Target analytes	Template	Matrices	Monomer/solvent	Sample pretreatment	Coupling	Date	Ref
	Terbutylazine	Water surface and sediment	MAA/CH_2Cl_2	Water: no. Soil: soxhlet extraction (MeOH)	off	2000	[142]
	Terbutylazine	Humic acid	MAA/toluene	SPE (RAM)	on	2001	[143]
	Terbutylazine	Surface water	MAA/toluene	SPE (C_{18})	off	2002	[144]
Triazines and metabolites	Terbutylazine	Surface water	MAA/CH_2Cl_2	No	off	2003	[145]
	Terbutylazine	Grape juice soil	MAA/CH_2Cl_2	No	off	2004	[146]
	Propazine	Water, soil, corn	MAA/toluene	Water: SPE (PS-DVB) Soil, corn: extraction in MeCN	off	2001	[147]
	Simazine	Humic acid, urine	MAA/CH_2Cl_2	SPE (C_{18})	on	1999	[148]
Trimethoprim	Trimethoprim	Urine, tablets	MAA/$CHCl_3$	Tablets: extraction with EtOH, Urine: dilution, filtration	off	2005	[149]
Tylosin and metabolites	Tylosin	Broth samples	Monomers mixture/ THF	Dilution in MeOH	off	2004	[150]
Verapamil	Verapamil	urine	MAA/$CHCl_3$	SPE (RAM)	on	2004	[151]

AA: acrylamide. AMPSA: 2-acryl-amido-2-methyl-1-propanesulfonic acid. DEAEM: diethylamino ethyl methacrylate. MAAM: methacrylamide. MAA: methacrylic acid. TFMAA: trifluoromethyl acrylic acid. UAEE: urocanic acid ethyl ester. VP: vinyl pyridine.

Choice of Reagents

In MISPE, the most common approach consists in a non covalent imprinting. In this case, the porogen solvent is one of the most important factors determining effective molecular recognition[152] because the accuracy of the assembly of the template and the monomer is related to the physical and chemical characteristics of the solvent. MIPs are generally prepared using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as cross-linker and a non polar and aprotic solvent (such as dichloromethane, chloroform or toluene) as porogen. Thus, hydrogen bonds and electrostatic interactions can strongly take place in this organic media. The same type of interactions is also involved when 2- or 4-VP are used as monomers and when acid analytes are used as templates. The polar nature of the interactions involved between the templates and these monomers explains the difficulty to apply MIP directly to aqueous samples. This point will be discussed below.

The choice of the template is also an important point. The structure and the functionalities of this molecule define the shape of the cavity and the nature of the selective interactions that should take place during the extraction procedure in order to obtain the expected selectivity. However, it is known that despite exhaustive washing steps with different types of solvents in acidic and/or basic conditions, trace amounts of the imprint molecule may remain in the MIP. A leaching of these molecules during the extraction step can thus be observed, leading to erroneous results. This problem can be circumvented by using a quite expensive approach based on the use of an isotope as it was done for bisphenol A.[96] Another approach consists in using a structural analogue of the target molecule as template such as what was done recently to prepare a MIP for several anaesthetics[98] or several cocaine metabolites.[153] In this case, the "dummy" molecule should possess a shape and a spatial arrangement of its functionalities very close to the whole group of compounds in order to obtain good recovery yields for each of them. The choice of the template is also very important for obtaining a class selective sorbent, i.e., an MIP that is able to selectively extract several structural analogues. As an example, the effect of the template was clearly demonstrated for the extraction of triazines (methoxy-, thiomethyl- and chloro-triazines) on two MIPs synthesized by using a chlorotriazine (terbutylazine) and a thiomethyltriazine (ametryn) as template molecules.[146] The ametryn MIP retained strongly the ten studied triazines because high extraction recoveries were in the 60–100% range for each analyte. In contrast, low extraction recoveries were obtained on the terbutylazine MIP for the triazines that did not belong to the chlorotriazines group. Those results were very similar to those obtained when using immunosorbents based on anti-chlorotriazine and anti-thiomethyltriazines antibodies,[154] showing the similarity of the behavior of these two types of molecular recognition-based sorbents.

Recently, the effect of the template was again demonstrated with results obtained for MIPs produced for phenylurea herbicides.[126] Good recoveries

were observed for most of the phenylureas using isoproturon as template, while the use of linuron led to very low extraction recoveries for all the studied compounds from this group.

Selectivity of an MIP for SPE

Many studies just reported some measurements of the retention of the template in liquid chromatography on MIP and NIP packed in columns that prove the presence of cavities when a difference of retention is observed. However, the presence of cavities in itself does not ensure that the procedure of extraction will be selective.

To obtain an optimal selectivity, the extraction procedure applied to a real sample should allow the target molecule to specifically interact with the monomers residues located in the cavities. These interactions should not be affected by the compounds constituting the sample (ions, proteins,...). Moreover, the procedure of extraction should allow the onset of interactions in the cavities while at the same time excluding interactions between the target analyte and the monomer residues that cover the surface of the polymers and are located outside of the cavities. Therefore, the procedure should be based on the use of a solvent for the percolation step and/or the washing step that possess an elution strength sufficiently high to disrupt the interactions that can take place with residual monomers at the surface of the polymer without affecting the overall retention in the imprints. This means that the procedure has to be optimized in order to eliminate low energy interactions at the surface without damaging specific interactions taking place in the cavities and that are of stronger energy due to the spherical recognition. To evaluate the risk of non specific interactions with the external surface of the MIP, the procedure of extraction has to be tested in parallel with the NIP.

