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Targeted extraction of active compounds from natural products by molecularly imprinted polymers

Review Article

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Abstract: One of the most promising separation techniques that have emerged during the last decade is based on the use of molecularly imprinted polymers (MIPs). MIPs are stable polymers that possess specific cavities designed for a template molecule, endowed with excellent selectivity compared to regular solid phase extraction techniques. Molecularly imprinted solid-phase extraction (MISPE) has already shown a high efficiency for the sample preparation from complex matrices. Natural products received huge attention in recent years. Indeed, the application of MISPE for the screening of natural products appears extremely interesting not only for the selective extraction of a target compound but also for the concomitant discovery of new drug candidates, promising sources of therapeutic benefits. In the present review, examples of recognition and separation of active components from natural extracts are emphasized. MIPs are very promising materials to mimic the recognition characteristics exhibited by enzymes or receptors although further developments are necessary to fully exploit their wide potential.

Keywords: *Molecularly imprinted polymers • Solid-phase extraction • Pharmaceutical analysis • Screening*

1. Introduction

Medicinal natural products can be considered as natural combinatorial chemical libraries with abundant diversities in chemical structures and pharmacological activities [1]. The mechanisms of action of natural medicinal products are often difficult to clarify, resulting from the lack of effective techniques for the separation or isolation of active components. As it is easily automated and flexible, solid phase extraction (SPE) is the most widely used technique to provide cleaner extracts prior to analysis [2]. Traditional SPE sorbents (*e.g.* silica-gel, polyamide, ion-exchange types and reverse-phase) however cannot separate analytes efficiently from complex matrices, due to their sometimes unsatisfying broad selectivity [3]. As promising alternatives to such sorbents, selective materials called molecularly imprinted polymers (MIPs) can be used. The concept of molecular imprinting technology as we know it today was introduced in 1972 by the laboratory of Wulff and Sarhan [4]. Their molecular imprinting procedure was based on a covalent attachment strategy between monomers containing functional groups and template molecules (Fig. 1). Copolymerization of the template monomer complex with an excess of a cross-linking agent in the presence of a porogenic solvent produced rigid macroporous polymers. Removal of the template molecules by chemical reaction rendered the polymers able to selectively rebind the template or the target molecules *via* the same covalent interactions. For the first time, binding sites with a structure complementary to the template structure were formed on synthetic organic polymers. In the early

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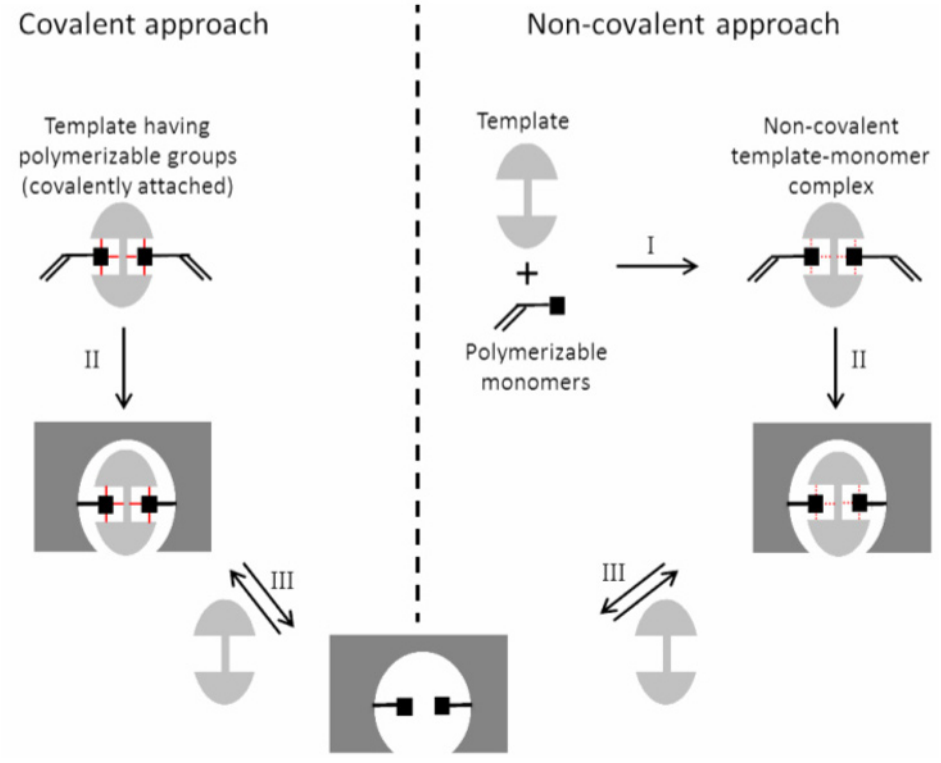


Figure 1. Schematic representations of two different molecular imprinting principles (adapted from [6]). Red line represents covalent bond and dotted red line symbolizes non-covalent bonds.

1980s, the group of Mosbach expanded the scope of molecular imprinting by introducing a general non-covalent approach that differs in the way the template is linked to the functional monomers and, subsequently, to the polymeric binding sites. The technique is based on a spontaneously non-covalent pre-assembly of the functional monomers around the template, free radical polymerization (FRP) with a cross-linker and then template extraction with solvents followed by rebinding *via* non-covalent interactions (Fig. 1) [5]. The main drawback of this approach is that the prepolymerization step is not an entirely-defined process. The weak stability of non-covalent interactions may lead to pre-polymerization complexes with different template/monomer stoichiometries resulting in binding sites with different affinities. In spite of that drawback, non-covalent imprinting is now the most widely applied strategy to generate MIPs. Tunable to a large panel of molecules, imprinted materials can be easily synthetized and evaluated within a reasonable time and are suitable for practical repetitive use since the cleavage and rebinding of templates are technically straightforward [6-8].

Such MIPs have been shown to exhibit recognition characteristics comparable to those exhibited by antibodies for their antigens [9-12]. MIPs can become a viable alternative to antibody-based highly specific molecular recognition techniques ("immunoassays") as they offer superior characteristics with respect to chemical, mechanical and thermal stability. In contrast with antibodies, MIPs can be used in a range of extreme environments (high temperatures, extremes of pH, ionic strength, organic solvents) [13]; and they can be reused and stored for several months without loss of performance [14]. Their synthesis is simple and contrasts with the often time-consuming generation of antibodies which involves immunization of animals or cell modifications. MIPs can thus be more easily generated than antibodies which are problematic due to the fact that different individuals and preparations may elicit antibodies with different properties [7]. Due to their outstanding advantages, MIPs have gained rapidly increasing attention from the scientific community as reflected by the continually growing number of published papers in this analytical field [7,15-17]. Most intensively studied are the MIPs used as recognition elements in chemosensors and as sorbents in chromatography. MIPs are also applicable in galenical pharmacy (MIPs as drug delivery systems or as trap systems for undesirable compounds) [18], in chemical or enzymatic catalysis and in the screening of combinatorial libraries.

The first application of molecularly imprinted solid phase extraction (MISPE) was reported in 1994 when Sellergren realized the direct extraction of pentamidine from urine [19]. This paper demonstrated the possibility

of decreasing analysis times with simpler instrumentation compared to conventional isolation with usual sorbents. It aroused a huge interest from scientists towards applications of MISPE in different matrices. Whereas the first MISPE applications have focused on biological and environmental samples, other matrices, such as drug, food and herbal products have received later attention [8]. The present review describes the methodology of molecularly imprinted solid phase extraction (MISPE), including the preparation of MIPs for SPE, and recent applications of MISPE to the targeted extraction of active compounds from natural products.

