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Review Article

Application of molecularly imprinted polymers in food sample analysis – a perspective

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Abstract: Since the introduction of the molecularly imprinting technology (MIT) in 1970s, it becomes an emerging technology with the potential for wide-ranging applications in food manufacturing, processing, analysis and quality control. It has been successfully applied in food microbiology, removal of undesirable components from food matrices, detection of hazardous residues or pollutants and sensors. Molecularly imprinted solid-phase extraction (MISPE) is the most common application so far. The review describes the methods of making the molecularly imprinted polymer systems, the application of the technology in food safety issues and the remaining challenges.

Keywords: Molecularly imprinted polymer, food safety, biosensor

Introduction

Molecular imprinting is an emerging technology which enables us to synthesize the materials with highly specific receptor sites towards the target molecules. Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer that can bind certain target compound with high specificity. The polymers are prepared in the presence of the target molecule itself as the template. The concept behind the formation of the selective binding sites is schematically shown in Figure 1. In brief, the template interacts with functional monomers before being cross-linked by cross-linker in polymerization process. The specific binding site complementary to the target analyte is generated upon the removal of the template from the solid polymers.

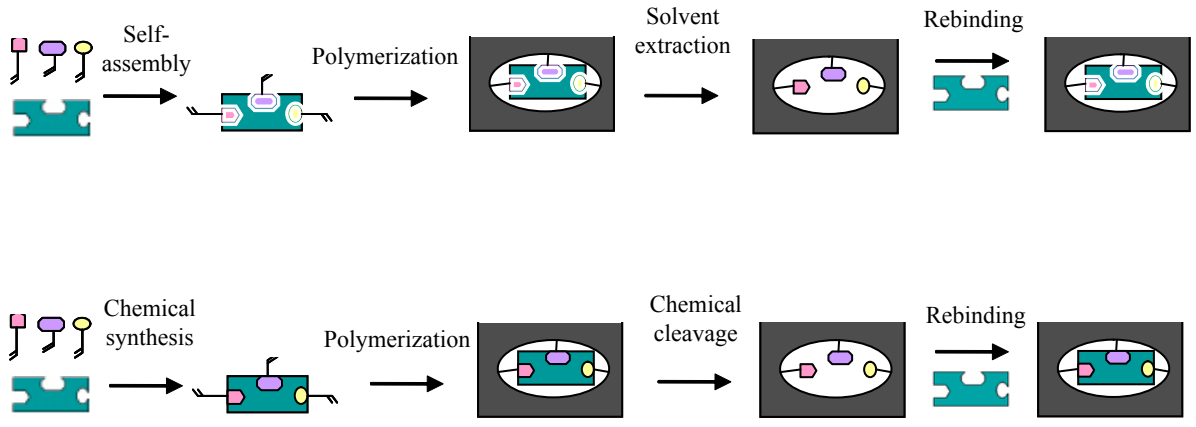

Figure 1. Schematic representation of non-covalent and covalent molecular imprinting procedures.

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The molecularly imprinting technology was first proposed by Wulff and Sarhan (1972). The technology was then expanded by the effort of Mosbach and co-workers in 1980s (Andersson *et al*., 1984). The MIPs possess several advantages over the conventional immunosorbent (IS). They show high selectivity and affinity, high stability and the ease of preparation (Piletsky *et al*., 2006). The MIPs can be used repeatly without loss of activity with high mechanical strength and durable to harsh chemical media, heat and pressure compared to biological receptors (Lavignac *et al*., 2004). Chemical stability studies demonstrated that the polymers kept >95% of their affinity even after 24 h of exposure to autoclaving treatment, triethylamine, 10M HCl acid and 25% NH_3. Heat treatment revealed that the polymers are thermally resilient and able to retain their chemical affinity, as the MIP will not degrade up to temperatures of 150°C (Svenson and Nicholls, 2001). They can be stored for years without loss of affinity for the target analyte. Researches in this technology have grown rapidly due to its potential application in various fields, ranging from chemical, pharmaceutical, engineering, material science and biotechnological industries. The synthesis approaches, analysis, characterization and application of MIP in food safety in the recent few years are discussed in this review, challenges of MIP are also been briefly discussed.

Synthesis of MIP

Covalent, semi-covalent and non-covalent

There are three different approaches to prepare MIPs: covalent (pre-organized approach), non-covalent (self-assembly approach) and semi-covalent approach. The covalent or pre-organized approach was introduced by Wulff and co-workers (1972), involves the formation of reversible covalent bonds between the template and monomers before polymerization. Then the template is removed by cleavage of the covalent bonds, which will be re-formed upon rebinding of the target molecule. Covalent approach leads to a homogenous population of binding sites due to the high stability of template-monomer. However, this approach is restrictive since the cleavages of covalent bonds always require a rather harsh condition.

The semi-covalent approach is an intermediate option (Sellergren and Andersson, 1990; Whitcombe *et al*., 1995) where the template is covalently bound to a functional monomer, but the rebinding is based on non-covalent interactions.

The non-covalent or self-assembly approach was introduced by Mosbach and co-workers (Arshady and Mosbach, 1981). This approach is based on the formation of relatively weak non-covalent interactions (e.g. hydrogen bonding, electrostatic interaction, hydrophobic interaction, Van der Waals forces and dipole-dipole bonds) between the template molecule and functional monomers before polymerization. The association and disassociation of the imprint occurs by plain diffusion in and out of the sites. This approach is the most used method for the preparation of MIPs, owing to its simplicity and availability of different monomers able to interact with almost any kind of template. However, it suffers from some drawbacks because the template-monomer interactions are governed by equilibrium process. A high amount of monomer is used in order to displace the equilibrium to form the template-monomer complex. As a result, the excess of free monomers is randomly incorporated to the polymeric matrix and leading to the formation of heterogeneous or non-selective binding sites.

Despite of the drawbacks, the non-covalent strategy is still the preferred method in preparing MIP. The non-covalent methodology is easily conducted and the template removal can be carried out simply through solvent extraction. This methodology is also found to be more versatile and the imprinting step is quite similar to the recognition pattern observed in nature. The apparent weakness can be overcome by allowing a multitude of interaction point simultaneously.

Preparation approach

There are a number of preparation methodologies reported so far, ranging from traditional bulk polymerization to multi-step swelling and emulsion core-shell polymerization. Many researchers used bulk polymerization technique since it requires simple apparatus and the reaction conditions can be easily controlled. However, this procedure is tedious and time-consuming. The grinding process creates irregular particles that can cause back pressure problems when packed as the stationary phases for chromatography. The useful fines are lost during the sieving process (Mahony *et al*., 2005) and resulted in low yield of MIPs. Only less than 50% of the resultant polymers will be usable for the chromatographic purposes (Mayes and Mosbach, 1996). These prevent their large-scale production and acceptance in analytical laboratories (Martín-Esteban, 2001). In spite of these obvious drawbacks, most of the MIPs reported in the literature are still prepared by bulk polymerization (Tamayo *et al*., 2007).