Optimization of a Selective Extraction Procedure on MIP

It has been largely demonstrated that MIPs offer the highest selectivity when samples are dissolved in the solvent used for the MIPs preparation.[152] As most of the MIPs are synthesised in non polar and aprotic solvents, MIPs seem to be perfectly adapted for the clean-up of complex matrices after a previous extraction step that allows the transfer of the target analytes into the appropriate solvent.[90,92,101,103,111,124–127,136,140–144,146–148,151] During the percolation of the non-aqueous sample, the target analyte can then develop specific interactions in the cavities, mainly hydrogen bonds and/or electrostatic interactions. However, a washing step is generally achieved by the same solvent or with the addition of a small proportion of a polar modifier in order to limit the interaction of the target analytes by non specific interactions with the external surface of the MIP. The desorption of

the analytes is then achieved by percolating a protic and polar solvent such as methanol with the possible addition of basic or acid compounds in order to break the hydrogen bonds and the residual electrostatic interactions. Over the last years, numerous papers have testified that aqueous samples such as natural waters or biological fluids can be applied directly to the MIP or after a simple dilution step (see Table 1). However, during this step, the retention is mainly ensured by non specific hydrophobic interactions between the target analytes and the polymeric matrix. Therefore, it is necessary to proceed to a washing with a solvent that will favour the development of the selective polar interactions between the analytes and the cavities.[92,98,110,145]

The importance of the washing and the difficulty to optimize this step have been largely reported.[98,100,110,112,117,149] The use of the NIP in parallel is essential to be sure of the improvement of selectivity obtained with the MIP.[91,92,98,110,112,117,126,145,146,153] As an example, Figure 8 reports the effect of the proportion of methanol in the washing fraction on the retention of diphenylphosphonate on the MIP and on the NIP. Without methanol, the retention on the NIP is the same as on the MIP. The addition of more than 50% of methanol causes a large decrease of the retention on the NIP due to the disruption of the non specific interactions on the NIP without affecting the retention on the MIP, i.e., the interactions of diphenylphosphonate with the cavities.

Extraction Recoveries

In SPE, the extraction recovery depends on the conditions of use of the sorbent: a 100% recovery can only be attained if the analyte is totally

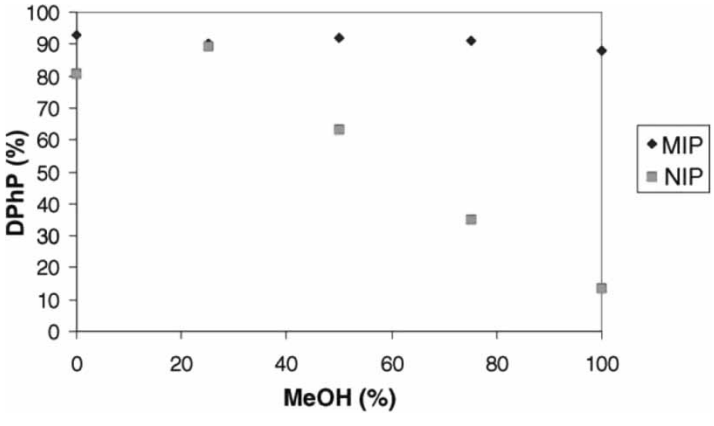

Figure 8. Recoveries of diphenyl phosphate extracted by MIP and NIP cartridges from 1 ml water washed with a 5 mM NH_3 solution containing different percentages of methanol. From 110.

retained during the percolation of the sample and of the washing solvent and totally recovered in the eluting fraction. As in all chromatographic processes, the behaviour of the compounds during these different steps depends on the affinity of the analyte for the sorbent, i.e., the MIP, and of the elution strength of the different percolated solvents. To reduce as much as possible the risk of non specific interactions, the introduction of a drastic washing step as previously mentioned can cause a partial elution of the analyte leading thus to a slight decrease of the recoveries. This is acceptable if a real selective procedure is obtained as a compensation.[98,110] In some cases, residual non specific interactions cannot be suppressed without affecting the retention on the MIP. As an example, for a MIP synthesized for (−)-ephedrine, the washing step was optimized using the NIP in parallel with the MIP. A recovery of 92% was obtained for the extraction of an Ephedra sample on the MIP while as much as 65 % of recovery was observed on the NIP despite the optimization of the washing step.[111] Nevertheless, the selectivity of the procedure was demonstrated by the reduction of the matrix peak when using the MIP as illustrated on Figure 9.

Due to the use of acidic or basic monomers, the effect of the pH on the recoveries has also been frequently described. Several studies reported the

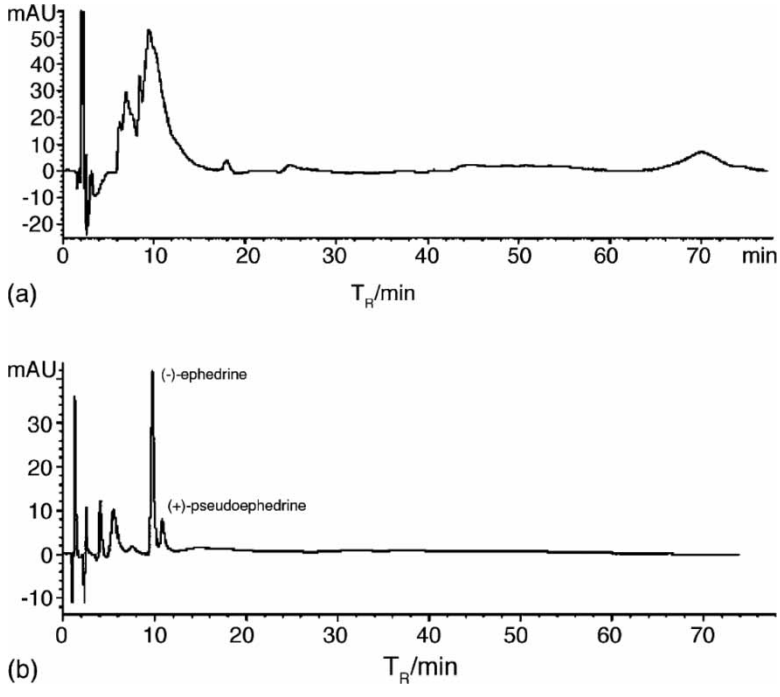

Figure 9. The chromatogram of Chinese Ephedra extracts before (b) and after MISPE (a). LC analysis and UV detection(220 nm). Adapted from 111.

