Applications of molecularly imprinted polymers in analytical and pharmaceutical chemistry

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SUMMARY

Application of molecularly imprinted polymers in analytical and pharmaceutical chemistry
The paper reviews recent developments in the use of molecularly imprinted polymers in several analytical techniques, such as solid-phase extraction, liquid chromatography, capillary electrophoresis, capillary electrochromatography, and as selective sorbents in chemical sensors. Molecular imprinting is achieved by the interaction between complementary groups in a template molecule and functional monomer units. The benefits of imprinted polymers are low cost, storage stability, high mechanical strength, repeated operations without loss of activity, and potential application to a wide range of target molecules.
Key words: molecularly imprinted polymers – solid-phase extraction – liquid chromatography – electrochromatography – membranes – sensors *Má*

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SÚHRN

Využitie polymérov s molekulovými odtlačkami v analytickej a farmaceutickej chémii
Tento súborný referát prezentuje prehľad využitia polymérov s molekulovými odtlačkami v rôznych analytických metódach, ako sú extrakcia na tuhej fáze, kvapalinová chromatografia, kapilárna elektroforéza, kapilárna elektrochromatografia, a ako selektívny sorbent v senzoroch. Odtláčanie molekúl sa dosahuje pomocou interakcií medzi komplementárnymi skupinami molekuly templátu (odtláčanej molekuly) a funkčného monoméru. Výhodou polymérov s molekulovými odtlačkami je nízka cena, stabilita pri skladovaní, vysoká mechanická pevnosť, možnosť opakovaného použitia bez straty aktivity, a možnosť využitia pre širokú škálu sledovaných molekúl.
Kľúčové slová: polyméry s molekulovými odtlačkami – extrakcia na tuhej fáze, kvapalinová chromatografia – elektrochromatografia – membrány – senzory

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Molecularly imprinted polymers (MIPs) are synthetic materials with artificially generated recognition sites able to specifically rebind a target molecule in preference to other closely related compounds. MIPs are obtained by polymerising functional and cross-linking monomers around a template molecule, leading to a highly cross-linked three-dimensional network polymer. The monomers are chosen considering their ability to interact with the functional groups of the template molecule. After polymerization the template molecule is removed and binding sites are established.

The idea of molecular specificity that could be tailored in sorbents has been around for a long time. In 1931, Polyakov [1] demonstrated that molecular specificity could be imprinted into silica gel by pretreating silicic acid with organic adsorbates before polycondensation. Later, Dickey [2, 3] performed more extensive investigations. Dickey precipitated silicic acid in the presence of alkyl orange dyes and found that after drying the hydrogel and removing as much of the excess imprinting agent as possible, the resulting xerogel had a greatly increased adsorption capacity for the particular dye present during gelation. In addition, the natural selectivity of silica gel for one dye in a series of azo dyes could be reversed by this pretreatment procedure. Modern molecular imprinting has its roots in Europe with the extensive investigation of Wulff [4, 5], Ramström [6] and Sellergren [7].

MIPs are stable towards a wide range of solvents, are highly thermostable, and can be used over a range of temperatures. They can be stored at ambient temperature and in dry state without loss of performance [8, 9]. MIPs can be easily and quickly prepared and can be applied to a wide range of target molecules [10, 11]. The selectivity is comparable to that of immunosorbents and the capacity of MIP is even higher. However, MIPs possess many disadvantages, for example, it is hard to completely remove the template from the polymer, the imprinted polymer is insoluble. The polymer contains many imprinted cavities of which only some are really good and match the template molecule [12]. There is no ideal of effective procedure for the design of MIPs and it might take several weeks of trial-and-error experiments using different formulations.

MIP preparation

Imprinting techniques

In general, molecular imprinting can be achieved in three ways:

1. The non-covalent procedure is based on the formation of a prepolymerization complex between monomers carrying suitable functional groups and the template, through noncovalent bonds such as ionic interactions or hydrogen-bonding. The template can be removed after polymerization simply by solvent extraction.

