

REVIEW ARTICLES

Molecularly imprinted polymers

Polyméry s molekulovými odtlačkami

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Summary

Nowadays about 28 million different simple or complex chemical entities are known. Today we cannot imagine the life without different kinds of drugs, cosmetics, pesticides, food additives or stimulants. In the recent period in the field of analytical chemistry or pharmacy more modern techniques or methodologies are required which will allow selective determination of different kinds of analytes, especially in complex biological matrices. The imprinted polymers are very often used for the preparation of samples before analysis and this procedure can reduce the possibilities of interferences. This paper deals with the characterization, preparation, properties and application of imprinted polymers in the field of drugs, cosmetics, food and biological materials.

Keywords: molecularly imprinted polymers • chromatography • extraction • drugs

Súhrn

V súčasnosti je známych približne 28 miliónov rôznych jednoduchých alebo komplikovaných zlúčenín. Dnes si ťažko môžeme predstaviť život bez rôznorodých druhov liečiv, kozmetických prípravkov, pesticídov, prísad do potravín, alebo povzbudzujúcich prostriedkov. V poslednom čase v oblasti analytickej chémie, alebo farmácie stále viac sa vyžaduje použiť moderné techniky alebo nové metodológie, ktoré umožňujú selektívne stanovenie rôznych analytov, zvlášť v zložitých biologických vzorkách. Stále častejšie na prípravu vzoriek k analýze sa využívajú polyméry s molekulovými odtlačkami, pomocou ktorých možno značne zabrániť interferenciám pri stanovení stopových koncentrácií analytov. Táto práca sa zaoberá charakterizáciou, prípravou, vlastnosťami a aplikáciou polymérov s molekulovými odtlačkami v oblas-

ti analýzy vzoriek liečiv, kozmetických prípravkov, potravín a biologických materiálov.

Kľúčová slova: polyméry s molekulovými odtlačkami • chromatografia • extrakcia • liečiva

Introduction

The most frequently used methods for sample preparation are liquid-liquid extraction (LLE) or solid-phase extraction (SPE). Due to a large waste of solvents, the LLE method is not employed today so often as its good alternative, the SPE method. The SPE is used in food, environmental, clinical or pharmaceutical chemistry to preconcentrate, cleanup complex matrices, analyte storage with high volatility or those not stable in a liquid medium or to carry out derivatization reactions between the reactive groups on the sorbent surface and those in the analyte^{1–3}.

The most common coupling of the SPE method is with high performance liquid chromatography (HPLC), capillary electrophoresis (CE) or gas chromatography (GC) where the solid phases are often based on silica or bonded silica. The SPE method is simple and fast, it does not require a high quantity of a solvent, it is cheap, effective and recoverable, and it can be automated in a simple way⁴.

However, despite the many qualities, this method has still limitations which play an important role in the extraction mechanisms. First of all the SPE is not so selective according to requirements in modern biological or environmental laboratories. Mostly, the problem is in incomplete end-capping processes or with the presence of interfering groups in complicated matrices which have similar or the same sorption mechanisms as the analyte of interest^{5, 6}.

For this reason the SPE has undergone changes, which have allowed a wider application in a sample preparation.

First, there was an application of the molecularly selective immunosorbents. Immunosorbents make use of special molecular recognition between antibodies and antigens. In spite of a big selectivity to the target molecules, they are very expensive to prepare, time-consuming, fragile and less stable. Moreover, antibodies are easily denatured in the presence of organic solvents and consequently difficult to isolate^{7–10}.

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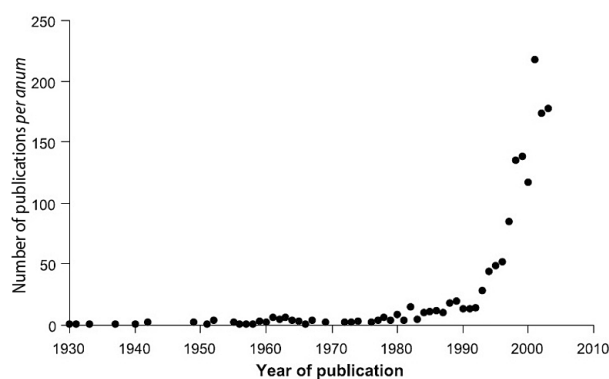