2. Molecularly imprinted polymers in solid phase extractions

2.1. MIP preparation

2.1.1. Influence of polymerization parameters

The synthesis of a MIP results in the formation of a polymer containing cavities complementary to the template in size, shape and position of the functional groups. Currently, the most common approach to synthesize molecularly imprinted sorbents is non-covalent imprinting. The stability of the non-covalent pre-polymerization complex is then crucial to increase the number of imprinted sites and to minimize non-specific binding. The synthesis reagents (functional monomers, template molecule, cross-linking agent, porogenic solvent and radical initiator) and the initiator system are factors involved in the non-covalent adduct stability.

2.1.1.1. Functional Monomers

In order to obtain materials with good recognition properties, the functional monomers must be able to form a strong complex with the template. The functional groups of the monomers have to be complementary to those of the template. It is preferable to choose acidic monomers such as methacrylic acid (MAA) if the template presents basic functional groups; whereas, basic monomers such as 4-vinylpyridine (4-VP) are preferred for templates bearing acidic groups. Uncharged monomers such as 2-hydroxyethylmethacrylate (HEMA) [20] or acrylamide (AA) can also provide good selectivity. For example, the use of AA led to materials showing high selectivity for templates with carboxylic [21], amides [22] or alcohol groups [23-27]. A vast choice (acidic, basic and neutral) of functional monomers is commercially available. From these, MAA is the most commonly used monomer; its broad applicability is related to its carboxylic acid group that can act as a hydrogen bond and proton donor as well as a hydrogen bond acceptor. MAA is thus able to interact either with acidic, basic or neutral compounds by establishing a strong hydrogen bond. Nevertheless, MAA and the other polar monomers are not suited to the imprinting of poorly polar or apolar templates which are unable to form hydrogen bonds. Although hydrophobic monomers (*e.g.* styrene) can be used in such cases, the best solution is to develop and synthesize monomers with a structure complementary to the template structure that will interact via hydrophobic and Van der Waals forces [28]. In other cases, it may be beneficial to synthesize monomers with the optimal functionalities to complement those of the template. The template-monomer complexes can then be stabilized by multiple hydrogen bonds which provide high affinity to MIP binding sites [29,30]. But, discovering new functional monomers is a very time-consuming process. Consequently, for water-soluble compound imprinting, Guo's group recently developed MIPs based on a co-polymer melamine-urea-formaldehyde (a hydrogel) as the functional monomer [31,32]. The noticeable advantages of MIP preparation with hydrogels lie in a relatively simple and fast procedure compared to the traditional methods due to (i) the functional monomer hydrophilicity, and (ii) ability from water-soluble compounds to be directly selected as the template molecule without cross-linker.

Another factor that will affect the number and recognition properties of imprinted cavities is the concentration of the functional monomer. The optimum template-monomer molar ratio (T/M) is often determined via an iterative scheme between synthesis and generated polymers assessment after each considered formulation. Such an optimization approach was carried out first by Sellergren *et al*. [33]. They synthetized MIPs for L-phenylalanine anilide (L-PA) using various concentrations of MAA. At a concentration of 25 mol% MAA (the rest being the cross-linking monomer), the ability to discriminate D- and L- PA reached a maximum. Based on these results, most of researchers chose a 1:4 T/M ratio. For templates offering more than 4 sites for interaction with the functional monomer, the T/M can be decreased and adapted to the template structure. However, a higher monomer concentration must be avoided; otherwise, the majority of the monomer units are not complexed to templates; and, instead, form a large number of nonselective background sites [33,34]. Zhang *et al*. [35] recently reported that monomer aggregation has the potential to reduce the number of these background sites due to the strong tendency of MAA to form hydrogen-bonded dimers. Binding sites formed from the dimerized monomer are deactivated as the recognition groups are blocked by self-association. The number of templated sites was also reduced, but the overall effect was beneficial as the number of background sites was reduced by a large percentage.

These results contribute to explain why MAA is such a versatile monomer in molecular imprinting.

2.1.1.2. Template molecule

The template molecule is selected along criteria such as cost, availability, stability, toxicity, solubility and functional groups that can provide interaction sites with the functional monomers [36]. This last parameter has a strong influence on the MIP selectivity. Indeed, pre-organization of functional groups as well as shape selectivity are both responsible for MIPs molecular recognition. Previous reports [37] indicated that templates offering multiple functional group interactions were likely to yield binding sites of higher specificity and affinity. This hypothesis was contradicted by the work of Simon *et al*. [38] which indicated that greater selectivity was found for templates with two or less functional groups, for which the dominant mode of molecular recognition is shape selectivity. The lowered performance for templates with three or more functional groups may result from a competition between shape selectivity and pre-organization of functional groups that does not appear to work in concert with each other during the imprinting process or in the rebinding behavior. Another interesting observation for templates with three or more functional groups is that the imprinting performance goes up as the distance between the functional groups increases [38]. The importance of shape selectivity was also highlighted by another work from the group of Spivak revealing two major contributions of cavity structure on MIP selectivity [39]. First, steric considerations play a dominant role in cases where a too large molecule structure does not fit into an imprinted site formed from a smaller template molecule. Secondly, molecular structures that are equal to or smaller than those of the template molecule are selected by maximizing Van der Waals interactions within the MIP binding site. Moreover, this work also confirmed the influence of template structure, revealing that unique branching architectures provide better selectivity *versus* straight-chain hydrocarbons, with complete loss of recognition by straight-chain groups with eight or more carbons.

In order to increase the number of pre-polymer complexes, researchers have tried to increase the template concentration while keeping the amount of monomer constant; however, maximizing the amount of template did not optimize the MIP's performance. One suggested undesirable effect of adding an excess of template is the loss of site integrity related to the extent of template self-association [40]. For templates with two functional groups, as the Template/Monomer ratio increases, the highly selective sites arising from 2-points binding were likely to be substituted by less selective 1-point binding sites [41]. However, similar results were later obtained for templates with one functional group for which only one interaction with the functional monomer was expected. Researchers suggested that the number of functional groups in the polymer binding site is not determined directly by the prepolymer complex solution phase; rather, it is hypothesized that during polymerization that phase separation phenomena that take place could allow for the aggregation of polar functional groups in binding site "pockets", resulting in binding sites with multiple functionalities. Thus, multiple functional monomer interactions in the final polymer, regardless of the pre-polymer stoichiometry, appear to be responsible for the high-affinity binding sites seen for non-covalently imprinted polymers [42]. According to these experimental considerations, a template amount of about 5% of the total amount of monomer is usually chosen as a starting working concentration and is further optimized. For MISPE preparation, the template must be available in preparative amount (often 1 mmol) [36]. Therefore, if the target molecule is expensive or difficult to synthesize, a useful solution is to substitute it *via* a structural analogue, called a *"dummy template"* [43]. The use of dummy templates can also prevent template *"bleeding"* [44] which results from the small amount of template that remains strongly bound to the polymer after post-synthesis washing. Template bleeding is the cause of false results when the material is used for sample preparation prior to analytical quantification. In order to reduce bleeding to acceptable levels, possible solutions consist of avoiding solvent switches, drying the sorbent or applying thermal post-treatment [45]. Ellwanger *et al*. preposed in 2001 to use microwave-assisted extraction with solvents mixture containing trifluoroacetic or formic acid and to replace MISPE by on-line coupled column techniques containing a minimum amount of MIP sorbent [46].