existence of an optimal range of pH, to favour electrostatic interactions between the ionized form of the monomer residues and the basic or acidic template, thus improving the recoveries.[92,93,98,110]

In MISPE, the percolation of highly contaminated samples can also cause a decrease of recoveries due to the overloading of the capacity of the MIP. This parameter depends on the procedure of extraction. For a procedure giving rise to an optimal selectivity, i.e., without any retention on the NIP, the retention is only ensured by the specific cavities. In this case, the capacity is directly defined by the number of specific cavities.

The capacity of a MIP can be estimated by binding assays in a given solvent (mainly the solvent used for the polymerisation step). This approach consists in measuring the amount of analyte retained by the MIP at the equilibrium and by subtracting the amount of analyte adsorbed in the same conditions by the NIP. However, this approach does not allow the determination of the real capacity corresponding to a given procedure of extraction involving different steps and different solvents. So, the measure of the capacity should take into account the extraction recovery. It can be evaluated by constructing a saturation curve obtained by percolating a constant volume of sample spiked with increasing amount of target analyte through the MIP. The amount of extracted analyte is plotted versus the amount of analyte present in the percolated sample. Similar experiments carried out on the NIP allow a verification of the selectivity of the applied procedure.

This approach was used to determine the capacities of MIPs.[91,92,111,112,139,146] The observed values are sometimes difficult to compare because they do not directly correspond to the real number of cavities when partial non-specific interactions still occur. This explains the large range of values (1 to 40 μmol/g) that can be found in the literature.[141,155–157] A MIP developed for the selective extraction of triazines was compared to an immunosorbent based on anti-triazines antibodies.[146] A capacity value 30-fold higher than the capacity of the immunosorbent was obtained, thus highlighting the advantage of MIPs particularly for their use in miniaturized devices.

Application to Real Samples: Selectivity and Matrix Effects

There are more and more applications of MIPs directly to real samples without a preliminary treatment (see Table 1). Generally, the extraction procedure is first developed on a synthetic sample, such as a spiked buffer or a pure water sample, which allows to check the selectivity of the procedure;[100,118] then the washing step is optimized as previously mentioned. However, matrix components such as proteins, humic acids or salts can dramatically disrupt the selective interactions, thus decreasing the extraction recoveries. Proteins can be easily removed from the biological fluid by a simple precipitation with acetonitrile.[109,115,130,135] The matrix effect can also be reduced by a simple dilution of the sample with a buffer

or by its acidification[89,97,98,105,106,110,116,118,120,132] before its percolation through the MIP. For the application of a MIP to the extraction of triazines from real waters, the matrix effects were attributed to the presence of divalent cations in real water (such as calcium ions) involving an ion-exchange between these cations and the hydrogen of the carboxyl groups of the MIP, thus preventing subsequent interactions (by H bond) between the triazines and the MIP.[145] By introducing an acidic washing step after the percolation of an industrial effluent from textile industry, the matrix effect has been suppressed. This type of matrix effect was also observed by Zhu[138] for the extraction of sulfonylureas and it was inhibited by the addition of EDTA to trap the calcium ions in water samples.

The enhancement of the selectivity provided by the MIP has been largely described in the literature. MIPs have often been compared to classical sorbents in order to demonstrate the possibility to obtain cleaner baseline when using the MIP than when using C_{18} silicas or hydrophobic polymers.[126,145,146] The selectivity was also demonstrated by spiking the sample with compounds belonging to the same range of polarity as the target analytes, the lack of retention of these compounds on the MIP thus demonstrating the selectivity of the extraction procedure on MIPs.[117,146]

The best result that can be expected using MISPE corresponds to the achievement of a 100% recovery on the MIP and no retention at all on the NIP after the percolation on both sorbents of a real sample containing the target analyte. Results close to this optimal situation were obtained for the selective extraction of triazines from real waters,[146] bupivacaine and analogs[98] or alfuzosine[92] from plasma. In the case of triazines, the potential of the MIP was compared with the one of an immunosorbent and the same improvement of selectivity was observed as illustrated on Figure 10: a very clean baseline was obtained for the two corresponding chromatograms thus facilitating the detection and the identification of the analytes.

Perspectives

Many successful above-mentioned applications proved that the use of MIPs for the solid phase extraction is a powerful method for the clean-up and the direct selective extraction of compounds at trace levels from various complex matrices. Nevertheless, this field of research still needs improvements in the synthesis of MIPs for the selective extraction of very polar molecules that are not well soluble in conventional solvents. There is also an increasing demand for the development of MIPs for high molecular weight compounds such as proteins or micro-organisms. A very interesting paper was recently published, concerning the selective extraction of bovine haemoglobin by using a MIP synthesized with polyacrylamide in water[158] that seems to present a great potential for this type of molecules.