2. The covalent imprinting, in which polymerizable derivatives of the template molecule are copolymerized with a crosslinking monomer. These derivatives are obtained by forming covalent bonds between the template and suitable polymerizable monomers. To remove the template from the polymer, these covalent bonds have to be chemically cleaved [13–15].

3. Semi-covalent approach is a hybrid of the two previous methods. Covalent bonds are established between the template and the functional monomer before polymerization, once the template has been removed from the polymer matrix, the subsequent re-binding of the analyte to the MIP exploits non-covalent interactions [14].

Polymer preparation

The procedure for the synthesis is as follows. The polymerization mixture usually contains a porogen, a template, a functional monomer, a cross-linker, and an initiator. The template molecule must contain functional groups that sufficiently interact with the functional groups of the monomer to form a stable complex. MAA or TFMAA are commonly used as monomers for templates containing basic groups. For analytes with an acid functional group, monomers containing basic functional groups, such as 2-VP, 4-VP or AA, are suitable. If the mentioned monomers are used, the monomer–analyte complexes are formed mainly by polar non-covalent interactions. The functional monomer usually includes two functional groups. At one end, it interacts with the template and at the other end of the monomer there is a group that is able to bind covalently with the cross-linker. The cross-linker polymerizes the monomers around the template with covalent binding and holds them in place after the template is removed. If the entire binding sites are covered with polymer, then it may be difficult or impossible to remove the template from the imprint. This problem could be minimized by selecting the appropriate cross-linker and concentration [16]. The solvent dissolves all components of the polymerization mixture and it also supplies the production of pores. Pores allow access to binding sites. Although polar solvents are better porogens, non-polar solvents (toluene, chloroform) are preferred in the non-covalent approach because they stabilize electrostatic interactions and/or hydrogen bonds between the analyte and monomer. More polar solvents can by used (acetonitrile) but the prepared polymer has lower sorption affinity of the analyte. Polar solvents such as methanol and water generally are not taken for suitable porogens because they prevent polymerization and also cancel the hydrogen bonding between the analyte and monomer [17]. But several articles indicate a possibility of using those solvents as porogens [18, 19].

Traditional bulk polymerization is the most widely used approach for the preparation of MIPs. Unfortunately, the chromatographic performance of these particles is usually unsatisfactory due to their irregular size and shape. Furthermore, the tedious and time-consuming process and low yield of MIPs prevent their industrial production and acceptance in analytical laboratories [20]. With passage of time other manners of MIP preparation were developed, for example: suspension

Tab. 1. Summary of studies in which MIPs have been applied to solid-phase extraction

Analyte	Sample	Method	Ref.
Alfuzosin	Human plasma	Off-line	54
Atrazine	Beef liver	Off-line	36
ß-Agonist	Calf urine	Off-line	61, 62
Caffeine	Human urine, coffee, beverages	On-line	75
Ceramide III	Yeast	On-line	76
Chloramphenicol	Honey	Off-line	63
Cholesterol	Human serum, cow milk, yolk, shrimp, pork, beef	Off-line	56
Enrofloxacin	Tissue sample	Off-line	57
(-)-Ephedrine	Chinese Ephedra	Off-line	66
Hyoscyamine	Human and calf urine and serum	Off-line	45
Pentycaine	Human plasma	Off-line	59
Pentycaine	Human plasma	On-line	73
Pharmaceutical compounds	Dog plasma	Off-line	55
Sinomenine	Sinomenium acutum Reht. et Wils	Off-line	58
Tetracycline, oxytetracycline	Pig kidney tissue	Off-line	60
Verapamil	Cell cultures, urine	On-line	74

polymerization [21, 22], multistep swelling polymerization [23, 24], dispersion polymerization [25], precipitation polymerization [26, 27], i*n situ* polymerization [28].

Applications of MIP

MIPs have been used as stationary phases in affinity chromatography, artificial antibodies, biomimetic recognition elements in biological sensors, and sorbents for SPE. It is possible to prepare MIPs with high enantio- and stereoselectivity for many chiral compounds, such as derivatives of aminoacids [29], peptides [30, 31], aminoalcohols [32], sugars [33], anti-inflammatory agents [34].