2.1.1.3. Cross-linker

The cross-linker copolymerizes the monomers around the template with covalent binding and fixes them in space in a stable arrangement complementary to the template. Its role is also to guarantee a high rigidity of the polymeric matrix. In most imprinting systems, the use of an excess amount of cross-linkers relative to the functional monomers is necessary for recognition [33]. However, a cross-linker percentage higher than 80% has generally adverse effects on MIPs performance; on one hand, binding sites are covered with polymer which renders difficult the removal and rebinding of the template [47] and, on the other hand, the interactions between cross-linkers and solvents are increased resulting in mobile binding sites which bring down the

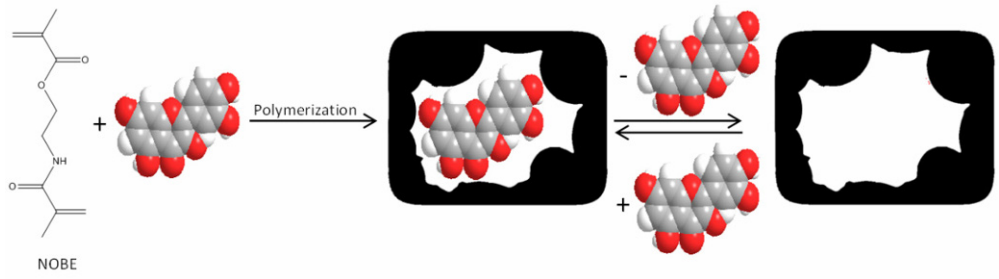


Figure 2. Outline of the OMNiMIP strategy. Adapted from [50].

recognition specificity of MIPs. The performance of a cross-linker is mainly related to its solvation propensity. For example, cross-linkers such as divinylbenzene (DVB) and ethylene glycol dimethacrylate (EDMA) are both better than pentaerythritol triacrylate (PTA) and N,N-methylenebisacrylamide (BisAA). Indeed, PTA and BisAA are highly solvated by many of the common solvents used in the synthesis and rebinding experiments. These cross-linkers are less available to interact with template molecules resulting in MIPs with lower affinity [34]. A minimum of wettability however, is necessary to allow the rebinding of template with MIP. In contrast with EDMA, DVB is too poorly solvated and too rigid to provide good recognition properties. Moreover, DVB exhibits lower thermal stability than EDMA which is, therefore, the most convenient cross-linker for a large variety of template molecules [37,48]. Gavrilovic *et al*. [49] recently reported that the addition of a "functional cross-linker" to the traditional polymerization reagents is able to enhance 'shape selectivity'. A functional cross-linker based on the steroidal template DHT (5-dihydrotestosterone, 17-hydroxy-5-androstan-3-one) was designed and synthesized to interact with the template in the pre-polymerization mixture *via* van der Waals stacking interactions. The developed polymer thus presented an improvement in terms of abundance and homogeneity of the binding sites which renders very promising the use of such "functional cross-linkers". All the factors described above show that optimization of MIP formulation components is complicated by many variables. To simplify the MIP design, Spivak and coworkers [50-52] have developed the "OMNiMIPs" (one-monomer molecularly imprinted polymers) strategy. This approach uses only one monomer, N,O-bismethacryloylethanolamine (NOBE), that incorporates the template-binding functionality with the necessary cross-linking features for molecular recognition and network formation (Fig. 2). This results in a much simpler MIP formation since it permits elimination of variables such as choice of functional monomer and cross-linker, ratio of functional monomer/cross-linker and ratio of functional monomer/template. Moreover, the utilization of NOBE alone often provides MIPs with higher performance than MIPs incorporating functional monomer (*e.g*. MAA). This may be attributed to a higher functional group density by the polymers in the vicinity of the template which may provide more positive binding interactions. NOBE also probably provides more rigid polymers with higher steric specificity of the binding pocket.

2.1.1.4. Porogenic solvent

The solvent determines the timing of the growing polymer phase separation during the polymerization step. If the solubility phase of the porogen is low, the precipitation occurs early which results in a material with larger pores and lower surface area. Higher solubility phase porogens will generally be preferred for an application in liquid media like SPE, as larger surface areas improve the accessibility to cavities [53]. A minimum volume of solvent is nevertheless necessary, otherwise the polymer becomes dense and impenetrable, leading to a low surface area [54]. Surprisingly however, Sellergren *et al*. [55] obtained the highest selectivity with dichloromethane which provides polymers with a weak porosity and reduced surface area. Spivak *et al*. [56] mentioned also the example of chloroform which often provides optimum results whereas the polymers obtained have little or no porosity. These two papers concluded that there is no evident correlation between polymer morphology and the recognition properties of the materials. In fact, it appears that porosity is not required if the solvation is sufficient to allow the diffusion of substrates through the MIP; the polarity of the solvent, therefore, seems to be the only parameter that influences molecular recognition. The solvent has to be chosen considering the stability of the monomer-template assemblies. In most cases, as template and monomers interact via hydrogen bonding, weakly polar and aprotic solvents are chosen; the most widely used solvents are acetonitrile, chloroform, dichloromethane and toluene [57]. Protic and/or polar solvents, such as mixtures of alcohols and water, can be used in the imprinting of hydrophilic templates and poorly

polar templates, which will interact with the functional monomer *via* strong electrostatic [58,59] or hydrophobic interactions [28], respectively.

2.1.1.5. Initiator system

The initiator is a factor critically important in creating high performance MIPs. In FRP, the free radical initiator can be generated thermochemically or photochemically. As each initiator has its own decomposition rate at a given temperature, the initiator is chosen with regard to its thermal resistance and the solubility of the template molecule. Azoinitiators (*e.g.* 2,2'-azobisisobutyronitrile, AIBN) and acetophenone derivatives (*e.g.* 2,2-dimethoxy-2-phenylacetophenone, DMPA) are the most commonly used initiators in molecular imprinting (Table 1). The general recommendation based on the work of Mijangos *et al.* [60] is that MIPs should be synthesized over a long period of time using low concentrations of initiators and low temperatures for higher recognition properties. A negative effect of high temperatures on the quality of the formed imprints was also demonstrated in previous works in which the best performances were obtained for polymers prepared by photo-initiation [33,61].

2.1.2. Polymerization techniques

2.1.2.1. Bulk polymerization

Table 2 shows that most of the MIPs intended to be used in SPE are produced by a bulk polymerization method in which all the components involved are simply mixed. The resultant monolith polymer is then ground and sieved to obtain particles of an adequate size range for subsequent use. Although simple, the bulk polymerization (BP) has its drawbacks which are reported in Table 3. To overcome such problems, other polymerization methods have been developed: principally *in situ*, precipitation, suspension, and multi-step swelling polymerizations. All these approaches are summarized in Table 3 with advantages and drawbacks for each method.