Later, Mayes and Mosbach (1996) proposed the suspension polymerization that used liquid perfluorocarbon in a continuous phase to yield MIP beads. This non-polar dispersant stabilizes the interactions between functional monomers and

templates required for the recognition process. The method yields are in 5 to 50 µm size ranges, depending on the amount of surfactant and stirring speed. This method is relatively fast and reliable but it is quite expensive.

Precipitation polymerization method was developed by Ye and co-workers (Ye and Mosbach, 2001) which can provide 0.3-10 µm size of particles. It is based on the precipitation of the polymeric chains out of the solvent in the form of particles as they grow more and more insoluble in an organic continuous medium (Pérez-Moral and Mayes, 2004). This method is found to be able to obtain uniform size and high yields of resultant polymers. Yet, it requires large amount of template and high dilution factor. A new precipitation polymerization method was developed for the one-step preparation of monodisperse MIP particles about 5 µm in diameter (Wang *et al*., 2003), which can be applied to HPLC and solid-phase extraction (SPE) separation as the stationary phase.

Two-step or multi-step swelling polymerization was developed by Hosoya *et al.* (1994) and further optimized by Haginaka and Sagai (2000). It requires several swelling steps before the polymerization process. This method enables to prepare monodisperse beads with controlled diameter (2-50 µm) which makes them the ideal particles for HPLC. But it requires complicated procedures and reaction conditions, while their performance in chromatographic separation is still unsatisfactory (He *et al.*, 2007).

Surface imprinting polymerization is another option to prepare chromatography-grade imprinted materials. In this method, the radical polymerization is performed on the porous silica for chromatography so that the imprinted materials will form a thin-layer coating at the surface of the beads (Sellergren *et al*., 2002; Ruckert *et al*., 2002).

In situ polymerization is a very simple method for preparing MIPs as it is a one-step for HPLC or SPE separation (Hosoya *et al*., 1996; Zhang *et al*., 2003; Lin *et al*., 2006) where the polymerization is carried out directly in a chromatographic column. Matsui and his co-workers first used the in-situ polymerization technique for preparation of molecularly imprinted monoliths (Matsui *et al*., 1993; Matsui *et al*., 1995). Its good porosity and permeability makes it a favorable method in preparing stationary phases for chromatography and SPE (Liu *et al*., 2005).

Pérez-Moral and Mayes (2004) compared five different synthetic methods which are the bulk, suspension, precipitation, two-step swelling, and emulsion core-shell polymerization to provide a general view of the behaviors of different polymerization procedures using a fixed composition and specific method of analysis.

Optimization

The optimization is a challenging task since the synthesis involves a lot of variables that can affect the molecular recognition properties of the polymer and their performance in latter application. The ideal polymer should be rigid to preserve the cavity after the removal of the template, and yet should be flexible to facilitate a fast equilibrium between uptake and release of the analyte. Thus, the optimization requires good understanding of chemical equilibrium, molecular recognition theory, thermodynamics and polymer chemistry in order to obtain polymer with desired properties (Mosbach, 1994; Andersson *et al*., 1996; Haupt and Mosbach, 1998; Cormack and Elorza, 2004; Spivak, 2005).

Computer simulation

Piletsky *et al*. (2001 (a), 2001 (b), 2002 and 2004) used computer software to simulate the polymer properties through molecular modeling and thermodynamic calculations. The molecular modeling is difficult due to their numerous possible structure and interactions with template, solvent and other molecules which require extremely large computational workload. Thus, they simplified the model by making assumption that the complexes formed in monomer mixture during the polymerization process will be preserved in the final products. Thereby, instead of modeling the polymer, a model of the monomer mixture and the interactions taking place in solutions between monomers, crosslinker, template and solvent was carried out to reduce the computational time. The simulation between monomers and template models can be quantified and used for rational selection of ideal monomers for polymer preparation. The molecular modeling has been used to predict which functional monomers are capable of forming effective polymer. Researchers can select the high affinity monomers that interacting strongly with the target analyte from the virtual library (Chianella *et al*., 2006; Dineiro *et al*., 2006; Breton *et al*., 2007).

Combinatorial screening

Combinatorial screening allows one to rapidly prepare a large range of products on a small scale for screening and optimization of MIP formulations. This strategy was proposed independently by Sellergen and co-worker (Lanza and Sellergren, 1999) and Takeuchi group (Takeuchi *et al*., 1999). A number of polymers are synthesized directly in HPLC vials as small monoliths and their rebinding capacity will

be evaluated by measuring the template release after incubation in the presence of a suitable solvent. Sellergen group synthesized triazine-targeted MIPs while Takeuchi targeted sulphonylurea herbicides; both experiments have successfully employed the combinatorial screening for rapid evaluation and selection for the best combination of MIP components. The selection of optimum formulation can be eased by the use of experimental design and multivariate analysis methods since such methods allow identifying the main factors affecting the properties of MIPs (Tamayo *et al*., 2007).

Application in food safety

Application of molecular imprinting has become attractive in many fields of chemistry, biology and engineering, particularly as an affinity material for sensors (Ansell *et al*., 1996; Kriz *et al*., 1997; Dickert *et al*., 2000; Haupt and Mosbach, 2000; Hirayama *et al*., 2002;), binding assays (Chianella *et al*., 2002), artificial antibodies (Ye and Mosbach, 2001; Lavignac *et al*., 2004), adsorbents for solid phase extraction (Mullett and Lai, 1998; Bereczki *et al*., 2001; Weiss *et al*., 2001; Molinelli *et al*., 2002; Martin *et al*., 2003), chromatographic stationary phase (Xie *et al*., 2001; Hwang and Lee, 2001; Peter *et al*., 2003; Liu *et al*., 2006), catalysis (Wulff, 2002), drug development and screening (Ye and Mosbach, 2001). Among these applications, the one most widely used is SPE, for which MIPs are commercialized (Haupt, 2003), where new applications of MIPs in SPE keep coming out constantly.

In food industry, the detection of contaminants is of utmost importance to ensure the food is safe for consumption. To get the required level of protection, food-producing industries and the regulatory agencies are interested in rapid, simple, accurate assays for contaminants or anti-microbial drugs in food. Anyhow, up to date, the applications of MIPs in food have been proposed in a lesser extent although it is an emerging area with promising developments. Table 1 lists the applications of MIPs to detect and determine the contaminants in food samples in the recent years. Solid-phase extraction is routinely used to clean up and pre-concentrate the biological samples since the residues may exist in very low concentration (Zhu *et al*., 2002). Molecularly imprinted solid-phase extraction (MISPE) offers an easy and effective pretreatment method in the food and food-related products (Pizzariello Xie *et al*., 2001., 2001; Blomgren *et al*., 2002).

Detection of antimicrobial residues

The detection of antibiotic residues is always a highlighted issue since many of them are potentially harmful to human being. The current detection methods available include mainly enzymatic, microbiological, chromatographic and immunological methods. Shi and co-workers (2007) prepared the MIPs through aqueous suspension polymerization method and packed them into the SPE cartridges to detect the chloramphenicol (CAP) in milk and shrimp samples. It was found that the recoveries of CAP were above 80%, which demonstrated they are potential to be applied for enrichment and pre-concentration the trace CAP from complex food matrices.