Fig. 1. The number of publications within the field of molecular imprinting science and technology per annum for the period 1931–2003¹⁴⁾

All these obstructions can be omitted by using molecularly imprinted polymers (MIPs). The MIPs are synthetic highly cross-linked polymers prepared in the presence of the target molecule, named the template^{11, 12)}. In the presence of this specific analyte special cavities are formed, tailor-made by copolymerization of functional and cross-linking monomers. After polymerization, the print molecule is removed leaving three-dimensional binding sites. Consequently, the resultant polymer possesses the abilities to recognize any molecule or groups of molecules on which it was designed¹³⁾.

Over many years, since Polyakov¹⁴⁾ in the 1930s published his first report about molecular imprinting, the interest for the MIPs was insignificant. The true growth started at the beginning of the 1990s and has been rapidly increasing since. In spite of their great similarity to the biological systems, they are still inexpensive and simple to prepare.

Schematically, the growing interest in the MIPs is shown in Figure 1.

Characterization of MIPs

It should be noted that simultaneously with MIP preparation, a non-imprinted polymer (NIP, a polymer without the presence of the template) is always prepared to compare nonspecific interactions of the target molecules with the non-imprinted cavities.

The MIPs, as very selective and sensitive materials, are perfect tools for pre-concentration or extraction of an analyte of interest, mainly in environmental or biological laboratories and even in cosmology^{15, 16)}.

Izenberg et al.¹⁶⁾ suggested employing of the MIPs in astrobiology missions as an excellent device for biological samples detection in multi-sensor microlaboratories.

However, another and more ordinary application of MIPs can include stationary phases and sorbents for HPLC, thin layer chromatography (TLC) or capillary electrochromatography (CEC) in analytical chemistry, catalysts, biomimetic sensors, binding assays, reusable protecting groups or polymer-supported reagents in organic synthesis^{17–19)}.

Molecular imprinting techniques

Generally MIPs can be synthesized by three different procedures^{10, 20, 21)}:

A. The non-covalent imprinting is the most commonly used procedure for the MIPs synthesis. This technique exploits *in situ* forming of the template and functional monomer complex by nonspecific interactions such as hydrogen bonding, van der Waals or electrostatic forces, ionic or hydrophobic interactions. The main advantage of this method is its simplicity, low costs of preparation, fast binding of the template, easy removal of the template from the polymer by Soxhlet extraction, for instance, and its potential application to a wide range of target molecules. However, the polymerization conditions have to be carefully chosen to minimize the nonspecific binding sites.

B. The covalent imprinting depends on covalent-linkage of the template with the functional monomer prior to the polymerization process. The template removal takes place in the way of a chemical reaction. The binding of the target molecules is proceeding in the same way and via the same covalent interactions as with the template. The main advantage of this approach is that a wider spectrum of polymerization conditions can be used and the template/monomer complex is stable and stoichiometric. In comparison with the non-covalent approach this method is much more exacting, more extensive, the template binding and its releasing is slower and due to the polymerization conditions there are some limits for the use of different molecules as a template.

C. The semi-covalent imprinting is the hybridization of both approaches, covalent and non-covalent. The polymerization process takes place on a covalent way but the following template binding is non-covalent. The combination of these two methods provides advantages from both of these. Firstly, during of the quick binding process of the template the production of stable and stoichiometric complex takes place and secondly, the target molecules are fast binding in a non-covalent way.