2.1.2.2. In-situ polymerization

For a typical in-situ polymerization procedure, the reagents for molecular imprinting are mixed and dispensed into the container that will be used for MISPE, either the cartridge (off-line mode) [62], the column (on-line or in-line mode) [62,63] or the glass vial (batch mode) [64,65]. Most often the polymerization is initiated by heating. The resultant monolith polymer can then be directly used for subsequent procedures. Recently, MIPs for sinomenine [63] and matrine [62] were prepared *in situ* in stainless steel columns [62]. These molecular imprinted monolithic stationary phases (MIMSP) were evaluated in a LC-system by comparing the retention of the template and some analogues in the MIP and a reference non-imprinted polymer (NIP) prepared without the template. The extraction of both target analytes from *Sinomenium acutum* Reht. et Wils *and Sophorea flavescentis* Ait., respectively, was performed by using conventional and *in-situ* prepared MIP-SPE cartridges. Both studies pointed to a successful result for the MIP-SPE cartridges prepared with the *in situ* technique; since, they show a high affinity for the template and a good workability in the herb matrix.

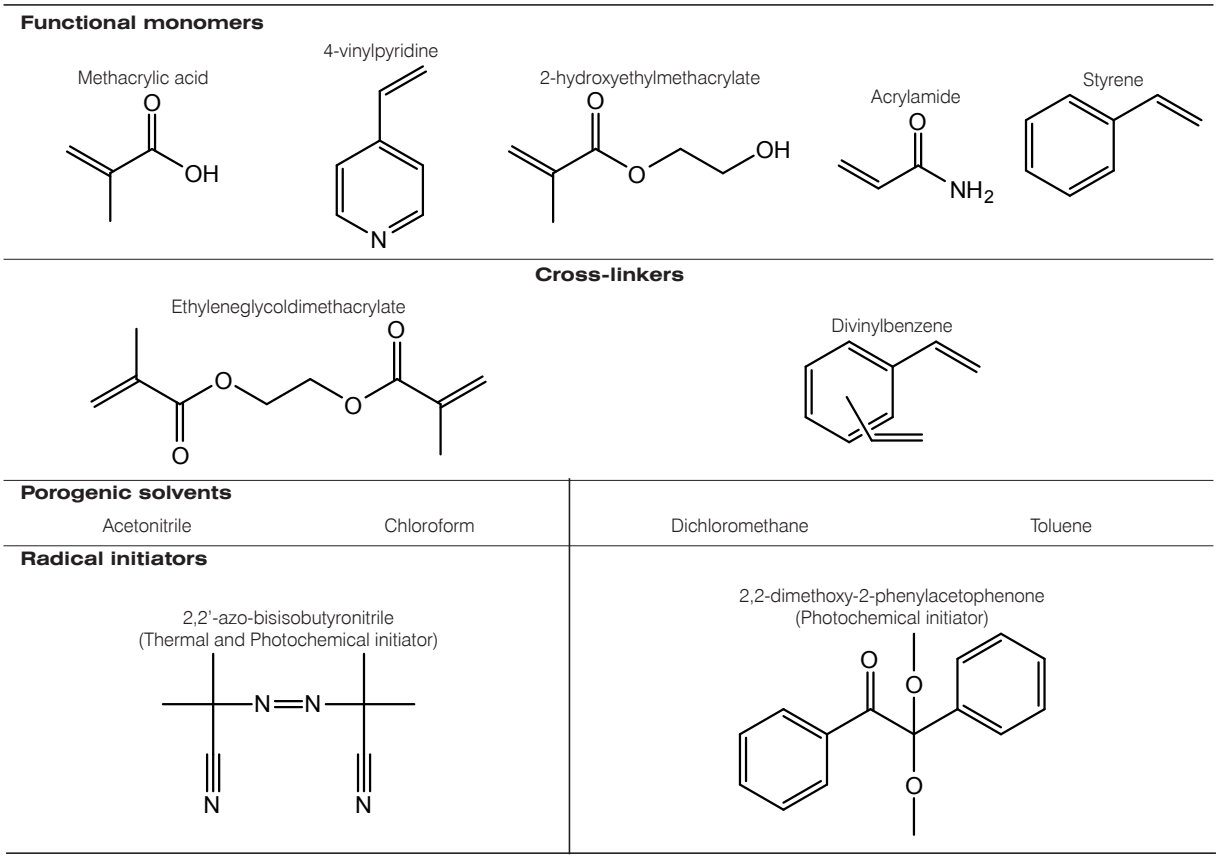
2.1.2.3. Precipitation polymerization

As shown in Table 3, precipitation polymerization (PP) is the most widely-used technique to overcome the drawbacks of BP. PP is carried out in a more dilute polymerization mixture which is initially homogeneous but becomes heterogeneous when the growing polymer chains reach a certain critical mass and precipitates as particles. PP has been successfully used to produce MISPE sorbents for the clean-up of plant extracts in the off-line mode aiming to selectively extract fenuron [66], derivates of p-hydroxybenzoic acid [21], andrographolide [67] and podophyllotoxin [68]. As reported by Yuan *et al.* [68], spherical beads of a given size can be obtained when the parameters governing the PP process are carefully controlled. In their comparative study, particles with irregular shapes were obtained by BP and monodisperse microspheres with ultra-homogeneous shapes by PP which could explain their improved recognition properties. Microwave heating was proposed to initiate the PP with the advantages of reducing polymerization time, saving energy and increasing imprinting effect compared with conventional heating.

2.1.2.4. Suspension polymerization

In this procedure, the organic-based imprinting mixture is mixed with an excess of an immiscible solvent (water or perfluorocarbon fluids) containing a suspension stabilizer. This produces droplets of dispersed phase in which the polymerization is induced. The stabilizer adsorbed at the surface of the droplets acts as a steric barrier, thereby preventing the droplets and the growing polymer chains from coalescencing to yield spherical beads. The final size of the beads is governed by the stirring speed, the amount of stabilizer and the ratio of the dispersed and the dispersant phases. It is worth noting that the suspension polymerization has not been as widely used as other polymerization techniques in recent years. Lai *et al.* [69] reported the synthesis of molecularly imprinted microspheres (MIMs) using matrine as template molecule by aqueous micro-

Table 1. Reagents most commonly used for molecular imprinting.



suspension polymerization [69]. The MIMs obtained were employed both as SPE sorbent and HPLC chromatographic stationary phase to purify and then determine matrine in *Sophora flavescens* Ait. Both the MISPE cartridge and the MIMs-HPLC column exhibited specific affinity to the template molecule.

2.1.2.5. Multi-step swelling polymerization

In the first step of this process, droplets of a radical initiator and a water insoluble 'activating' solvent (a low molecular weight solvent such as dibutyl phthalate or chlorodecane) are emulsified in water thanks to a stabilizer (sodium dodecylsulfate). Preformed, uniformly-sized polystyrene 'seeds' are then added and stirred for a number of hours until the emulsion droplets are adsorbed onto the seeds. Further additions of activating solvent are often needed to obtain seeds swollen to the desired size. This dispersion is then mixed for several hours with a second dispersion containing the imprinting mixture in the presence of a polymeric stabilizer (polyvinyl alcohol). Once the droplets of imprinting mixture are adsorbed on the swollen seeds, the polymerization is induced and held for 24 h by maintaining gentle stirring. Beaded polymer particles are finally obtained with a size 5-100 times larger than the original seeds depending on the level of activating solvent and the ratio of the various dispersion phases. This polymerization technique is more complex than others and is, therefore, less commonly used. Nevertheless, Kubo *et al*. have obtained imprinted materials for extracting domoic acid from shellfish samples. Domoic acid being a toxic compound, o-phtalic acid (o-ph), a structural analogue, was selected as dummy template. The polymer particles obtained in this study were about 5 µm in diameter with excellent size uniformity which enabled their use as the stationary phase in a LC system. The separation of domoic acid from the 50% aqueous methanol (v/v) extract of blue mussels using the *o*-ph-MIP column was successfully achieved [70].