De Prada *et al*. (2005) developed the on-line preconcentration through solid-phase extraction, and coupled to square wave voltammetry for quantification to selectively detect sulfamethazine in milk samples. The MISPE enabled the enrichment to achieve the factor of 45, which was sufficient to analyze the antibacterial compound at the maximum level permitted by the Codex Alimentarius Commision in milk (25 mg/L). The milk samples were spiked with low concentration levels of sulfamethazine and the recoveries of practically 100% were achieved.

MIP targeting the tetracycline (TC) and oxytetracycline (OTC) was developed by Caro *et al*. (2005) to selectively remove the antibiotic and several tetracycline analogues from pig-kidney tissue. The polymers were packed into SPE tubes for the sample clean-up for HPLC analysis. The sample extract was spiked with 600 ppb of TC and OTC and the analysis showed good recoveries of both antibiotics. Suedee and co-workers (2004) synthesized MIP that targeting a class of tetracycline. It was used in the affinity membrane to selectively remove the antibiotics from water. The study demonstrated that it is useful to use MIPs with broad selectivity to isolate TC-degradation compounds.

Detection of dye residues

Industrial dyes that used illegally in food have raised concern from the consumers as well as the authority since they are viewed as genotoxic or carcinogenic or both. The illegal dyes that detected in food samples so far are Sudan I to IV, Para Red, Rhodamine B, Orange II, Acid Red, Sudan Red 7B, Metanil Yellow, Auramine, Congo Red, Butter Yellow, Solvent Red I, Naphthol Yellow, Malachite Green, Leucomalachte Green, Ponceau 3R, Ponceau MX and Orange SS. Puoci *et al*. (2005) synthesized Sudan I-specific MIP through bulk polymerization method using methacrylic acid (MAA-MIPs) and 4-vinylpyridine (4VP-MIPs) as the functional monomers and packed them into MISPE. The group succeeded to significantly concentrate the traces of Sudan I (10 ppm) in the spiked red chili powder for HPLC detection by using the MISPE, where

Table 1. Application of MIPs in food samples

Target analyte	Matrices	Template	Monomer/CL/solvent	Analytical system	Reference
Atrazine	onion,rice seed	atrazine	MAA/EDMA/MeCN	GC, GC-MS	Djozan and Ebrahimi, 2008
b-agonist	milk replacer,livers bovine muscle, duck,fishes,liver, rabbit,turkey	clenbuterol	MAA/EDMA/MeCN	HPLC LC-MS	Brambilla *et al*., 2001 Kootstra *et al*., 2005
Bisphenol A	canned-food	BPA-*d*16, *p-tert*butylphenol	MAA or 4-VP/EDMA or TRIM/MeOH or MeCN or toluene		Martin-Esteban and Tadeo, 2006
Caffeine	soft drink	caffeine	MAA or 2-VP/EDMA/ MeCN or chloroform	HPLC	Farrington *et al*., 2006
Chloramphenicol	milk,shrimp	chloramphenicol	DEAEM/EDMA/ octanol-chloroform	HPLC	Shi *et al*., 2007
Fenuron	barley,carrot, potato,wheat	fenuron	MAA or 4-VP/EDMA/ toluene	HPLC	Tamayo *et al*., 2003
Malachite green	fish	malachite green	MAA/EDMA/MeCN	HPLC	Yan *et al*., 2007; Li *et al*., 2008
Mycotoxin	red wine	OTA-mimic	Q-MAA:tBu-MAA/ EDMA/chloroform	HPLC	Maier *et al*., 2004
Sudan I	red chili powder	Sudan I	MAA or 4-VP/EDMA/ chloroform	HPLC	Puoci *et al*., 2005
Sulfamethazine	milk	sulfamethazine	MAA or 4-VP or HEMA or MAA:4-VP or MAA: HEMA/EDMA/MeCN	voltammetry	De Prada *et al.,* 2005
Tetracycline	pig kidney tissue	tetracycline, oxytetracycline	MAA/EDMA/MeCN	HPLC	Caro *et al*., 2005; Suedee *et al*., 2004
Triazine	corn,potato	propazine	propazine methacrylate/ EDMA/toluene	HPLC	Cacho *et al*., 2006

BPA, bisphenol A; DEAEM, 2-(diethylamino) ethyl methacrylate; EDMA, ethylene glycol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MAA, methacrylic acid; MeCN, acetonitrile; MeOH, methanol; OTA, ochratoxin A; Q-MAA, tertiary amine-methacrylic acid; tBu-MMA, *tert*-butyl group-methacrylic acid; TRIM, trimethlolpropane trimethacrylate; 2-VP, 2-vinylpyridine; 4-VP, 4-vinylpyridine.

4VP-MIPs demonstrated better affinity toward the target analyte. The purification step is considered a practicable solution for sample preparation when traces of Sudan I are not detectable using HPLC alone.

Yan and co-workers (2007) synthesized Malachite Green (MG)-templated MIP through precipitation method. The dye has been used illegally in treating fungal infection in fishes since 1930s (Halme *et al*., 2004). The resultant polymer was proved to selectively bind the dye in preference to other closely related compounds, with apparent maximum number of MG at 2.33 mmol/g for the MIPs. Su *et al*. (2007) packed the MIP into the HPLC column and concluded that the column was able to separate MG with its analogue (crystal violet) efficiently. Li and co-workers (2008) developed a group selective MISPE for malachite green. They found that in spite of high rebinding activity toward malachite green, the MISPE also displayed 83.0% and 87.5% binding of leuco-malachite green and crystal violet in the selectivity test. The MISPE was used as the sample pretreatment for spiked tap water before being analyzed with HPLC. Two pet fishpond water samples were tested and the presence of target compound was detected at 1.5 ng/mL and 0.67 ng/mL respectively.

Detection of chemical residues

Mycotoxins are natural occurring toxins and probably the most important food contaminants in terms of toxicity and widespread diffusion. Maier *et al*. (2004) developed a new analytical method to detect mycotoxin ochratoxin A (OTA) in red wines with two-dimensional solid-phase extraction (SPE) clean-up protocol on C18-silica and the target-specific MIP. They utilized OTA-mimic template, basic functional monomer, sterically demanding tertiary amine (Q-MAA) and the highly hydrophobic *tert*-butyl group (tBu-MAA) in the studies. Spiked samples (0.033-1.0 ng OTA/mL) provided >90% recoveries and R.S.D. <10% with LOD and LOQ value at 0.01 and 0.033 ng/mL respectively. The researchers have successfully reproduced three batches of polymers with consistent performance to show its excellent reproducibility. The reusability analysis demonstrated the recoveries after five reuse cycles, were practically identical with the unchallenged polymers. The corresponding chromatograms neither show any interfering matrix components nor increasing baseline signal. However, similar favorable performance characteristic was observed in control experiments in which the MIP was replaced by the corresponding NIP.

Tamayo and colleagues (2003) prepared MIP through precipitation polymerization to detect fenuron, a phenylurea herbicide in plant samples. The polymers prepared with methacrylic acid (MAA) as the functional monomer was consisted of homogenous binding site distribution when fitted the rebinding isotherm to the Langmuir-Freundlich isotherm. The MISPE synthesized was able to recover 95-115% of spiked fenuron in potato, carrot, wheat and barley using HPLC-UV. The interferences in the samples were being cleaned up by MISPE and the peak can be detected clearly in the chromatograms compared to the samples without MISPE treatment. The proposed procedure allowed the determination of fenuron at concentration below the maximum residue levels (MRLs) recommended by the legislation.