Moreover, MIPs present numerous advantages over immunosorbents such as their stability and their lower cost of development for most of the

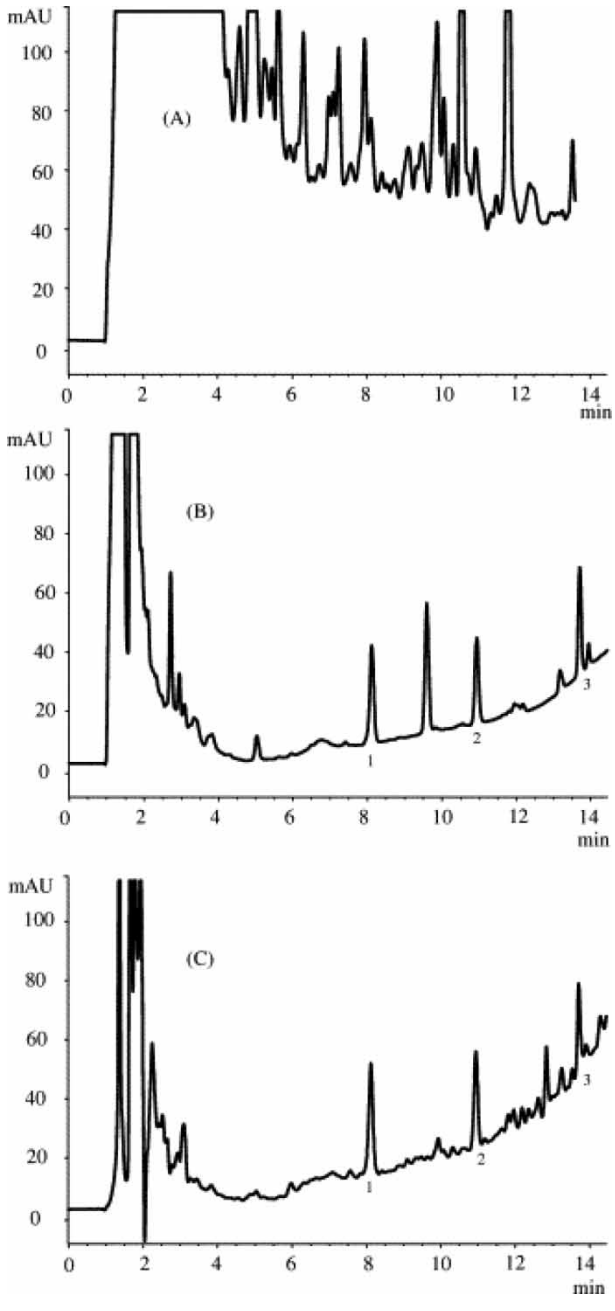

Figure 10. Chromatograms obtained after the injection of a soil extract containing 20 ng/g of triazines (A) without and (B) with a clean-up on the terbutylazine MIP and (C) on the anti-triazines immunosorbent. (1) atrazine; (2) simazine; (3) terbutylazine. UV detection at 220 nm. From 145.

molecules. Their higher capacity also makes the MIPs easier to implement in miniaturized devices. Their coating on SPME fibres was already described,[159] even if some improvements in selectivity are still required. At least, MIPs certainly present a high potential in miniaturized separation systems developed on chips for the selective treatment of low-volume samples. For all these future developments, it is important to keep in mind that MIPs are not intrinsically selective. It is the procedure of extraction (conditions of percolation, washing and elution) that will confer the MIP its selectivity by helping in the development of the selective interactions in the cavities.

SUMMARY AND CONCLUSIONS

MIPs have already been used in several applications, and are close to commercialization in some, such as solid-phase extraction. However, more work needs to be performed to make these a real alternative or complement to affinity supports that use biomolecules as ligands. In particular, work is needed in the development of MIPs that contain a more homogeneous binding site population, have a higher affinity for targets, and can be routinely used in aqueous solvents. Much of the current research efforts with MIPs is already dealing with these problems. In the meanwhile, the outstanding stability of MIPs, their low price, and the fact that they can be tailor-made for analytes for which a biological receptor cannot be found are all properties that make these attractive and suitable for many possible applications in affinity separations.

REFERENCES

1. Sellergren, B. *Molecularly Imprinted Polymers—Man-Made Mimics of Antibodies and Their Applications in Analytical Chemistry*; Elsevier: Amsterdam, 2001.
2. Komiyama, M.; Takeuchi, M.; Mukawa, T.; Asanuma, H. *Molecular Imprinting-From Fundamentals to Applications*; Wiley-VCH: Weinheim, 2002.
3. Arshady, R.; Mosbach, K. Makromol. Chem. **1981**, *182*, 687–692.
4. Wulff, G.; Sarhan, H. Angew. Chem. Intl. Ed. **1972**, *11*, 341.
5. Yilmaz, E.; Mosbach, K.; Haupt, K. Anal. Commun. **1999**, *36*, 167–170.
6. Vlatakis, G.; Andersson, L.I.; Müller, R.; Mosbach, K. Nature **1993**, *361*, 645–647.
7. Umpleby, R.J.; Bode, M.; Shimizu, K.D. Analyst **2000**, *125*, 1261–1265.
8. McNiven, S.; Yokobayashi, Y.; Cheong, S.H.; Karube, I. Chem. Lett. **1997**, *12*, 1297–1298.
9. Umpleby, R.J.; Rushton, G.T.; Shah, R.N.; Rampey, A.M.; Bradshaw, J.C.; Berch, J.K.; Shimizu, K.D. Macromolecules **2001**, *34*, 8446–8452.
10. Steinke, J.H.G.; Dunkin, I.R.; Sherrington, D.C. Trend Anal. Chem. **1999**, *18*, 159–164.