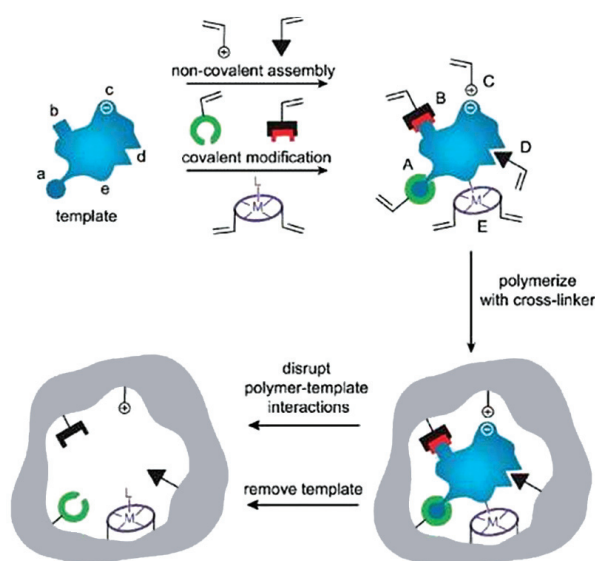


Fig. 2. The molecular imprinting process¹⁴⁾

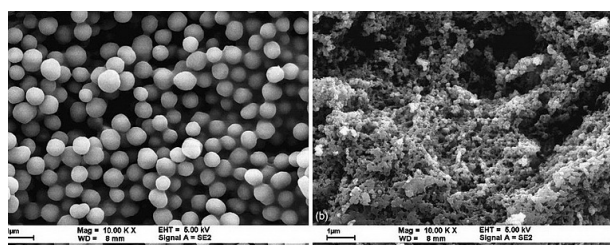


Fig. 3. The morphology of MIP particles prepared by (a) precipitation polymerization, (b) traditional bulk polymerization²⁶⁾

The scheme of the imprinting process is shown in Figure 2.

Production methodologies of MIPs

Presently the MIPs are prepared by the six following techniques:

A. Bulk polymerization is the most widely used procedure for the MIPs preparation^{22–24)}. This method does not require any complicated devices or particular skills. However, in spite of its simplicity and universality, this method is time-consuming, wasteful, needs the crushing, grounding and sieving processes, which affects the large loss of the product. Moreover, the particles produced by this method have low capacity, are irregular in shape and size, which causes peaks tailing and broadening because of their heterogeneity.

B. Precipitation polymerization is a modification of bulk polymerization and employs the largest amount of porogen, usually more than 95% (w/v). From this reason, precipitation polymerization disables particles aggregation and provides the microspheres with diameters in scales ranging from 0.3 to 10 μm , suitable for chromatographic applications. Except the limitations typical of bulk polymerization, this approach uses the largest volume of porogen and the MIP particles have the binding sites which are inside their networks, causing a slow mass transfer of target molecules^{14, 25)}. The morphology of MIPs beads prepared by bulk polymerization and precipitation polymerization is shown in Figure 3.

C. Suspension polymerization permits to obtain spherical beads in a fast and simple way and is mainly used for chromatography and electrochromatography-grade imprinted materials. This methodology has been carried out in liquid organic solvents or in water as the continuous phase and provides highly reproducible results. Suspension polymerization has proceeded in the UV irradiation only by less than 2 h. The particles diameter achieved by this method depends on the amount of the surfactant and the stirring speed. The presence of the surfactant in the mixture can cause some problems because of its interfering with the template-monomer interactions. Moreover, when water as a suspension phase is used, the non-covalent approach is not possible^{22, 27, 29)}.

D. Two-steps swelling polymerization method has used water as the suspension medium. This technique offers monodisperse beads ranging from 2 to 50 μm , ideal for HPLC use. These particles possess good separating abilities and yielded better column

efficiencies and peak shapes than the particles achieved by bulk polymerization. Main limitations of this method are complications due to the reaction conditions and procedures^{21, 28, 30, 31)}.

E. Surface imprinting polymerization is the method where the MIP layers are grafting onto the surface of preformed beads. The surface imprinting polymerization takes place in the presence of common substrates, used in other types of polymerization and in the presence of an emulsion stabilizer with a polymer matrix-forming comonomer. This method has found an application in separation, medical uses or sensing. The methodology is easy, yields monodisperse, thin imprinted layers but needs a complicated system and is time-consuming^{21, 32)}.

F. In situ polymerization is achieved by a direct one-step polymerization of a polymer mixture in stainless steel columns. After the polymerization, the template and porogen are washed out by a methanol-acetic acid mixture. The monolithic-MIP preparation is simple and the results demonstrate high selectivity, sensitivity, reproducibility and fast mass transport. The time consuming character of the preparation of each new template system is the main limitation of this approach²¹⁾.