Cacho *et al*. (2006) prepared triazine-specific semi-covalent MIP through precipitation polymerization to detect the triazinic herbicides in spiked corn and potato. They found that the semi-covalent polymers demonstrated better performance compared with non-covalent polymer of their previous work (Turiel *et al*., 2001; Cacho *et al*., 2003). The semi-covalent MISPE was useful to clean up the sample extracts and allow the triazines to be detected at concentration levels below the established maximum residue limits (MRLs) by current legislation, which was not possible for some of the triazines studied using the non-covalent MIP. The semi-covalent approach also showed a more homogenous binding site distribution and reduction of non-specific interaction. Agostino and co-workers (2006) developed the potentiometric sensor for atrazine detection with molecularly imprinted membrane. The membrane was rigid enough to bear the filling solution in contact with the internal reference electrode to give good potentiometric response, with detection limit of around 2×10^{-5} mol/L. The response time was less than 10 second and the sensor could be used for more than 2 months without any changes of the potentiometric response.

Djozan and Ebrahimi (2008) proposed the monolithic molecularly imprinted solid phase micro-extraction (SPME) to be coupled with GC and GC-MS for extraction and analysis of triazine herbicides. SPME is widely used for sample preparation in analytical laboratories. It is a two-step process contributing to simultaneous extraction and pre-concentration of analytes (Lord and Pawliszyn, 1998). The fiber was synthesized with atrazine as the template and placed in the home-made SPME syringe, which then to be inserted directly into GC and GC-MS injection port. The onion and rice seeds were spiked with atrazine and analogues of atrazine (simazine, propazine, cyanazine, ametryn, terbutryn and prometryn) and even structurally unrelated compounds to investigate its selectivity. They proved

that the SPME showed high selectivity towards the triazines compared with structurally related or unrelated compounds.

Kootstra *et al*. (2005) used MIP for detection of beta-agonists, a type of feed additive for growth promoter in bovine muscle with liquid chromatography-mass spectrometry (LC-MS). The MIP4SPE was commercialized by MIP Technologies. The result showed that eight compounds (cimaterol, ractopamine, clenproperol, clenbuterol, brombuterol, mabuterol, mapenterol and isoxsuprine) meet the requirements for a quantitative determination using MIPs for sample clean-up. The decision limit (CCα), detection capability (CCβ), repeatability, reproducibility and accuracy were calculated for validation of the analysis. The method was also found to be suitable for samples from rabbit, duck, turkey, liver and various kinds of fish. It is suggested that the combination of MIPs with LC-MS has promised a robust and rapid procedure for detection of the drug.

A combinatorial approach was used to develop the MIP-based extraction of bisphenol A (BPA) from canned-food samples (Martin-Esteban and Tadeo, 2006). The optimization was simplified by assessing the methacrylic acid (MAA) and 4-vinylpyridine (4-VP) as functional monomers, ethylene glycol dimethacrylate (EDMA) and trimethylolpropane trimethacrylate (TRIM) as cross-linkers, and methanol, acetonitrile and toluene as porogen. Isotope labeled compounds BPA-*d*16 (Sambe *et al*., 2006) and BPA's structure analogue *p-tert*butylphenol (Watabe *et al*., 2005), have also been used as templates in other approaches to avoid undesirable template leakage. The optimal components selected were 4-VP (4 mmol) and TRIM (12 mmol), which provided a higher degree of cross-linking, AIMN (0.88 mmol) and toluene (150 mL), which gave lower non-specific binding results. The MIP-based method allowed the determination of BPA with 78% recovery in canned-food samples.

Analysis of MIP

UV

UV spectroscopic analysis is used to confirm the template could complex with the functional monomer by electrostatic interaction (ionic interaction and hydrogen bonding). The results provide general insights into the nature of the pre-polymerization self-assembly phase. It was used for evaluating different complexes between a template and a monomer and for selecting the monomer (Zhu *et al*., 2006 (a)) and optimizing the ratio template/monomer (Zhu *et al*., 2006 (b)).

This analysis also allows us to estimate the number of medium to high affinity recognition sites in the synthesized polymer and a means for the rapid evaluation of molecular imprinting systems (Andersson and Nicholls, 1997). This approach was also used to verify the inert nature of the cross-linker and for the screening of ideal functional monomers (Svenson *et al*., 1998). This technique is widely applied in MIP analysis due to is its simplicity of use and the possibility to control monomers-template complex formation in aqueous media (Striegler and Tewes, 2002; Guo and He, 2000).

NMR

The NMR spectroscopy is a useful tool to investigate the complexation process and characterize the interaction between functional monomer and template in the pre-polymerization mixture (Tanabe *et al*., 1995; Katz and Davis, 1999; Idziak *et al*., 2001; Sanbe *et al*., 2002; Lu *et al*., 2003; Svenson *et al*., 2004; O'Mahony *et al*., 2005). In most of these studies, it is possible to determine the exact composition of the complex. NMR data was also combined with a molecular modeling approach for predicting the template/monomer ratio and also for selecting the porogen (Farrington *et al*., 2006). The chemical shift studies allow the calculation of dissociation constants and provide a potential means for predicting the binding capacities of MIPs (Whitcombe *et al*., 1998). Quaglia *et al*. (2001) investigated the significance of hydrogen bonding in achieving imprinting effects with NMR. O'Mahony *et al*. (2005) used NMR to determine the types of interactions occurring in pre-polymerization mixture in two different pre-polymerization complexes. They also correlate the observations from NMR with the final properties of the MIP by evaluating its selectivity, providing evidence that the efficiency of the non-covalent imprinting process is directly influenced by subtle binding interactions.

SEM and BET

The scanning electron microscope (SEM) is commonly used to examine the structure and surface morphology of the MIPs. Its excellent resolution makes it one of the best tools for this purpose. González *et al*. (2006) carried a comparative study on digoxin-templated MIP through "bulk" polymerization under different synthesis conditions to observe their morphology difference. It was found that the analyte binding capacity, binding specificity and chemical and thermal capacities were dependent directly on the characteristics of their surface morphology.

FTIR

FT-IR provides quantitative analysis of the binding modes of a substrate molecule to the polymer site by empirical calibration of FT-IR and ^{13}C cross polarization-magic angle spinning (CP/MAS) NMR data. The technique gives a consistent representation in which the target analyte binds to the polymer site. The analysis also provides an opportunity to quantify site isolation within the polymer and the fidelity with which the functionalized site is maintained by the network polymer (Shea and Sasaki, 1991). The imprinting process generally begins with a complexation between a functional monomer and a template that involves hydrogen bonding. The formation of this bond can be identified using FTIR since the stretching frequency of hydroxyl or amino groups (hydrogen bond donors) and carbonyl groups (hydrogen bond acceptors) are displaced and an observable shift can be observed (Katz and Davis, 1999; Duffy *et al*., 2002).