11. Spivak, D.; Shea, K.J. J. Org. Chem. **1999**, *64*, 4627–4634.
12. Wulff, G.; Gross, T.; Schönfeld, R. Angew. Chem. Intl. Ed. **1997**, *36*, 1962–1964.
13. Yano, K.; Tanabe, K.; Takeuchi, T.; Matsui, J.; Ikebukuro, K.; Karube, I. Anal. Chim. Acta **1998**, *363*, 111–117.
14. Lübke, C.; Lübke, M.; Whitcombe, M.J.; Vulfson, E.N. Macromolecules **2000**, *33*, 5098–5105.
15. Mallik, S.; Johnson, R.D.; Arnold, F.H. J. Am. Chem. Soc. **1994**, *116*, 8902–8911.
16. Hart, B.R.; Shea, K.J. J. Am. Chem. Soc. **2001**, *123*, 2072–2073.
17. Kempe, M.; Glad, M.; Mosbach, K. J. Mol. Recog. **1995**, *8*, 35–39.
18. Klein, J.U.; Whitcombe, M.J.; Mulholland, F.; Vulfson, E.N. Angew. Chem. Intl. Ed. **1999**, *38*, 2057–2060.
19. Takeuchi, T.; Fukuma, D.; Matsui, J. Anal. Chem. **1999**, *71*, 285–290.
20. Lanza, F.; Sellergren, B. Anal. Chem. **1999**, *71*, 2092–2096.
21. Panasyuk, T.L.; Mirsky, V.M.; Piletsky, S.A.; Wolfbeis, O.S. Anal. Chem. **1999**, *71*, 4609–4613.
22. Piletsky, S.; Piletska, S.V.; Chen, B.; Karim, K.; Weston, D.; Barrett, G.; Lowe, P.; Turner, A.P.F. Anal. Chem. **2000**, *72*, 4381–4385.
23. Malitesta, C.; Losito, I.; Zambonin, P.G. Anal. Chem. **1999**, *71*, 1366–1370.
24. Peng, H.; Liang, C.; Zhou, A.; Zhang, Y.; Xie, Q.; Yao, S. Anal. Chim. Acta **2000**, *423*, 221–228.
25. Dickert, F.L.; Tortschanoff, M.; Bulst, W.E.; Fischerauer, G. Anal. Chem. **1999**, *71*, 4559–4563.
26. Deore, B.; Chen, Z.; Nagaoka, T. Anal. Chem. **2000**, *72*, 3989–3994.
27. Dai, S.; Shin, Y.; Barnes, C.E.; Toth, L.M. Chem. Mater. **1997**, *9*, 2521–2525.
28. Glad, M.; Norrlöw, O.; Sellergren, B.; Siegbahn, N.; Mosbach, K. hromatogr. **1985**, *347*, 11–23.
29. Makote, K.; Collinson, M. Chem. Commun. **1998**, *3*, 425–426.
30. Sasaki, D.Y.; Rush, D.J.; Daitch, C.E.; Alam, T.M.; Assink, R.A.; Ashley, C.S.; Brinker, C.J.; Shea, K.J. ACS Symp. Ser. **1998**, *703*, 314–323.
31. Markowitz, M.A.; Kust, P.R.; Deng, G.; Schoen, P.E.; Dordick, J.S.; Clark, D.S.; Gaber, B.P. Langmuir **2000**, *16*, 1759–1765.
32. Sasaki, D.Y.; Alam, T.M. Chem. Mater. **2000**, *12*, 1400–1407.
33. Katz, A.; Davis, M.E. Nature **2000**, *403*, 286–289.
34. Lulka, M.F.; Chambers, J.P.; Valdes, E.R.; Thompson, R.G.; Valdes, J.J. Anal. Lett. **1997**, *30*, 2301–2313.
35. Lulka, M.F.; Iqbal, S.S.; Chambers, J.P.; Valdes, E.R.; Thompson, R.G.; Goode, M.T.; Valdes, J.J. Mat. Sci. Eng. C-Bio. S. **2000**, *11*, 101–105.
36. Iqbal, S.S.; Lulka, M.F.; Chambers, J.P.; Thompson, R.G.; Valdes, J.J. Mat. Sci. Eng. C-Bio. S. **2000**, *7*, 77–81.
37. Lahav, M.; Kharitonov, A.B.; Katz, O.; Kunitake, T.; Willner, I. Anal. Chem. **2001**, *73*, 720–723.
38. Lee, S.W.; Ichinose, I.; Kunitake, T. Langmuir **1998**, *14*, 2857–2863.
39. Lee, S.W.; Ichinose, I.; Kunitake, T. Chem. Lett. **1998**, *12*, 1193–1194.
40. Kato, M.; Nishide, H.; Tsuchida, E.; Sasaki, T. J. Polym. Sci. Polym. Chem. **1981**, *19*, 1803–1809.
41. Kido, H.; Miyama, T.; Tsukagoshi, K.; Maeda, M.; Takage, B. Anal. Sci. **1992**, *8*, 749–753.
42. Chen, H.; Olmstead, M.M.; Albright, R.L.; Devenyi, J.; Fish, R.H. Angew. Chem. Intl. Ed. **1997**, *36*, 642–645.