MIP in solid-phase extraction

MIP can be packed in disposable cartridges for off-line SPE or in columns for on-line SPE. Molecularly imprinted solid-phase extraction (MISPE) is based on the conventional SPE procedure, therefore it contains 4 basic steps: conditioning, loading, clean-up and elution. During the conditioning step, the cavities (binding sites) of the MIP are activated in order to maximise the interactions with the target analyte present in the sample. In the loading step, if the sample is percolated through the MIP in a low-polarity solvent, a selective loading step can be achieved, in which only the target analyte is selectively retained on the MIP while the sample matrix is non-retained [35, 36]. However, when the analyte of interest is present in an aqueous medium, the analyte and other interfering compounds are retained non-specifically on the polymer. Consequently, to achieve a selective extraction, a clean-up step with an organic solvent is introduced prior to the elution step. This clean-up is more critical in MISPE procedures than in conventional SPE. When water samples are percolated through MIPs, the MIP can be dried after sample percolation [37, 38]. The clean-up step must be optimised in terms of pH, nature, and volume of the washing solvent in order to exploit the MIP's ability to be selective in recognising the target molecule. Clean-up solvents should suppress the non-specific interactions without disrupting the selective interactions between the MIP and the target molecule. Recognition is often better when the porogen is used as the solvent because the environment established during the synthesis is reproduced [39, 40] (solvent memory effect). To obtain high enrichment factors, it is necessary to use small volumes of the eluting solvent, but the interactions between the MIP and the analyte are sometimes so strong that the volume of the eluting solvent has to be increased. To avoid this, mixtures of organic solvents or an organic solvent with a modifier, such as acetic acid or pyridine, can be used. When a selective elution is performed, the MISPE can be coupled directly to a specific detection system [7, 41, 42].

In some polymers it is difficult to remove the last traces of template, even after washing the polymer several times. It is caused by strong binding of template to the polymer. The best manner to obviate the template bleeding is to use an analogue of the target analyte as the template. Therefore, the bleeding of the template does not interfere in the quantification of the target analyte [43–45].

However, the use of template analogues is not always to solution. For this reason, other methods, such as thermal annihilation, microwave-assisted extraction and desorption of the template with supercritical fluids have been developed to remove the template from the MIP [46]. A very interesting alternative is the use of a stable isotope-labelled compound as the template molecule [47, 48].

MISPE procedures can be performed in off-line and on-line modes coupled with a chromatographic technique, mainly HPLC.

Off-line MISPE

In the off-line mode, polyethylene, polypropylene or glass cartridges are packed with 15–500 mg of polymer. After conditioning, sample loading and clean-up steps,

Tab. 2. Summary studies in which MIPs have been applied to other analytical methods

Analyte	Sample	Method	Ref.
Atrazine	Standard solutions	Sensor	101, 102
Atropine	Scopolia extract	LC	80
Cephalexin	Human plasma, serum	Pulsed elution	41
Cortisol	Tetrahydrofuran standards	Sensor	94
Diazepam	Human serum	Sensor	93
Ephedrine, Pseudoephedrine		LC	77
(+)-Ephedrine, (+)-Pseudoephedrine (+)-Norephedrine	Standard solutions	TLC	98
Levamisole hydrochloride	Pharmaceutical tablet	Sensor	92
Local anaesthetics	Standard solutions	CEC	89
Metformin hydrochloride	Human plasma	Pulsed elution	42
Morphine	Aqueous standards	Sensor	95
N-CBZ-phenylalanine	Standard solutions	LC	84
Propanolol	Standard solutions	CE	99
Propanolol	Standard solutions	CEC	100
(-)-Pseudoephedrine, (-)-Norephedrine	Standard solutions	TLC	97
Quinine	Standard solutions	TLC	104
Steroids	Standard solutions	LC	78
Sulfamethoxazole	Standard solutions	LC	81
(S)-nilvadipine	Standard solutions	LC	83
(S)-timolol	Standard solutions	TLC	105
Thiabendazol	Fruit sample	LC	82
1,1'-binaphtyl-2,2'-diamine	Standard solutions	CEC	103
Histamine, Ephedrine	Standard solutions	LC	79

the extract collected from the elution step is injected into the analytical system, such as liquid chromatography, gas chromatography or capillary electrophoresis.