Characterization of binding site

Equilibrium batch rebinding is one of the most common methods to evaluate the presence of cavities. A known amount of template is introduced in a vial with a given amount of MIP or NIP. Once the system has come to equilibrium, the amount of free template in solution is measured to calculate the amount of adsorbed template. This amount is compared to the one bound on the NIP, the number of cavities being correlated to the difference between the amounts adsorbed on both sorbents (Pichon, 2007). Although the imprinting concept suggests a homogeneous binding site distribution, experimental works have demonstrated that a heterogeneous distribution is common situation. Linearization is the most common method employed to fit the binding data to the adsorption model. Since the groups of Guiochon and Sellergren started to characterize the MIP through binding isotherms in the late 1990's, there are different adsorption models used in the analysis. García-Calzón and Díaz-García discussed the different binding isotherm models, including Langmuir isotherm, Jovanovic isotherm, Freundlich isotherm, Langmuir-Freundlich model, Jovanovic-Freundlich model, Allosteric isotherm in a review paper in detail by highlighting the their advantages and limitations (García-Calzón and Díaz-García, 2007).

Challenges/disadvantages

Although the technology receives much interest from the researchers, there are few drawbacks associated with it. Most of the MIPs are prepared by the non-covalent imprinting approach, which give a relatively low yield of specific binding sites and high non-specific binding. Thus, it is necessary to improve the synthesis methodology to obtain MIPs with a homogeneous population of binding sites, similar to monoclonal antibodies. Until present, the general procedure for MIP preparation cannot be determined and the optimization is normally being done by trial-and-error experiment using different ratio of template: monomer: cross-linker. The optimization of the synthesizing procedure is made complication when it involves a lot of variables that potentially affect the properties of the imprinted materials. Fortunately, it is possible to predict how a particular variable may impact upon the resultant polymers (Katz and Davis, 1999; Lübke *et al*., 2000; Turner *et al*., 2004; Oral and Peppas, 2004).

Template bleeding is considered as one of the main drawbacks especially in quantification of trace compound in complex samples. The traces of template remain in the polymer even after tedious repeated washing step, because the imprinted sites are formed not only on the surface but also deeply in the cross-linked polymer network structure, where organic solvent can hardly reach (Haginaka and Sambe, 2000). Attempts have been made to use "dummy" or analogue of target molecule to synthesize the MIP in order to avoid the interference during analysis. But this method is usually leads to reduced selectivity toward that target analyte. Zander *et al*. (1998) heated the MIPs and eluted them with strong polar solvent in order to reduce or eliminate the leakage of the template.

Polymers that have been developed are generally imprinted only for small molecules. The next stage in the design of interesting polymers may be polymers capable of recognizing macromolecules such as enzymes as can be observed with biological systems. In fact, it is found that the large molecular weight compounds are not easy to be imprinted. The concept of fragment imprinting technique is considered to be able to expand the applicable range of molecular imprinting (Hosoya *et al*., 1998; Kubo *et al*., 2004). Instead of the whole large molecule, a fragment of the target is imprinted as the pseudo-template molecule.

Conclusion

The findings of different research groups in the molecular imprinting field during the past few years show that MIP is an exciting and powerful technique compared with traditional detection materials for its selectivity, stability, robustness and low cost of preparation. As shown in this review, MIPs can be successfully used as selective sorbents to clean up

and pre-concentrate contaminants in different food samples matrices. Although there are drawbacks accompanied with this technology, there are also proposed ways to minimize or even overcome them. Many of the successful applications in various fields, especially in solid-phase extraction for sample clean-up have proved the potential of MIP. There are MIP-based SPE cartridges that have been commercialized by the companies, such as ELIPSA (Germany) and MIP Technologies (Sweden), for examples, clenbuterol-selective, triazine-selective and chloramphenicol-selective MISPE. It is expected that molecular imprinting will continue receiving enormous attention and research will be growing exponentially in its application in food safety field. The future trend will not only to improve its selectivity and sensitivity in contaminant detection, but also for selective extraction or removal of undesired components in the food.

References

Agostino, G.D., Alberti, G., Biesuz, R. and Pesavento, M. 2006. Potentiometric sensor for atrazine based on a molecular imprinted membrane. Biosensors and Bioelectronics 22: 145-152.

Andersson, L., Sellergren, B. and Mosbach, K. 1984. Imprinting of amino acid derivatives in macroporous polymers. Tetrahedron Letter 25: 5211-5214.

Andersson, L.I., Nicholls, I.A. and Mosbach, K. 1996. Molecular imprinting: the current status and future development of polymer-based recognition systems. Advances in Molecular and Cell Biology 15: 647-666.

Andersson, H.S. and Nicholls, I.A. 1997. Spectroscopic evaluation of molecular imprinting polymerisation systems. Bioorganic Chemistry 25: 203-211.

Ansell, R.J., Kriz, D. and Mosbach, K. 1996. Molecularly imprinted polymers for bioanalysis: chromatography, binding assays and 'biomimetic sensors'. Current Opinion in Biotechnology 7: 89-94.

Arshady, R. and Mosbach, K. (1981) Synthesis of substrate-selective polymers by host-guest polymerization. Macromolecular Chemistry and Physics 182: 687-692.

Bereczki, A., Tolokan, A., Horvai, G., Horvath, V., Lanza, F., Hall, A.J. and Sellergren, B. 2001. Determination of phenytoin in plasma by molecularly imprinted solid-phase extraction. Journal of Chromatography A 930: 31-38.

Blomgren, A., Berggren, C., Holmberg, A., Larsson, F., Sellergren, B. and Ensing, K. 2002. Extraction of clenbuterol from calf urine using a molecularly imprinted polymer followed by quantitation by high-performance liquid chromatography with UV detection. Journal of Chromatography A 975: 157-164.

Breton, F., Rouillon, R., Piletska, E.V., Karim, K., Guerreiro, A., Chianella, I. and Piletsky, S.A. 2007. Virtual imprinting as a tool to design efficient MIPs for photosynthesis-inhibiting berbicides. Biosensors and Bioelectronics 22: 1948-1954.

Cacho, C., Turiel, E., Martin-Esteban, A., Perez-Conde, C., and Camara, C. 2003. Cleanup of triazines in vegetable extracts by molecularly-imprinted solid-phase extraction using a propazine-imprinted polymer. Analytical and Bioanalytical Chemistry 376: 491-496.

Cacho, C., Turiel, E., Martín-Esteban, A., Ayala, D. and Pérez-Conde, C. 2006. Semi-covalent imprinted polymer using propazine methacrylate as template molecule for the clean-up of triazines in soil and vegetable samples. Journal of Chromatography A 1114: 255-262.

Caro, E., Marcé, R.M., Cormack, P.A.G., Sherrington, D.C. and Borrull, F. 2005. Synthesis and application of an oxytetracycline imprinted polymer for the solid-phase extraction of tetracycline antibiotics. Analytica Chimica Acta 552: 81-86.

Chianella, I., Lotierzo, M., Piletsky, S.A., Tothill, I.E., Chen, B., Karim, K. and Turner, A.P.F. 2002. Rational design of a polymer specific for microcystin-LR. Analytical Chemistry 74: 1288-1293.