43. Aherne, A.; Alexander, C.; Payne, M.J.; Perez, N.; Vulfson, E.N. J. Am. Chem. Soc. **1996**, *118*, 8771–8772.
44. D'Souza, S.M.; Alexander, C.; Carr, P.W.; Waller, A.M.; Whitcombe, M.J.; Vulfson, E.N. Nature **1999**, *398*, 312–316.
45. Shi, H.Q.; Tsai, W.B.; Garrison, M.D.; Ferrari, S.; Ratner, B.D. Nature **1999**, *398*, 593–597.
46. Ye, L.; Weiss, R.; Mosbach, K. Macromolecules **2000**, *33*, 8239–8245.
47. Ye, L.; Cormack, P.A.G.; Mosbach, K. Anal. Comm. **1999**, *36*, 35–38.
48. Surugiu, I.; Ye, L.; Yilmaz, E.; Dzgoev, A.; Danielsson, B.; Mosbach, K.; Haupt, K. Analyst **2000**, *125*, 13–16.
49. Biffis, A.; Graham, N.B.; Siedlaczek, G.; Stalberg, S.; Wulff, G. Macromol. Chem. Phys. **2001**, *202*, 163–171.
50. Ishi-i, T.; Nakashima, K.; Shinkai, S. Chem. Comm. **1998**, *9*, 1047–1048.
51. Ishi-i, T.; Iguchi, R.; Shinkai, S. Tetrahedron **1999**, *55*, 3883–3892.
52. Zimmerman, S.C.; Wendland, M.S.; Rakow, N.A.; Zharov, I.; Suslick, K.S. Nature **2002**, *418*, 399–403.
53. Xia, Y.; Whitesides, G.M. Angew. Chem. Intl. Ed. **1998**, *37*, 550–575.
54. Yan, M.; Kapua, A. Anal. Chim. Acta **2001**, *435*, 163–167.
55. Piletsky, S.A.; Matuschewski, H.; Schedler, U.; Wilpert, A.; Piletska, E.V.; Thiele, T.A.; Ulbricht, M. Macromolecules **2000**, *33*, 3092–3098.
56. Sulitzky, C.; Ruckert, B.; Hall, A.J.; Lanza, F.; Unger, K.; Sellergren, B. Macromolecules **2002**, *35*, 79–91.
57. Panasyuk-Delaney, T.; Mirsky, V.M.; Ulbricht, M.; Wolfbeis, O.S. Anal. Chim. Acta **2001**, *435*, 157–162.
58. Schmidt, R.H.; Haupt, K. Chem. Mater. **2005**, *17*, 1007–1016.
59. Schmidt, R.H.; Mosbach, K.; Haupt, K. Adv. Mater. **2004**, *16*, 719–722.
60. Pérez, N.; Whitcombe, M.J.; Vulfson, e.n. Macromolecules **2001**, *34*, 830–836.
61. Yilmaz, E.; Haupt, K.; Mosbach, K. Angew. Chem. Intl. Ed. **2000**, *39*, 2115–2118.
62. Matsui, J.; Nicholls, I.A.; Takeuchi, T. Tetrahedron Asymmetr. **1996**, *2*, 1357–1361.
63. Ramström, I.; Nicholls, I.A.; Mosbach, K. Tetrahedron Asymmetr. **1994**, *5*, 649–656.
64. Mayes, A.G.; Andersson, L.I.; Mosbach, K. Anal. Biochem. **1994**, *222*, 483–488.
65. Sellergren, B.; Shea, K.J. J. Chromatogr. A **1995**, *690*, 29–39.
66. Kempe, M. Anal. Chem. **1996**, *68*, 1948–1953.
67. Mayes, A.G.; Mosbach, K. Anal. Chem. **1996**, *68*, 3769–3774.
68. Hosoya, K.; Yoshihako, K.; Shirasu, Y.; Kimata, K.; Araki, T.; Tanaka, N.; Haginaka, J. J. Chromatogr. **1996**, *728*, 139–148.
69. Nilsson, K.G.I.; Lindell, J.; Norrlöw, O.; Sellergren, B. J. Chromatogr. A **1994**, *680*, 57–61.
70. Vallano, P.T.; Remcho, V.T. J. Chromatogr. A **2000**, *887*, 125–135.
71. Schweitz, L.; Andersson, L.I.; Nilsson, S. Anal. Chem. **1997**, *69*, 1179–1183.
72. Lin, J.M.; Nakagama, T.; Uchiyama, K.; Hobo, T. J. Pharmaceut. Biomed. Anal. **1997**, *15*, 1351–1358.
73. Lin, J.M.; Nakagama, T.; Uchiyama, K.; Hobo, T. Chromatographia **1996**, *43*, 585–591.
74. Spégel, P.; Schweitz, L.; Nilsson, S. Electrophoresis **2001**, *22*, 3833–3841.
75. Kriz, D.; Berggren-Kriz, C.; Andersson, L.I.; Mosbach, K. Anal. Chem. **1994**, *66*, 2636–2639.