2.2. MISPE applications

MISPE protocols need to be carefully optimized in order to fully exploit the MIP's selectivity and to minimize non-specific interactions. To evaluate the rate of non-specific retention on a MIP, a NIP is usually used in parallel since the retention on this sorbent, prepared without the template, only results from non-specific interactions. As reported in Table 2, several MISPE modes have been

Table 2. MISPE protocols applied for the extraction and determination of active compounds from natural products [a].

Template molecule	Polymerization reagents[b]	MIP synthesis[c]	Sample	Analyte	Washing solvent[d]	Eluting solvent[d]	MISPE Mode	Analytical system[e]	Performance Parameter[f]	Ref.
Quercetin	AA, EDMA, AIBN, THF	Non covalent, bulk	*Gingko biloba* leaves	Quercetin and kaempferol	MeOH	MeOH/acetic acid (9:1, v/v)	*Off-line*	LC/UV and LC/MS	$R_{Quercetin}$: 89%	[23]
Harman	MAA, EDMA, AIBN, toluene/acetonitrile (1:1, v/v)	Non covalent, bulk	Seeds of *Peganum nigellastrum* Bunge	Harmaline and harmine	MeOH		*In-line*	LC/MS	n.a.	[88]
Matrine	MAA, EDMA, AIBN, $CHCl_3$	Non covalent, suspension	*Roots of Sophora flavescens* Aiton	Matrine	MeOH/water (3:7, v/v)	MeOH/glacial acetic acid (9:1, v/v)	*Off-line*	LC/UV	n.a.	[69]
Quercetin	AA, EDMA, AIBN, THF	Non covalent, bulk	*Roots of Caragana jubata* (Pall.) Poir.	Inhibitors of epidermal growth factor receptor	MeOH	MeOH/acetic acid (9:1, v/v)	*Off-line*	LC/UV and LC/MS	R_{Butein}: 76% $R_{Piceatannol}$: 80%	[25]
Fenuron	MAA, EDMA, AIBN, toluene	Non covalent, precipitation	Potato, carrot, wheat and barley	Fenuron	Toluene	Acetonitrile/ acetic acid (90:10, v/v)	*Off-line*	LC/UV	$R_{Fenuron}$: 99% from potato, 98% from carrot, 115% from wheat, 98% from barley	[66]
(E)-Piceatannol	4-VP, EDMA, AIBN, acetonitrile/THF (3:1, v/v)	Non covalent, bulk	*Roots of Caragana jubata* (Pall.) Poir.	Inhibitors of epidermal growth factor receptor	MeOH		*In-line*	LC/UV	$IF_{(E)\text{-piceatannol}}$: 4.74 IF_{Butein}: 2.69 $IF_{quercetin}$: 2.05	[24]
Esculetin	AA, EDMA, AIBN, ethanol	Non covalent, bulk	Ash bark of Chinese traditional medicine	Esculetin	Water (pH 8), ethanol/ water (1:9, v/v) and ether/ chloroform (1:9, v/v)	Water/ethanol/ DMF (5:4:1, v/v/v) and ethanol	*Off-line*	LC/MS	n.a.	[26]
(−)-Ephedrine	MAA, EDMA, AIBN, acetonitrile	Non covalent, bulk	*Stems of Ephedra sinica* Stapf.	(−)-Ephedrine	Acetonitrile	MeOH/TFA (20 :1, v/v)	*Off-line*	LC/UV	$R_{(-)\text{-Ephedrine}}$: about 100%	[74]
Protocatechuic Acid	AA, EDMA, AIBN, acetonitrile	Non covalent, precipitation	*Melissa officinalis* L.	Derivates of p-hydroxybenzoic acid	Water and Acetonitrile	Methanol/acetic acid (9:1, v/v)	*Off-line*	LC/UV	$R_{Gallic\ acid}$: 56.4% (RSD: 7.0%) $R_{Protocatechuic\ acid}$: 77.1% (RSD: 4.2%) $R_{P\text{-hydroxybenzoic acid}}$: 70.0% (RSD: 3.8%) $R_{Vanillic\ acid}$: 82.1% (RSD: 3.3%) $R_{Syringic\ acid}$: 16.5% (RSD: 7.5%)	[21]
Sinomenine	MAA, EDMA, AIBN, toluene and dodecanol	Non covalent, *in situ*	*Sinomenium acutum* (Thunb.) Rehd. et Wils	Sinomenine	MeOH	MeOH/acetic acid (4:1, v/v)	*Off-line*	LC/UV	n.a.	[63]
Podophyllotoxin	AA, EDMA, AIBN, $CHCl_3$	Non covalent, bulk	Roots of *Sinopodophyllum emodi* Wall.	Podophyllotoxin	Ethanol/acetic acid (9:1, v/v)		*Batch*	HPCE	$R_{Podophyllotoxin}$: 32.1%	[72]
Resveratrol	AA, EDMA, AIBN, acetonitrile	Non covalent, bulk	*Polygonum cuspidatum* Sieb. et Zucc.	Resveratrol	Water and ethanol	MeOH/acetic acid (20:1, v/v)	*Off-line*	LC/UV	Purity of resveratrol: >99.21%	[27]
o-phthalic acid	4-VP, EDMA, 2,2'-azobis-(2,4-dimethyl-valeronitrile), toluene	Non covalent, two-step swelling	Blue mussels	Domoic acid	Acetonitrile/0.05% aqueous acetic acid (7:3, v/v)		*In-line*	LC/UV	n.a.	[70]
Trans-resveratrol	4-VP, EDMA, AIBN, acetone	Non covalent, bulk	Rootstock of *Polygonum cuspidatum* Sieb. et Zucc.	Trans-resveratrol	MeOH/H_2O (80:20, v/v)		*In-line*	LC/UV	$R_{Trans\text{-resveratrol}}$: 82.9% (RSD: 1.2 %) Purity of Trans-resveratrol: 80.1% (RSD: 0.6 %)	[14, 80]
18-β-glycyrrhetinic Acid	MAA, EDMA, AIBN, $CHCl_3$	Non covalent, bulk	*Roots of Glycyrrhiza glabra* L.	18-β-glycyrrhetinic acid	$CHCl_3$	MeOH	*Off-line*	LC/UV	$R_{18\text{-}\beta\text{-glycyrrhetinic}}$: 98%	[93]
Ligustrazine	Melamine-urea-formaldehyde	Non covalent, bulk	*Ligusticum chuanxiong* Hort.	Ligustrazine	Sodium hydroxide solution (5 mol L^{-1})	Acetone	*Off-line*	GC/MS	n.a.	[32]

Continued **Table 2.** MISPE protocols applied for the extraction and determination of active compounds from natural products [a].