Chianella, I., Karim, K., Piletska, E.V., Preston, C. and Piletsky, S.A. 2006. Computational design and synthesis of molecularly imprinted polymers with high binding capacity for pharmaceutical applications - model case: adsorbent for abacavir. Analytica Chimica Acta 559: 73-77.

Cormack, P.A.G. and Elorza, A.Z. (2004) Molecularly imprinted polymers: synthesis and characteristion. Journal of Chromatography B 804: 173-182.

de Prada, A.G-V., Martínez-Ruiz, P., Reviejo, A.J. and Pingarrón, J.M. 2005. Solid-phase molecularly imprinted on-line preconcentration and voltammetric determination of sulfamethazine in milk. Analytica Chimica Acta 539: 125-132.

Dickert, F.L., Lieberzeit, P., and Tortschanoff, M. 2000. Molecular imprints as artificial antibodies - a new generation of chemical sensors. Sensors and Actuators B 65: 186-189.

Dineiro, Y., Menendez, M.I., Blanco-Lopez, M.C., Lobo-Castanon, M.J., Miranda-Ordieres, A.J., Tunon-Blanco, P. 2006. Computational predictions and experimental affinity distributions for a homovanillic acid molecularly imprinted polymer. Biosensors and Bioelectronics 22: 364-371.

Djozan, D. and Ebrahimi, B. 2008. Preparation of new solid phase micro extraction fiber on the basis of atrazine-molecular imprinted polymer: application for GC and GC/MS screening of triazine herbicides in water, rice and onion. Analytical Chimica Acta 616: 152-159.

Duffy, D.J., Das, K., Hsu, S.L., Penelle, J., Rotello, V.M. and Stidham, H.D. 2002. Binding efficiency and transport properties of molecularly imprinted polymer thin films. Journal of the American Chemical Society 124: 8290-8296.

Farrington, K., Magner, E. and Regan, F. 2006. Predicting the performance of molecularly imprinted polymers: selective extraction of caffeine by molecularly imprinted solid phase extraction. Analytica Chimica Acta 566: 60-68.

García-Calzón, J.A. and Díaz-García, M.E. 2007. Characterization of binding sites in molecularly imprinted polymers. Sensors and Actuators B 123: 1180-1194.

González, G.P., Hernando, P.F. and Alegría, J.S.D. 2006. A morphological study of molecularly imprinted polymers using the scanning electron microscope. Analytica Chimica Acta 557: 179-183.

Guo, H. and He, X. 2000. Study of the binding characteristics of molecular imprinted polymer selective for cefalexin in aqueous media, Fresenius Journal of Analytical Chemistry 368: 461-465.

Haginaka, J. and Sagai, Y. 2000. Uniform-sized molecularly imprinted polymer material for (S)-propranolol. Journal of Pharmaceutical and Biomedical Analysis 22: 899-907.

Haginaka, J. and Sanbe, H. 2000. Uniform-sized molecularly imprinted polymers for 2-arylpropionic acid derivatives selectively modified with hydrophilic external layer and their applications to direct serum injection analysis. Analytical Chemistry 72: 5206-5210.

Halme, K., Lindfors, E. and Peltonen, K. 2004. Determination of malachite green residues in rainbow trout muscle with liquid chromatography and liquid chromatography coupled with tandem mass spectrometry. Food Additives and Contaminants 21: 641-648.

Haupt, K. and Mosbach K. 1998. Plastic antibodies: developments and applications. Tibtech November 16: 468-475.

Haupt, K. and Mosbach, K. 2000. Molecularly imprinted polymers and their use in biomimetic sensors. Chemical Reviews 100: 2495-2504.

Haupt, K. 2003. Imprinted polymers-tailor-made mimics of antibodies and receptors. Chemical Communications 171-178.

He, C., Long, Y., Pan, J., Li, K. and Liu, F. 2007. Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples. Journal of Biochemical and Biophysical Methods 70: 133-150.

Hirayama, K., Sakai, Y., Kameoka, K., Noda, K. and Naganawa, R. 2002. Preparation of a sensor device with specific recognition sites for acetaldehyde by molecular imprinting technique. Sensors and Actuators B 86: 20-25.

Hosoya, K., Yoshizako, K., Tanaka, N., Kimata, K., Araki, T. and Haginaka, J. 1994. Uniform-size macroporous polymer-based stationary phase for HPLC prepared through molecular imprinting technique. Chemistry Letters 1437-1438.

Hosoya, K., Yoshizako, K., Shirasu, Y., Kimata, K., Araki, T., Tanaka, N., *et al*. 1996. Molecularly imprinted uniform-size polymer-based stationary phase for high-performance liquid chromatography: structure contribution of crosslimked polymer network on specific molecular recognition. Journal of Chromatography A 728: 139-147.

Hosoya, K., Yoshizako, K., Sasaki, H., Kimata, K. and Tanaka, N. 1998. Molecular recognition towards coplanar polychlorinated biphenyls based on the porogen imprinting effects of xylenes. Journal of Chromatography A 828: 91-94.

Hwang, C.C. and Lee, W.C. 2001. Chromatographic resolution of the enantiomers of phenylpropanolamine by using molecularly imprinted polymer as the stationary phase. Journal of Chromatography B 765: 45-53.

Idziak, I., Benrebouh, A. and Deschamps, F. 2001. Simple NMR experiments as a means to predict the performance of an anti-17hethynylestradiol molecularly imprinted polymer. Analytica Chimica Acta 435: 137-140.

Katz, A. and Davis, M.E. 1999. Investigation into the mechanisms of molecular recognition with imprinted polymers. Macromolecules 32: 4113-4121.

Koostra, P.R., Kuijpers, C.J.P.F., Wubs, K.L., van Doorn, D., Sterk, S.S., van Ginkel, L.A. and Stephany, R.W. (2005) The analysis of beta-agonists in bovine muscle using molecular imprinted polymers with ion trap LCMS screening. Analytica Chimica Acta 529: 75-81.

Kriz, O., Ramstrom, O. and Mosbach, K. 1997. Molecular imprinting: new possibilities for sensor technology. Analytical Chemistry 69: 345A-349A.

Kubo, T., Hosoya, K., Watabe, Y., Ikegami, T., Tanaka, N., Sano, T. and Kaya, K. 2004. Recognition of hepatotoxic homologues of microcystin using a combination of selective adsorption media. Journal of Separation Science 27: 316-324.

Lavignac, N., Allender, C.J. and Brain, K.R. 2004. Current status of molecularly imprinted polymers as alternatives to antibodies in sorbent assays. Analytica Chimica Acta 510: 139-145.

Li, Y-H., Yang, T., Qi, X-L., Qiao, Y-W., Deng A-P. 2008. Development of a group selective molecularly imprinted polymers based solid phase extraction of malachite green from fish water and fish feed samples. Analytica Chimica Acta 624: 317-325.

Lin, L.Q., Zhang, J., Fu, Q., He, L.C. and Li, Y.C. 2006. Concentration and extraction of sinomenine from herb and plasma using a molecularly imprinted polymer as the stationary phase. Analytica Chimica Acta 561:178-182.