76. Suedee, R.; Songkram, C.; Petmoreekul, A.; Sangkunakup, S.; Sankasa, S.; Kongyarit, N. J. Planar Chromat. **1998**, *11*, 272–276.
77. Suedee, R.; Songkram, C.; Petmoreekul, A.; Sangkunakup, S.; Sankasa, S.; Kongyarit, N. J. Pharmaceut. Biomed. Anal. **1999**, *19*, 519–527.
78. Mathew-Krotz, J.; Shea, K.J. J. Am. Chem. Soc. **1996**, *118*, 8154–8155.
79. Dzgoev, A.; Haupt, K. Chirality **1999**, *11*, 465–469.
80. Wang, K.; Kobayashi, T.; Fujii, N. Langmuir **1996**, *12*, 4850–4856.
81. Hedborg, E.; Winquist, F.; Lundström, I.; Andersson, L.I.; Mosbach, K. Sens. Actuators A **1993**, *36–38*, 796–799.
82. Sergeyeva, T.A.; Piletsky, S.A.; Brovko, A.A.; Slinchenko, E.A.; Sergeeva, L.M.; Panasyuk, T.L.; Elskaya, A.V. Analyst **1999**, *124*, 331–334.
83. Kochkodan, V.; Weigel, W.; Ulbricht, M. Analyst **2001**, *126*, 803–809.
84. Hong, J.-M.; Anderson, P.E.; Qian, J.; Martin, C.E. Chem. Mater. **1998**, *10*, 1029–1033.
85. Hennion, M.-C.; Pichon, V. J. Chromatogr. A **2003**, *1000*, 29–52.
86. Hage, D.S. J. Chromatogr. B: Biomed. Sci. Applic. **1998**, *715*, 3–28.
87. Pichon, V.; Delaunay-Bertoncini, N.; Hennion, M.-C. *Immunosorbents in Sample Preparation*; Elsevier: Amsterdam, 2002.
88. Svenson, J.; Nicholls, I.A. Anal. Chim. Acta **2001**, *435*, 19–24.
89. Sellergren, B. Anal. Chem. **1994**, *66*, 1578–1582.
90. Zi-Hui, Z.; Qin, L. Anal. Chim. Acta **2001**, *435*, 121–127.
91. Le Moullec, S.; Bégos, A.; Pichon, V.; Béllier, B. J. Chromatogr. Asubmitted.
92. Chapuis, F.; Mullot, J.-U.; Pichon, V.; Tuffal, G.; Paolucci, F.; Hennion, M.-C. J. Chromatogr. Asubmitted.
93. Nakamura, M.; Ono, M.; Nakajima, T.; Ito, Y.; Aketo, T.; Haginaka, J. J. Pharmaceut. Biomed. Anal. **2000**, *37*, 231–237.
94. Lai, J.-P.; Niessner, R.; Knopp, D. Anal. Chim. Acta **2004**, *522*, 137–144.
95. Kubo, T.; Hosoya, K.; Watabe, Y.; Ikegami, T.; Tanaka, N.; Sano, T.; Kaya, K. J. Chromatogr. A **2003**, *987*, 389–394.
96. Sanbe, H.; Hoshina, K.; Haginaka, J. Analyst **2005**, *130*, 38–40.
97. Dirion, B.; Cobb, Z.; Schillinger, E.; Andersson, L.I.; Sellergren, B. J. Amer. Chem. Soc. **2003**, *125*, 15101–15109.
98. Andersson, L.I.; Hardenborg, E.; Sandberg-Stall, M.; Moller, K.; Henriksson, J.; Bramsby-Sjostrom, I.; Olsson, L.-I.; Abdel-Rehim, M. Anal. Chim. Acta **2004**, *526*, 147–154.
99. Theodoridis, G.; Zacharis, C.K.; Tzanavaras, P.D.; Themelis, D.G.; Economou, A. J. Chromatogr. A **2004**, *1030*, 69–76.
100. Tarley, C.x.S.R.T.; Kubota, L.T. Anal. Chim. Acta **2005**, *548*, 11–19.
101. Lai, E.P.C.; Wu, S.G. Anal. Chim. Acta **2003**, *481*, 165–174.
102. Wu, S.G.; Lai, E.P.C.; Mayer, P.M. J. Pharmaceut. Biomed. Anal. **2004**, *36*, 483–490.
103. Zhang, M.; Xie, J.; Zhou, Q.; Chen, G.; Liu, Z. J. Chromatogr. A **2003**, *984*, 173–183.
104. Mena, M.L.; Agui, L.; Martinez-Ruiz, P.; Yanbez-Sedeno, P.; Reviejo, A.J.; Pingarron, J.M. Anal. Bioanal. Chem. **2003**, *376*, 18–25.
105. Caro, E.; Marce, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F. J. Chromatogr. A **2003**, *995*, 233–238.
106. Baggiani, C.; Giovannoli, C.; Anfossi, L.; Tozzi, C. J. Chromatogr. A **2001**, *938*, 35–44.
107. Brambilla, G.; Fiori, M.; Rizzo, B.; Crescenzi, V.; Masci, G. J. Chromatogr. B **2001**, *759*, 27–32.

108. Berggren, C.; Bayoudh, S.; Sherrington, D.; Ensing, K. J. Chromatogr A **2000**, *889*, 105–110.
109. Venn, R.F.; Goody, R.J. Chromatographia **1999**, *50*, 407–414.
110. Moller, K.; Nilsson, U.; Crescenzi, C. J. Chromatogr. B **2004**, *811*, 171–176.
111. Dong, X.; Wang, W.; Ma, S.; Sun, H.; Li, Y.; Guo, J. J. Chromatogr. A **2005**, *1070*, 125–130.
112. Hu, S.-G.; Li, L.; He, X.-W. J. Chromatogr. A **2005**, *1062*, 31–37.
113. Xie, J.; Zhu, L.; Xu, X. Anal. Chem. **2002**, *74*, 2352–2360.
114. Haginaka, J.; Sanbe, H. Anal. Chem. **2000**, *72*, 5206–5210.
115. Feng, S.Y.; Lai, E.P.C.; Dabek-Zlotorzynska, E.; Sadeghi, S. J. Chromatogr. A **2004**, *1027*, 155–160.
116. Chianella, I.; Piletsky, S.A.; Tothill, I.E.; Chen, B.; Turner, A.P.F. Biosens. Bioelectr. **2003**, *18*, 119–127.
117. Caro, E.; Marce, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F. J. Chromatogr. A **2004**, *1047*, 175–180.
118. Caro, E.; Marce, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F.J. J. Chromatogr. B **2004**, *813*, 137–143.
119. Zander, A.; Findlay, P.; Renner, T.; Sellergren, G.; Swietlow, A. Anal. Chem. **1998**, *70*, 3304–3314.
120. Masque, N.; Marce, R.M.; Borrull, F.; Cormack, P.A.G.; Sherrington, D.C. Anal. Chem. **2000**, *72*, 4122–4126.
121. Yu, J.C.C.; Krushkova, S.; Lai, E.P.C.; Dabek-Zlotorzynska, E. Anal. Bioanal. Chem. **2005**, *382*, 1534–1540.
122. Hu, S.G.; Wang, S.W.; He, X.W. Analyst **2003**, *128*, 1485–1489.
123. Bereczki, A.; Tolokan, A.; Horvai, G.; Horvath, V.; Lanza, F.; Hall, A.J.; Sellergren, B. J. Chromatogr A **2001**, *930*, 31–38.
124. Martin-Esteban, A.; Turiel, E.; Stevenson, D. Chromatographia **2001**, *53*, S434–S437.
125. Tamayo, F.G.; Casillas, J.L.; Martin-Esteban, A. J. Chromatogr. A **2005**, *1069*, 173–181.
126. Carabias-Martínez, R.; Rodríguez-Gonzalo, E.; Herrero-Hernández, E.; Díaz-García, M.E. J. Sepn. Sci. **2005**, *28*, 453–461.
127. Tamayo, F.G.; Casillas, J.L.; Martin-Esteban, A. Anal. Chim. Acta **2003**, *482*, 165–173.
128. Mena, M.L.; Martinez-Ruiz, P.; Reviejo, A.J.; Pingarron, J.M. Anal. Chim. Acta **2002**, *451*, 297–304.
129. Sanbe, H.; Haginaka, J. Analyst **2003**, *128*, 593–597.
130. Martin, P.D.; Jones, G.R.; Stringer, F.; Wilson, I.D. J. Pharmaceut. Biomed. Anal. **2004**, *35*, 1231–1239.
131. Molinelli, A.; Weiss, R.; Mizaikoff, B. J. Agric. Food Chem. **2002**, *50*, 1804–1808.
132. Xie, J.; Chen, L.; Li, C.; Xu, X. J. Chromatogr. B **2003**, *788*, 233–242.
133. Zhu, L.; Chen, L.; Xu, X. Anal. Chem. **2003**, *75*, 6381–6387.
134. Andersson, L.I.; Paprica, A.; Arvidsson, T. Chromatographia **1997**, *46*, 57–62.
135. Theodoridis, G.; Kantifes, A.; Manesiotis, P.; Raikos, N.; Soukali-Papadopoulou, H. J. Chromatogr. A **2003**, *987*, 103–109.
136. Puoci, F.; Garrefa, C.; Iemma, F.; Muzzalupo, R.; Spizzirri, U.G.; Picci, N. Food Chem. **2005**, *93*, 349–353.
137. Guzman-Vazquez de Prada, A.; Martinez-Ruiz, P.; Reviejo, A.J.; Pingarron, J.M. Anal. Chim. Acta **2005**, *539*, 125–132.