Off-line MISPE has been applied to the extraction of several compounds from different matrices, such as environmental [49–53], biofluids [54–59], tissue samples [57, 60–62], food samples [63–65], and plants [39, 58, 66, 67].

It is known that the concentration of analytes in environmental samples is usually in low levels, so large volumes of the sample have to be percolated through the MIP. This can be a problem because of restricted flow rates and plugging, which can lead to long analysis time. However, in the last few years it has been demonstrated in several studies that it is possible to percolate large sample volumes of environmental samples through the MIP (after the filtration step) with a good flow rate and an ability to re-utilise the cartridge several times [50].

On-line MISPE

On-line systems allow the entire sample passed through the MIP to be injected directly onto a subsequent analytical system. The on-line approach overcomes the drawbacks of the off-line approach, reducing the loss of analytes and the risk of contamination, and improving accuracy and reproducibility. The first application of an on-line MISPE procedure coupled to HPLC was described by Masqué and co-workers [67] for selective extraction of 4-nitrophenol.

On-line MISPE have been applied to the extraction of a few compounds from matrices such as environmental [14, 68–72], biological [47, 73, 74] and food samples [75, 76].

MIP in LC

The advantages of MIP such as high selectivity and stability have made fast development of MIP used as chromatographic stationary phases, particularly in HPLC [77–81]. This approach was originally performed by Sellergren in 1994 [7].

The main drawback of these methods is the large volume of washing solutions necessary to remove non-specifically bound matrix compounds leading to a quite long analysis time. The main reason might be attributed to the fact that a large volume of aqueous samples is loaded and thus a high amount of matrix compounds is retained on MIP columns by hydrophobic interactions. An alternative would be the injection of sample extracts in organic solvents [82].

MIPs are also used as chiral stationary phases in HPLC [83, 84] and TLC [97, 98, 104, 105]. The use of MIPs as chiral stationary phases in HPLC leads to higher enantioselectivity in comparison with standard chiral stationary phases. Typical values of the selectivity coefficient > are 1.5–5.0, although higher values were achieved. The problem is that the resolution towards the selectivity is commonly insufficient because of the peak broadening and tailing.

MIP in CE and CEC

MIPs have been used as the chiral selective matrix in CE and CEC. CEC is considered to combine the advantage of the high separation efficiency of capillary electrophoresis and high selectivity offered by HPLC.

CEC-based MIPs have shown higher efficiency than HPLC-based MIPs. There are three MIP formats used for CEC: the particles [85, 86], the coating [87, 88] and the monolith [89, 90].

MIP as membranes

Membranes can be synthesized by polymerization of a mixture containing a monomer, an analyte and a cross-s-linker on a silanized glass plate or in the pores of a carrier. It is possible to prepare the membrane without a cross-linker. Recently alternative approaches of monofunctional and bifunctional membranes preparation are researched [91, 92].

MIP as sensors

Biomolecules such as enzymes, antibodies and receptors are used as specific recognition elements in MIP sensors preparation [93–95]. MIPs are attached to a suitable surface to form a thin layer. The physical-chemical properties of the layer change because of analyte binding to the MIP and this change can be measured [96].

CONCLUSIONS

Presently, almost an exponential growth in the literature published each year is an indicator of a growing interest in molecular imprinting technology. The present paper was focused on the applications of molecularly imprinted polymers. In recent years, also Šubert [106] and Ansell [107] are interested in many theoretical and practical aspects of MIPs in their reviews. Due to MIPs' advantages, including the ease and low cost of preparation, durability to heat and pressure, and predetermined recognition ability, MIPs are successfully used as sorbents in SPE for cleaning-up and enriching of analytes, as stationary phases in liquid chromatography, capillary electrochromatography, in electrophoresis, etc. MIPs are used in bioanalytical, pharmaceutical, environmental and other fields.

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