Liu, H.Y., Row, K.H. and Yang, G.L. 2005. Monolithic molecularly imprinted columns for chromatographic separation. Chromatography 61: 429-432.

Liu, F., Liu, X., Ng, S.C. and Chan, H.S. 2006. Enantioselective molecular imprinting polymer coated QCM for the recognition of l-tryptophan. Sensors and Actuators B 113: 234-240.

Lord, H.L. and Pawliszyn, J. 1998. Recent advances in solid-phase microextraction. LC GC: Liquid Chromatography, Gas Chromatography 16: 41.

Lu, Y., Zhang, H. and Liu, X. 2003. Study on the mechanism of chiral recognition with molecularly imprinted polymers. Analytica Chimica Acta 489: 33-43.

Lübke, C., Lübke, M., Whitcombe, M.J. and Vulfson, E.N. 2000. Imprinted polymers prepared with stoichiometric template-monomer complexes: efficient binding of ampicillin from aqueous solutions. Macromolecules 33: 5098-5105.

Mahony, J.O., Nolan, K., Smyth, M.R and Mizaikoff, B. 2005. Molecularly imprinted polymers - potential and challenges in analytical chemistry. Analytica Chimica Acta 534: 31-39.

Maier, N.M., Buttinger, G., Welhartizki, S., Gavioli, E. and Lindner, W. 2004. Molecularly imprinted polymer-assisted sample clean-up of ochratoxin A from red wine: merits and limitations. Journal of Chromatography B 804: 103-111.

Martin, P.D., Jones, G.R., Stringer, F. and Wilson, I.D. 2003. Comparison of normal and reversed-phase solid phase extraction methods for extraction of β-blockers from plasma using molecularly imprinted polymers. Analyst 128: 345-350.

Martin-Esteban, A., Tadeo, J.L. 2006. Selective molecularly imprinted polymer obtained from a combinatorial library for bisphenol A. Combinatorial Chemistry and High Throughput Screening 9: 747-751.

Martín-Esteban, A. 2001. Molecularly imprinted polymers: new molecular recognition materials for selective solid-phase extraction of organic compounds. Fresenius Journal of Analytical Chemistry 370: 795-802.

Matsui, J., Kato, Y., Takeuchi, T., Yokoyama, K., Tamiya, E. and Karube, I. 1993. Molecular recognition in continuous polymer rods prepared by a molecular imprinting technique. Analytical Chemistry 65: 2223-2224.

Matsui, J., Miyoshi, Y. and Matsui, R. 1995. Rod-type affinity media for liquid chromatography prepared by in-situ molecular imprinting. Analytical Sciences 11: 1017-1019.

Mayes, A.G. and Mosbach, K. 1996. Molecularly imprinted polymer beads: suspension polymerization using a liquid perfluorocarbon as the dispersing phase. Analytical Chemistry 68: 3769-3774.

Molinelli, A., Weiss, R. and Mizaikoff, B. 2002. Advanced solid phase extraction using molecularly imprinted polymers for the determination of quercetin in red wine. Journal of Agricultural and Food Chemistry 50: 1804-1808.

Mosbach, K. 1994. Molecular imprinting. Trends in Biochemical Sciences 19: 9-14.

Mullett, W. and Lai, E. (1998) Determination of theophylline in serum by molecularly imprinted solid-phase extraction with pulsed elution. Analytical Chemistry 70: 3636-3641.

O'Mahony, J., Molinelli, A., Nolan, K., Smyth, M.R. and Mizaikoff, B. 2005. Towards the rational development of molecularly imprinted polymers: 1H NMR studies on hydrophobicity and ion-pair interactions as driving forces for selectivity, Biosensors and Bioelectronics 20: 1884-1893.

Oral, E. and Peppas, N.A. 2004. Dynamic studies of molecular imprinting polymerizations. Polymer 45: 6163-6173.

Pérez-Moral, N. and Mayes, A.G. 2004. Comparative study of imprinted polymer particles prepared by different polymerization methods. Analytica Chimica Acta 504: 15-21.

Peter, S., Schweitz, L. and Nilsson, S. 2003. Molecularly imprinted polymers in capillary electrochromatography: recent developments and future trends. Electrophoresis 24: 3892-3899.

Pichon, V. 2007. Selective sample treatment using molecularly imprinted polymers. Journal of Chromatography A 1152: 41-53.

Piletsky, S.A., Karim, K., Piletska, E.V., Day, C.J., Freebairn, K.W., Legge, C. and Turner, A.P.F. 2001a. Recognition of ephedrine enantiomers by molecularly imprinted polymers designed using a computational approach. Analyst 126: 1826- 1830.

Piletsky, S.A., Subrahmanyam, S., Piletska, E.V., Chen, B.N., Karim, K. and Turner, A.P.F. 2001b. 'Bite-and-switch' approach using computationally designed molecularly imprinted polymers for sensing of creatine, Biosensors and Bioelectronics 16: 631-637.

Piletsky, S.A., Chianella, I., Lotierzo, M., Tothill, I.E., Chen, B.N., Karim, K. and Turner, A.P.F. 2002. Rational design of a polymer for microcystin-LR using a computational approach. Analytical Chemistry 74: 1288-1293.

Piletsky, S.A., Turner, N.W., Piletska, E.V., Karim, K., Whitcombe, M., Malecha, M., Magan, N. and Baggiani, C. 2004. Effect of the solvent on recognition properties of molecularly imprinted polymer specific for ochratoxin A. Biosensors and Bioelectronics 20: 1060-1067.

Piletsky, S.A., Turner, N.W. and Laitenberger, P. 2006. Molecularly imprinted polymers in clinical diagnostics - future potential and existing problems. Medical Engineering and Physics 28: 971-977.

Pizzariello, A., Stred'ansky, M., Stred'anska, S. and Miertus, S. 2001. A solid binding matrix/molecularly imprinted polymer-based sensor system for the determination of clenbuterol in bovine liver using differential-pulse voltammetry. Sensors and Actuators B 76: 286-294.

Puoci, F., Garreffa, C., Iemma, F., Muzzalupo, R., Spizzirri, U.G. and Picci, N. 2005. Molecularly imprinted solid phase extraction for detection of Sudan I in food matrices. Food Chemistry 93: 349-353.

Quaglia, M., Chenon, K., Hall, A.J., De Lorenzi, E., Sellergren, B. 2001. Target analogue imprinted polymers with affinity for folic acid and related compounds. Journal of the American Chemical Society 123: 2146-2154.

Ruckert, B., Hall, A.J. and Sellergren, B. 2002. Molecularly imprinted composite materials via iniferter-modified supports. Journal of Materials Chemistry 12: 2275-2280.

Sambe, H., Hoshina, K., Hosoya, K. and Haginaka, J. 2006. Simultaneous determination of bisphenol A and its halogenated derivatives in river water by combination of isotope imprinting and liquid chromatography-mass spectrometry. Journal of Chromatography A 1134: 16-23.

Sanbe, H., Hoshina, K., Haginaka, J., Kunimoto, K.-K. and Fu, Q. 2002. Chiral recognition based on (S)-nilvadipine-imprinted polymers and chiral recognition mechanism. Chromatography 23: 65-66.