138. Zhu, Q.-Z.; Degelmann, P.; Niessner, R.; Knopp, D. Environ. Sci. Technol. **2002**, *36*, 5411–5420.
139. Dong, X.; Wang, N.; Wang, S.; Zhang, X.; Fan, Z. J. Chromatogr. A **2004**, *1057*, 13–19.
140. Mullett, W.M.; Lai, E.P.C. Anal. Chem. **1998**, *70*, 3636–3641.
141. Muldoon, M.T.; Stanker, L.H. Anal. Chem. **1997**, *69*, 803–808.
142. Ferrer, I.; Lanza, F.; Tolokan, A.; Horvath, V.; Sellergren, B.; Horvai, G.; Barcelo, D. Anal. Chem. **2000**, *17*, 3934–3941.
143. Koeber, R.; Fleischer, C.; Lanza, F.; Boos, K.-S.; Sellergren, B.; Barcelo, D. Anal. Chem. **2001**, *73*, 2437–2444.
144. Pap, T.; Horvath, V.; Tolokan, A.; Horvai, G.; Sellergren, B. J. Chromatogr. A **2002**, *973*, 1–12.
145. Chapuis, F.; Pichon, V.; Lanza, F.; Sellergren, B.; Hennion, M.-C. J. Chromatogr. A **2003**, *999*, 23–33.
146. Chapuis, F.; Pichon, V.; Lanza, F.; Sellergren, B.; Hennion, M.-C. J. Chromatogr. B **2004**, *804*, 93–101.
147. Turiel, E.; Martin-Esteban, A.; Fernandez, P.; Perez-Conde, C.; Camara, C. Anal. Chem. **2001**, *73*, 5133–5141.
148. Bjarnason, B.; Chimuka, L.; Ramstrom, O. Anal. Chem. **1999**, *71*, 2152–2156.
149. Hu, S.-G.; Li, L.; He, X.-W. Anal. Chim. Acta **2005**, *537*, 215–222.
150. Piletsky, S.; Piletska, E.; Karim, K.; Foster, G.; Legge, G.; Turner, A. Anal. Chim. Acta **2004**, *504*, 123–130.
151. Mullett, W.M.; Walles, M.; Levsen, K.; Borlak, J.; Pawliszyn, J. J. Chromatogr. B **2004**, *801*, 297–306.
152. Yoshizako, K.; Hosoya, K.; Iwakoshi, Y.; Kimata, K.; Tanaka, N. Anal. Chem. **1998**, *70*, 386–389.
153. Zurutuza, A.; Bayoudh, S.; Cormack, P.A.G.; Dambies, L.; Deere, J.; Bischoff, R.; Sherrington, D.C. Anal. Chim. Acta **2005**, *542*, 14–19.
154. Delaunay-Bertoncini, N.; Pichon, V.; Hennion, M.-C. J. Chromatogr. A **2003**, *999*, 3–15.
155. Ensing, K. LC-GC Europe **2002** (January), 16–25.
156. Matsui, J.; Miyoshi, Y.; Doblhoff-Dier, O.; Takeuchi, T. Anal. Chem. **1995**, *67*, 4404–4408.
157. Umpleby, R.J.; Baxter, S.C.; Bode, M.; Berch, J.K., Jr.; Shah, R.N.; Shimizu, K.D. Anal. Chim. Acta **2001**, *435*, 35–42.
158. Hawkins, D.M.; Stevenson, D.; Reddy, S.M. Anal. Chim. Acta **2005**, *542*, 61–65.
159. Koster, E.H.M.; Crescenzi, C.; Den Hoedt, C.; Ensing, K.; de Jong, G.J. Anal. Chem. **2001**, *73*, 3140–3145.

Received November 4, 2005
Accepted December 22, 2005
Manuscript 6670J