Template molecule	Polymerization reagents[b]	MIP synthesis[c]	Sample	Analyte	Washing solvent[d]	Eluting solvent[d]	MISPE Mode	Analytical system[e]	Performance Parameter[f]	Ref.
Catharanthine	MAA, EDMA, AIBN, acetonitrile	Non covalent, bulk	*Leaves of Catharanthus roseus L.*	Catharanthine	Acetonitrile/ glacial acetic acid (99:1, v/v)	MeOH/acetic acid (90:10, v/v)	*Off-line*	LC/UV	$R_{catharantine}$: 101%	[94]
Valnemulin	MAA, EDMA, AIBN, acetonitrile	Non covalent, bulk	Feed samples	Valnemulin	Water and acetonitrile	MeOH/ammonia hydroxide (95:5, v/v)	*Off-line*	LC/UV	$R_{Valnemulin}$ in three kinds of feed samples: 76.0-94.4% (RSD: <9%)	[95]
Glycyrrhizic acid	HEMA, EDMA, AIBN, DMF	Non covalent, bulk	Roots of *Glycyrrhiza glabra* L.	Glycyrrhizic acid	Ethanol/ water (9:1 v/v)	Hot water	*Off-line*	LC/UV	$R_{Glycyrrhizic\ acid}$: 93-107%	[20]
Matrine	MAA, EDMA, AIBN, toluene and dodecanol	Non covalent, *in situ*	*Sophorea flavescentis Ait.*	Matrine	Acetonitrile	Acetonitrile/ glacial acetic acid (93:7, v/v)	*Off-line*	LC/UV	$R_{Matrine}$: 89.2%	[62]
Andrographolide	AA, EDMA, AIBN, acetonitrile/toluene (3:1, v/v)	Non covalent, precipitation	*Andrographis paniculata (Burm.f.)*	Andrographolide and dehydroandro-grapholide	MeOH		*Off-line*	LC/UV	$R_{Andrographolide}$: 96.6-104.0% (RSD: 3.1-4.3%) $R_{Dehydroandrographolide}$: 95.8-104.0% (RSD: 2.9-3.7%)	[67]
1,3,5-pentanetricar-boxylic acid	4-VP, EDMA, AIBN, toluene	Non covalent, bulk	Blue mussels	Domoic acid	Water and acetonitrile	MeOH/acetic acid (20:1, v/v)	*Off-line*	LC/UV	$R_{Domoic\ acid}$: 93.4% (RSD: 4.9%)	[78]
Berberine	AA, EDMA, AIBN, DMSO	Non covalent, bulk	Cortices of *Phellodendron wilsonii* Hayata & Kanch	Berberine	MeOH/$CHCl_3$ mixture (1:60, v/v)		*Off-line*	LC/UV	$R_{Berberine}$: 96.1%	[96]
Podophyllotoxin	AA, EDMA and DVB, AIBN, acetonitrile	Non covalent, precipitation	*Dysosma versipellis* (Hance) M. Cheng, *Sinopodophyllum hexandrum* Royle and *Diphylleia sinensis* Li	Podophyllotoxin	MeOH	MeOH/acetic acid (9:1, v/v)	*Off-line*	LC/UV	LOD : 0.12-0.18 μg/ml $R_{Podophyllotoxin}$: 89.5-91.1% (RSD: <3.7%)	[68]
Matrine	Melamine-urea-formaldehyde	Non covalent, bulk	Root of *Sophorea tonkinensis* Gagnep	Matrine	Water	Acetone	*Off-line*	GC/MS	n.a.	[31]
Ferulic acid	1-Allyl-3-ethylimidazolium bromide, EDMA, AIBN, n-butanol/H_2O	Non covalent, bulk	Salicornia herbacea L.	Phenolic acids	Water and methanol	Aqueous HCl (0.5 mol/L)	*Batch and off-line*	LC/UV	$R_{Ferulic\ acid}$: 96.0% $R_{Protocatechuic\ acid}$: 82.1% $R_{Caffeic\ acid}$: 83.6%	[89]
Propyl gallate	4-VP, EDMA, AIBN, ethyl acetate	Non covalent, bulk	Radix Salviae Miltiorrhizae	Antiplatelet active ingredients	Acetonitrile (mobile phase)	MeOH/glacial acetic acid (9:1, v/v)	*On-line*	LC/MS/MS	n.a.	[85]

[a] MISPE: Molecularly imprinted solid phase extraction;

[b] AA: Acrylamide, AIBN: 2,2' – Azobisisobutyronitrile, DMF: N,N-dimethylformamide, DMSO: Dimethyl sulfoxide, DVB: Divinylbenzene, EDMA: Ethylene glycol dimethacrylate, HEMA: 2-hydroxyethylmethacrylate, MAA: Methacrylic acid, THF: Tetrahydrofuran, 4-VP: 4-vinylpyridine

[c] Bulk: Bulk Polymerization; suspension: suspension polymerization; precipitation: precipitation polymerization; in situ: in situ polymerization; two-step swelling: two-step swelling polymerization

[d] DMF: N,N-dimethylformamide, MeOH: Methanol, TFA: Trifluoroacetic acid

[e] GC: Gas chromatography, HPCE: High-Performance Capillary Electrophoresis, LC: Liquid chromatography, MS: Mass spectrometry, UV: Ultraviolet

[f] R: Recovery, n.a.: not available, IF: Imprinting effect defined by IF=k'(MIP)/ k'(NIP), where k'(MIP) is the capacity factor of the molecularly imprinted polymer and k'(NIP) is that of the nonimprinted polymer (the capacity factor is defined by $k'=(t_R - t_0)/t_0$, where t_R is the retention time of a sample, and t_0 the time to elute the void marker acetone), RSD: Relative standard deviation, LOD: Limit of detection

developed in different formats which are described below.

2.2.1. MISPE in batch mode

Batch use of imprinted polymers is particularly applied in the evaluation of polymers by equilibrium binding experiments. In such procedures, MIP particles are mixed with the sample solution and then separated from it in order to determine the concentration of free substrate in the solution [34,71]. More rarely, MISPE in batch mode is applied for sample preparation. This was realized by Zhu *et al*. [72] for the extraction of podophyllotoxin directly from the ethanol extract of the traditional Tibetan medicine, *Sinopodophyllum emodi* Wall. As widely reported for hydrogen bonding based polymers, the solvent used during polymerization is often the most convenient for the rebinding of templates [8,57,73]; chloroform, used for the podophyllotoxin MIP synthesis, was effectively confirmed the best solvent for rebinding experiments. The authors, however, selected ethanol, a more common solvent for the extraction of traditional Chinese medicines, as rebinding solvent even if it slightly decreased the adsorption capacity; indeed, the stability of hydrogen bonds decreases when the

Table 3. Comparison of imprinted polymerization methods.

Polymerisation methods	Morphology and size	Advantages	Drawbacks	Ref.
Bulk Polymerisation	Irregular particles with a size between 20 and 50 μm	Simple and versatile Convenient for SPE in off-line mode	Time consuming, labor intensive and wasteful in material Low loading capacity due to partial destruction of binding sites during grinding Particles of irregular shape and size The particles poorly pack in columns and create large void volumes limiting their use in on-line and in-line modes	[57, 66, 73, 97-100]
In situ Polymerisation	Continuous porous monolith polymer	Simple and fast Crushing and sieving steps are avoided	Stationary phases exhibiting higher back pressure and peak broadening Restriction in the selection of polymerisation solvents since high porosity of the MIP is needed to allow permeation by the eluent	
Precipitation Polymerisation	Micro- to nanospheres with a size between 0.1 and 10 μm	Simple and fast High reaction yield (>85%) Good control of the final size, shape and number of particles High recognition and loading capacity	More porogen needed Formation of colloidal particles slightly irregular in shape due to the presence of the template molecule Not robust in terms of "imprintability" (restrictions in the template, monomers and porogens that can be used)	
Suspension Polymerisation	Spherical beads with a size between 5 and 100 μm	Simple, reliable and fast (one-step polymerisation in less than 2h) High reaction yield Robust in terms of "imprintability" Improved chromatographic characteristics Perfluorocarbon fluids: dispersant phase inert, stable, largely immiscible with organic compounds, easily recyclable.	High molar concentration of template and monomers used to compensate the partial loss of the reagents in the dispersant phase Aqueous dispersant phase may interfere with the interactions established between template and monomers	
Multi-step swelling Polymerisation	Monodispersed spherical particles over a size range 2-50 μm	Good control of the size distribution and the shape of particles High reaction yield (>88%) Robust in terms of "imprintability"	Laborious and complex Heterogeneity of binding sites not improved Aqueous dispersant phase Polymers prone to swelling	