Sellergren, B. and Andersson, L.I. 1990. Molecular Recognition in Macroporous Polymers Prepared by a Substrate Analogue Imprinting Strategy. Journal of Organic Chemistry 55: 3381-3383.

Sellergren, B., Ruckert, B. and Hall, A.J. 2002. Layer-by-layer grafting of molecularly imprinted polymers via iniferter modified supports. Advanced Materials 14: 1204-1208.

Shea, K.J. and Sasaki, D.Y. 1991. An analysis of small-molecule binding to functionalized synthetic polymers by 13C-CP/MAS NMR and FT-IR spectroscopy. Journal of the American Chemical Society 113: 4109-4120.

Shi, X., Wu, A., Zheng, S., Li, R. and Zhang, D. (2007) Molecularly imprinted polymer microspheres for solid-phase extraction of chloramphenicol residues in foods. Journal of Chromatography B 850: 24-30.

Spivak, D.A. 2005. Optimization, evaluation, and characterization of molecularly imprinted polymers. Advanced Drug Delivery Reviews 57: 1779-1794.

Striegler, S., Tewes, E. 2002. Investigation of sugar-binding sites in ternary ligand-copper(II)-carbohydrate complexes. European Journal of Inorganic Chemistry (2): 487-495.

Su, L.Q., Qiao, S. and Zhang W.B. 2007. Studies on the synthesis and properties of malachite green imprinted polymer. Chinese Chemical Letters 18: 229-232.

Suedee, R., Srichana, T., Chuchome, T. and Kongmark, U. 2004. Use of molecularly imprinted polymers from a mixture of tetracycline and its degradation products to produce affinity membranes for the removal of tetracycline from water. Journal of Chromatography B 811: 191-200.

Svenson, J. and Nicholls, I.A. 2001. On the thermal and chemical stability of molecularly imprinted polymers. Analytica Chimica Acta 435: 19-24.

Svenson, J., Karlsson, J.G. and Nicholls, I.A. 2004. 1H nuclear magnetic resonance study of the molecular imprinting of (-)-nicotine: template self-association, a molecular basis for cooperative ligand binding. Journal of Chromatography A 1024: 39-44.

Takeuchi, T., Fakuma, D. and Matsui, J. (1999) Combinatorial molecular imprinting: an approach to synthestic polymer receptors. Analytical Chemistry 71: 285-290.

Tamayo, F.G., Casillas, J.L. and Martin-Esteban, A. 2003. Highly selective fenuron-imprinted polymer with a homogeneous binding site distribution prepared by precipitation polymerization and its application to the clean-up of fenuron in plant samples. Analytica Chimica Acta 482: 165-173.

Tamayo, F.G., Turiel, E. and Martín-Esteban, A 2007. Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: recent developments and future trends. Journal of Chromatography A 1152: 32-40.

Tanabe, K., Takeuchi, T., Matsui, J., Ikebukuro, K., Yano, K. and Karube, I. 1995. Recognition of barbiturates in molecularly imprinted copolymers using multiple hydrogen bonding, Journal of the Chemical Society-Chemical Communications 2303-2304.

Turiel, E., Martin-Esteban, A., Fernández, P., Pérez-Conde, C. and Cámara, C. 2001. Molecular recognition in a propazine-imprinted polymer and its application to the determination of triazines in environmental samples. Analytical Chemistry 73: 5133-5141.

Turner, N.W., Piletska, E.V., Karim, K., Whitcombe, M., Malecha, M., Magan, N., Baggiani, C. and Piletsky, S.A. 2004. Effect of the solvent on recognition properties of molecularly imprinted polymer specific for ochratoxin A. Biosensors and Bioelectronics 20: 1060-1067.

Wang, J.F, Cormack, P.A.G., Sherrington, D.C. and Khoshdel, E. 2003. Monodisperse, Molecularly imprinted polymer microspheres prepared by precipitation polymerization for affinity separation applications. Angewandate Chemie-International Edition in English 42: 5336-5338.

Watabe, Y., Hosoya, K., Tanaka, N., Kubo, T. and Morita, M. 2005. Novel surface modified molecularly imprinted polymer focused on the removal of interference in environmental water samples for chromatographic determination. Journal of Chromatography A 1073: 363-370.

Weiss, R., Molinelli, A., Jakusch, M. and Mizaikoff, B. 2001. Molecular imprinting and solid phase extraction of flavonoid compounds. Bioseparation 10: 379-387.

Whitcombe, M.J., Rodriguez, M.E., Villar, P. and Vulfson, E. 1995. A new method for the introduction of recognition site functionality into polymers prepared by molecular imprinting: Synthesis and characterization of polymeric receptors for cholesterol. Journal of the American Chemical Society 117: 7105-7111.

Whitcombe, M.J., Martin, L. and Vulfson E.N. 1998. Predicting the selectivity of imprinted polymers. Chromatographia 47: 457-464.

Wulff, G.. and Sarhan, A. 1972. Use of polymers with enzyme-analogous structures for the resolution of racemates. Angewandate Chemie-International Edition in English **11**: 341-344.

Wulff G. 2002. Enzyme-like catalysis by molecularly imprinted polymers. Chemical Reviews 102: 1-27.

Xie, J., Zhu, L., Luo, H., Zhou, L., Li, C. and Xu, X. 2001. Direct extraction of specific pharmacophoric flavonoids from ginko leaves using a molecularly imprinted polymer for quercetin. Journal of Chromatography A 934: 1-11.

Yan, S., Gao, Z., Fang, Y., Cheng, Y., Zhou, H. and Wang, H. 2007. Characterization and quality assessment of binding properties of malachite green molecularly imprinted polymers prepared by precipitation polymerization in acetonitrile. Dyes and pigments 74: 572-577.

Ye, L. and Mosbach, K. (2001) Molecularly imprinted microspheres as antibody binding mimics. Reactive and Functional Polymers 48: 149-157.

Zhang, M.L., Xie, J.P., Zhou, Q., Chen, G.Q. and Liu, Z. 2003. On-line solid-phase extraction of ceramides from yeast with ceramide III imprinted monolith. Journal of Chromatography A 984: 173-183.

Zhu, Q.Z., Degelmann, P., Niessner, R. and Knopp, D. 2002. Selective trace analysis of sulfonylurea herbicides in water and soil samples based on solid-phase extraction using a molecularly imprinted polymer. Environmental Science and Technology 36: 5411-5420.

(a) Zhu, X., Su, Q., Cai, J., Yang, J. and Gao, Y. 2006. Molecularly imprinted polymer membranes for substance-selective solid-phase extraction from aqueous solutions. Journal of Applied Polymer Science 101: 4468-4473.

(b) Zhu, X., Cao, Q., Hou, N., Wang, G. and Ding, Z. 2006. The preparation and the recognition property of molecularly imprinted polymer of podophyllotoxin. Analytica Chimica Acta 561: 171-177.

Zander, A., Findlay, P., Renner, T., Sellergren, B. and Swietlow, A. 1998. Analysis of nicotine and its oxidation products in nicotine chewing gum by a molecularly imprinted solid-phase extraction. Analytical Chemistry 70: 3304-3314.