MIPs preparation

Briefly, the procedure for the MIP synthesis is as follows. The template molecule with a functional monomer, cross-linking monomer, porogenic solvent (porogen) and an initiator have to be mixed. In order to initiate polymerization, the mixture is heated or irradiated with UV light. During the polymerization process a stable complex develops between the functional groups of the template and the functional monomer. In order to maximize interactions, the functional monomer has to possess complementary groups with the template as much as is possible. Finally the product in the form of a rigid and highly cross-linked polymer is crushed, sieved and submitted to an extraction process in order to remove the template. After that, the three-dimensional cavities can recognize any target molecules with a complementary shape and chemical properties to those of the template.

The most common functional monomers described in the literature are: methacrylic acid (MAA)^{17, 33–42)}, acrylamide (AA)^{35, 43–46)}, 2-vinylpyridine (2-VP)^{38–40)}, 4-vinylpyridine (4-VP)^{17, 35, 43, 44, 47)}, trifluoromethacrylic acid (TFMAA)^{14, 31, 41)}, styrene¹⁷⁾ and methacrylamide (MAAM)^{48, 49)}.

Another essential element of MIP polymerization is a cross-linker which has three major functions: firstly, it controls the morphology of the polymer matrix. Secondly, it is necessary to stabilize the imprinted binding sites, so the amount of the cross-linker should be sufficient to keep the stability of them. Finally, its presence provides the stability of the polymer matrix. Usually, an 80% excess of a cross-linker is used in the polymerization mixture.

The commonly used cross-linking monomers are: ethylene glycol dimethacrylate (EGDMA)^{17, 31, 33–35, 37, 40–43)}, trimethylolpropane trimethacrylate (TRIM)^{42, 50, 51)},

N,N'-1,3-phenylene bismethacrylamide (PBMA)⁵²⁾ and bisacrylamide (BAAM)⁵²⁾.

The role of a porogen is to dissolve all components present in a polymerization mixture and to make it possible to produce large pores in order to allow an access to the binding sites, which directly influences the imprinted polymer performance. The nature and level of porogenic solvents determines the strength of the non-covalent interactions. The porogens should have relatively low polarity, in order to reduce the interferences during the template-monomer complex formation.

The most widely used organic porogens are: methanol, acetonitrile, toluene, dodecanol, dichloromethane or chloroform. All of them enhance ionic interactions between the template and the functional monomer. Also water can be used as a porogen, which can support the formation of hydrophobic interactions^{14, 21)}.

Advantages and drawbacks of MIPs

Molecularly imprinted polymers in comparison with biomolecules (receptors, enzymes) have many advantages but also a few properties which need to be improved or completely eliminated. Receiving the best polymer may take a few years and hundreds bad sorbents but they are still the most universal materials in modern laboratories.

The advantages of MIPs include^{8, 19, 33, 52–57)}:

- Low cost and simple preparation;
- High selectivity;
- Possibility of use in aggressive media (concentrated bases or acids, organic solvents);
- Mechanical strength;
- Durability to heat and pressure;
- Possibility to repeat analyses without loss of their activities;
- Potential applications for a wide range of target molecules.

The disadvantages of MIPs can be enumerated as follows^{58, 59)}:

- The imprinted polymers are insoluble;
- It is difficult to completely remove the template from the polymer;
- Cavities which are non-imprinted are always present in the polymer;
- There is no ideal effective procedure for the design of MIPs.

Application of MIPs

Many papers describe an application of MIPs as sorbents in SPE, SPME or TLC, stationary phases in LC, the chiral selective matrix in CE and CEC, membranes, drug delivery systems, sensors, in immunoassays or catalysis^{7, 20, 60–62)}.

The MIPs have found a wide application in analyses of different kinds of samples. The most common investigative matrices are:

- *Food samples*: caffeine in beverages and coffee⁶³⁾, simultaneous determination of caffeine and theophylline

in green tea and human plasma³⁶⁾, quercetin in red wine⁶⁴⁾, sulfamethazine in milk⁶⁵⁾, triazines in food samples^{60, 66)}, clenbuterol in animal feeds^{67, 68)}, tetracycline antibiotics in egg sample²³⁾ or nerve agent degradation products in rice samples¹²⁾.