polarity of the solvent increases. In this case, ethanol provided acceptable selectivity which was explained by the partial suppression of non-specific adsorptions by this more polar solvent. The MISPE of podophyllotoxin from *S. emodi* was realized in ethanol by mixing the plant extract and the MIP particles during 12 hours. MIP particles were then separated by filtration and eluted with ethanol/acetic acid (9:1, v/v). As a control, the same procedure was applied to corresponding NIP particles. The ratios of adsorption (ratio of the amount adsorbed by polymers to that in the sample solution) were calculated as 35.0% and 14.9% for MIP and NIP, respectively, and the adsorption recovery (ratio of the amount released from polymer to that in the sample solution) as 32.1% and 13.6% for MIP and NIP, respectively. The concentration of one analogue (4'-demethylpipodophyllotoxin) present in the plant extract was also determined in the filtrates and the eluates revealing its low adsorption on the polymers. These results show that MISPE used in batch mode can selectively extract a target analyte from other related compounds with a moderately satisfactory recovery [72].

2.2.2. MISPE in off-line mode

In the off-line mode, the MIP is used as sorbent in a cartridge format, and the extraction procedure is similar to the extraction with conventional SPE sorbents. Conditioning, loading sample, washing and elution steps are carried out. This allows the isolation of the target compound(s) from the complex sample for final determination by mainly chromatographic techniques. A successful application of MISPE in the off-line mode was reported by Dong *et al*. [74] who developed a method for the selective extraction of (−)-ephedrine from its very close structural analogues contained in Chinese Ephedra (*Ephedra sinica* Stapf.). The MISPE was developed by a careful selection of solvents with regard to the porogen and the type of template-monomer interactions that take place during polymerization. Both MeCN and $CHCl_3$, previously used as porogens for (−)-ephedrine imprinted polymers syntheses [75], can stabilize the interactions between template and MIP. Henceforth, MeCN, the porogen for the MIP synthesis and $CHCl_3$, the solvent for herb extraction, were considered as possible solvents for SPE samples. However, it was found that, with $CHCl_3$, the loading capacity of the MIP column was decreased after several SPE experiments. This was explained by a much more important swelling of the MIP in $CHCl_3$ than in MeCN; indeed, as reported by Zander *et al*. [45], swelling leads to changes in site accessibility. Therefore, since a stable column capacity was obtained with MeCN, this was retained as the SPE conditioning and sample solvent. Indeed, conditioning the cartridge prior to the loading step activates the MIPs binding sites, maximizing their interactions with the target analyte. The washing step is also critical in MISPE procedures due to the complexity of sample matrices. To find a proper washing solvent for *E. sinica* assays, Dong *et al*. examined four solvents with different polarities (MeOH, MeCN, $CHCl_3$ and *n*-hexane). Criteria for the selection of a washing solvent were then defined. Firstly, the solvent must be able to elute the interfering components eventually present in the polymer matrix; *n*-hexane could not elute yellowish matrix components of *E. sinica* from the MISPE column, indicating that nonpolar solvent has a weak washing ability for some matrix compounds. Secondly, the solvent has to maximize the specific interactions between the analyte and MIP; MeOH eluted (−)-ephedrine from MIP cartridges and is not a proper washing solvent. Because the structure of the MIP was stable in MeCN, whereas in $CHCl_3$, MIPs swelled and gradually lost binding capacity, MeCN was finally chosen as the washing solvent in the MISPE process. Therefore, it appears that the porogen used during polymerization is the better alternative for both the loading and washing steps when electrostatic interactions dominate. However, when non-specific interactions are important, it is necessary to increase the polarity of the solvent. Sometimes, a small amount of a polar additive, such as acetonitrile, alcohol, water, or a weak acid, is used to improve the washing selectivity (Table 2). For elution, Table 2 shows that polar solvents (acetonitrile), protic solvents (methanol), or their mixtures are sometimes enough to elute the analyte(s). But in most cases, a small amount (1–10%) of modifier, such as water, weak acids or weak bases must be added to disrupt the strong interactions between the MIP and analyte. The maximum amount of modifier is fixed to 10% based on the results of Zander *et al*. [45] which reported a swelling of the polymer when the amount of water was increased over 10%. Therefore, to efficiently release (−)-ephedrine from the column, Dong's group tested different concentrations (0.05-5%) of TFA in MeOH; the volume of elution solution required for the complete elution of the (−)-ephedrine decreased when TFA proportion increase. Using as small a volume of elution solution as possible can reduce the experiment time and lead to a higher enrichment of purify analyte, 5% TFA in MeOH was selected as the optimal elution solvent. Under these optimized SPE conditions, a clean analytical HPLC baseline was obtained for *Ephedra* extracts, indicating that the sample pre-treatment was efficient. Moreover, good recovery and precision were obtained in the assessment of the MISPE–HPLC procedure, leading to a reliable method applicable to the determination of (−)-ephedrine in herbal *Ephedra* [74].

In general, sample is loaded onto the MIP cartridge in a low polarity solvent to stabilize electrostatic interactions and rebind analytes to specific sites. By contrast, when aqueous samples are percolated through the MIP, target analytes and matrix components are mainly retained by non-selective hydrophobic interactions with the polymeric matrix [76,77]. Another solution is to select a washing solvent able to disrupt non-specific hydrophobic bonds and to enhance selective polar interactions between the analytes and the cavities. In this context, matrix components should be removed and non-specifically bound analytes redistributed to the selective imprints. This was successfully realized in a recent study of Zhou *et al*. [78] who aimed to develop a MISPE off-line method for the determination of domoic acid residues in aqueous seafood samples [60]. They performed the washing step by using water and acetonitrile successively, resulting in a successful clean-up of the matrix and a high selective extraction of domoic acid. As mentioned in Table 2, the off-line mode is the most widely used MISPE procedure for the pretreatment of complex matrices. Easily automated, any solvent and additive can be used in this set-up if

they don't influence the subsequent chromatographic analysis, and consequently a lower limit of detection can be reached. However, the off-line mode involves a manipulation between the pre-concentration and analysis steps which leads to increased time analysis, loss of analytes and risk of contamination [8,57,73,79]. To avoid this sample manipulation and to obtain higher enrichment factors, it is possible to couple the MISPE in-line or on-line with the analytical system as discussed below.