- *Environmental samples*: triazines and their metabolites⁶⁹⁾, anti-inflammatory drugs⁷⁰⁾, catechol⁷¹⁾ in river water, benzo(a)pyrene in tap water, lake water or instant coffee samples⁷²⁾, β -blockers⁷³⁾, bisphenol A^{51, 74–76)}, ciprofloxacin⁷⁷⁾, triazines⁷⁸⁾ and naphthalene mono- and disulfonates⁷⁹⁾ in water samples, 4-chlorophenols and 4-nitrophenol in river water⁸⁰⁾, nerve agent degradation products in aqueous soil extracts⁸¹⁾, organophosphorus pesticides in water and soil⁸²⁾ or sulfonylurea in water and soil samples⁸³⁾.

- *Drugs and biological samples*: caffeine in human urine⁶³⁾, β -agonists in the porcine⁶⁷⁾ and bovine muscle^{82, 85)} and the liver⁶⁷⁾, calves urine⁸⁶⁾, and biological materials⁶⁸⁾ or in the pork liver⁸⁷⁾, propranolol⁸⁸⁾, albuterol⁸⁹⁾, sulpiride and atenolol⁹⁰⁾, Cd(II)⁹¹⁾, Fe(III)⁹²⁾, trimethoprim⁹³⁾, phenytoin⁹⁴⁾, ropivacaine, mepivacaine and bupivacaine⁹⁵⁾ and derivatives of phenylcarbamic acid⁹⁶⁾ in human plasma, methotrexate⁹⁷⁾, clenbuterol in calves urine⁹⁸⁾, theophylline⁹⁹⁾ and degradation products of nerve agents¹¹⁾ in human serum, tramadol in human plasma^{100, 101)} and urine samples¹⁰⁰⁾, cotinine¹⁰¹⁾ and naproxen¹⁰³⁾ from urine samples, morphine¹⁰⁴⁾, quercetin from rats plasma¹⁰⁵⁾, verapamil and its metabolites in urine, plasma and cell culture¹⁰⁶⁾, 17 β -estradiol⁴⁵⁾, pentamidine¹⁰⁷⁾, L-theanine from plant material¹⁰⁸⁾, mycophenolic acid in maize¹⁰⁹⁾, phenylcarbamic acid derivatives in rat serum and human plasma^{110, 111)}, diphenyl phosphate¹¹²⁾ and tamoxifen¹¹³⁾ in human urine, scopolamine in urine and serum samples¹¹³⁾, atropine and scopolamine in pharmaceutical preparations containing *Scopolia* extract¹¹⁵⁾, estrogens in fishery samples¹¹⁶⁾, phenobarbital in human urine and medicines¹¹⁷⁾, nateglinide and its enantiomer¹¹⁸⁾, ciprofloxacin and enrofloxacin in urine and tissues samples¹¹⁹⁾, cholesterol¹²⁴⁾, tetracycline antibiotics in pig kidney tissue extract¹²⁰⁾, ceramides in yeast lipid extracts¹²¹⁾, (–)-ephedrine in herbal ephedra¹²²⁾, anti-EGFR inhibitors in extract and whole *Caragana jubata* plant¹²³⁾, chloramphenicol in ophthalmic solutions and spiked milk¹²⁴⁾, (S)-nicotine in cigarette smoke extract¹²⁵⁾.

Conclusion

Presently molecularly imprinted polymers are the most promising and popular research objects in chemistry. They are used as sorbents in SPE and SPME, as stationary phases in HPLC or CEC, as sensors, catalysts, binding assays, reusable protecting groups or polymer-supported reagents in organic synthesis. Due to their properties they are excellent materials for sample pre-concentration, cleaning or extraction, especially for complex matrices.

Among all available sorbents, they are distinguished by their durability on harsh media, heat, pressure or mechanical strength. They are highly selective for different target molecules, nature friendly because of a high level of regeneration, and the big advantage is also

low costs of MIPs preparation. Working with MIPs, in most cases, does not require complicated instruments or special skills of the operator.

The MIPs, depending on the necessity or laboratory equipment, can be prepared by different ways: by the most popular bulk polymerization, suspension polymerization, precipitation polymerization, two-steps swelling polymerization, *in situ* polymerization or by surface imprinting polymerization.

Conflict of interest: none.

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