2.2.3. MISPE in-line mode

This mode involves the direct coupling of a MIP-column in-line with the detection system. In some cases the protocols have only been applied to assess the retentivity and selectivity of the sorbents [23,25,62,74], in others they have been fully developed to both pre-concentrate and separate analytes from complex matrices. Recently, Ma *et al*. [14,80] reported the use of a pyridyl MIP-column in a HPLC system to selectively extract trans-resveratrol from *Polygonum cuspidatum* Sieb. et Zucc [14,80] . In the in-line extraction, the mobile phase should stabilize the analyte-MIP interactions and also facilitate the separation between the analyte and matrix components. For the extraction of trans-resveratrol, experiments revealed the negative influence of two types of solvent added in the major mobile phase (methanol). The adding of an acidic solvent (acetic acid) decreased the retention of trans-resveratrol and the MIP selectivity, likely by interaction with the MIP pyridyl groups, which is in competition with the trans-resveratrol binding. The use of an organic solvent (acetonitrile), depending on its concentration, differently influenced the retention of trans-resveratrol. At low concentration, the retention was decreased because acetonitrile interrupted the hydrophobic interactions between MIP and template molecules. At higher concentration, MIP trans-resveratrol hydrogen-bonding became the dominating interaction resulting in a more efficient retention of the template molecules. Nevertheless, mobile phases containing acetonitrile did not give a satisfying separation of trans-resveratrol from its structurally related compound emodin, also identified in the *P. cuspidatun* extract. The mobile phase MeOH/water (80:20, v/v) appeared the best optimization able to separate efficiently trans-resveratrol and emodin from their initial matrix, performing a one step in-line extraction. To remove non-selectively bound matrix components, it is sometimes necessary by insertion of wash procedures leading to a quite long analysis time [76,77]. An alternative, namely MISPE with pulsed elution (MISPE-PE), exists and overcomes this disadvantage. The concept is based on the use of a small volume of elution solvent (generally 20 µL) instead of solvent switching [81]. The application of several successive 20 µL pulses of different solvents (MISPE with differential pulsed elution), permits to quantitatively recover both matrix components and analytes bound to the imprinted polymer [82].

2.2.4. MISPE on-line mode

In this process, a pre-column is slurry packed with a small amount of MIP particles and located in the design of a six-port switching valve connected to a LC system. The sample is first loaded in the MIP-pre-column (valve in the load position), and after first washing-out interfering compounds, the analytes are eluted by the mobile phase and transferred to the chromatographic system (valve in the inject position) [83]. If the mobile phase is not appropriate to elute the analytes from the MIP pre-column, another eluting solvent can be introduced. In such cases, the eluted analytes flow to the injection loop prior to be injected in the chromatographic set-up and are carried off by the mobile phase [84,85]. Compared with the off-line mode, the on-line mode presents some major advantages such as solvent saving and low cost. In particular cases, a higher concentration factor is obtained by collecting the analytes in a very small volume of solvent after loading the whole sample extract through the MIPs bed. MISPE on-line mode looks mainly appropriate for multianalyte determinations where the MIP recognizes several structural analogs [85,86]. For that reason, its use is less widely achieved than the other modes but seems to be interesting in the drug discovery process discussed hereunder [85].

2.3. Potential of MISPE in the drug discovery process

MIPs prepared with dummy templates are designed to selectively extract the analytes of interest from complex matrices [21,70,78,87-89]. In recent years, this faculty of MIPs to recognize analogs of the templates has also attracted attention in the drug discovery process [24,25,85]. The first application of MIP to the screening of lead compounds in natural products was reported by Zhu *et al*. in 2003 [25]. In this work, a MISPE was used to discover anti-tumor components in *Caragana jubata* (Pall.) Poir. This traditional Tibetan medicine was chosen for its inhibitory activity towards the epidermal growth factor receptor (EGFR), a mediator hyperactivated in numerous tumors. In order to identify the anti-EGFR inhibitors contained in *C. jubata*, the MIP was prepared with quercetin (IC_{50} 15µ*M*), a known inhibitor of EGFR. After loading an active extract of *C. jubata* on the MIP, two novel active anti-EGFR inhibitors were found to be selectively retained and identified as (*E*)-piceatannol (IC_{50} 4.9 µ*M*) and butein (IC_{50} 10 µ*M*). This work

demonstrated the feasibility of using MISPE to mimic enzymes or receptors for screening different potent inhibitory components directly from natural products. The strength with which the analytes bind to the imprinted sites appears as a measure of their molecular similarity to the template in terms of size, shape and functional groups disposition. These MIPs characteristics determine not only their selectivity, but can also play important roles in selecting bioactive analytes. For this type of drug discovery application, therefore, absolute specificity is not really required. An appropriate cross-reactivity to molecules with a similar size and shape comparing to the template is preferable, pointing out a likely compatible pharmacophore. The initial template choice reveals itself to be of crucial importance to create a MIP with relevant selection characteristics [90]. According to Zhu *et al.* publications [24,25], the MIP based on the EGFR inhibitor possessing the highest activity more successfully simulates the receptor. As the MIP imprinted with quercetin was not able to retain the different inhibitors according to their bioactivities, they prepared a second MIP imprinted with piceatannol which presents higher anti–EGFR activity. As expected, the piceatannol-MIP binding affinity effectively correlated with the molecular activity. These results highlight, once again, the importance of the template molecule choice for mimicking enzyme experiments to identify different inhibitors.

In parallel, others published methods defining different strategies using MIPs in drug discovery. The first one, developed by Sreenivasan *et al*. [91], used several template molecules, all known as substrates for a given receptor. They aimed to obtain a hybrid MIP which could more specifically mimic the structure/shape of the receptor. These hybrid MIPs exhibited high selectivity for several compounds having a common pharmacophore indicating such an innovative approach is useful to select molecules with a particular biological activity amongst a general set of compounds. In a second strategy, Rathbone et al. [92] designed and synthesized a set of composite templates based on the known agonists structure. The subsequently produced MIPs are supposed to mimic the targeted receptor. The series of prepared polymers thus were exposed to a panel of drugs comprising known compounds binding the studied receptor as well as its isoforms. The MIP imprinted with the most relevant template molecule showed more proficiency in isolating new receptor substrates.

3. Conclusions

In the field of molecular recognition, MIP synthesis appears as an extremely powerful technique. Indeed, their inherent advantages including high selectivity, stability, reusability, ease and low cost of preparation, provide good alternatives to biological recognition agents. In this review, we detailed the highly promising applications of MIPs for solid phase extraction. Factors involved in the recognition properties of the polymers such as synthesis reagents, polymerization techniques and application modes were discussed including the importance of their optimization for a particular combination of analyte and application. Through the numerous studies referred to here, the superiority of MISPE over traditional SPE sorbents could be confirmed. In addition, recent research works generated MIP with activity in aqueous samples which is an improvement compared with the original MISPE protocols. This review demonstrated the wide potential of using MISPE in the study of natural products for the selective extraction of target compounds and in discovery of new drug candidates. It offers a promising aspect in drug development demonstrated by the identification of *C. jubata* proposed for various anti-cancer compounds [24,25] in 2003. However, some features have still to be improved prior to revolutionizing the drug discovery process. Efforts should especially be carried out to produce MIPs which exhibit cross-reactivity instead of absolute specificity. From our point of view, less rigidity of the imprinted cavities should allow a more successful simulation of receptor binding pockets, and further research should be done in that direction. Moreover, the on-line mode of MISPE on monolithic columns appears to be the most appropriate mode for natural products drug screening. Indeed, the structural analogues retained on the MIP pre-column could be directly transferred and separated in the analytical column of the LC system which is not feasible with the other MISPE modes. In conclusion, given the possibility to fully exploit their wide potential, molecularly imprinted solid phase extractions may result in faster and more cost-effective identification of lead compounds from natural resources as well as from synthetic combinatorial libraries.

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