Molecularly Imprinted Polymers: Providing Selectivity to Sample Preparation

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Introduction

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 (1). As depicted in Figure 1, these materials are obtained by polymerizing functional and cross-linking monomers around a template molecule, leading to a highly cross-linked three-dimensional network polymer. Once polymerization has taken place, the template molecule is extracted and binding sites with shape, size, and functionalities complementary to the target analyte are established. Therefore, the behavior of MIPs emulates the interactions established by natural receptors to selectively retain a target molecule (i.e., antibody–antigen) but without the associated stability limitations.

MIPs have been employed in fields where a certain degree of selectivity is required, such as sensors (2), chromatography (3), and catalysis (4). However, currently, their use in sample preparation, especially in solid-phase extraction, so-called molecularly imprinted solid-phase extraction (MISPE), is by far the most advanced technical application of MIPs. The use of MIPs as selective sorbent materials allows the performance of customized sample treatment step prior to the final determination. This is of special interest when the sample is complex and the presence of interferences could prevent final quantification by typical chromatographic techniques coupled to common detectors. Due to the inherent selectivity provided by MIPs, past years have seen a growing interest in this area, and it has been extensively reviewed (5–10).

In this paper, the different uses of MIPs in sample preparation will be briefly discussed, including their advantages and drawbacks as well as the further expected developments in the near future.

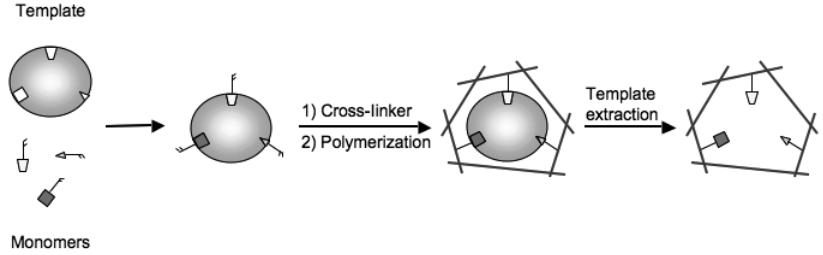

Figure 1. Schematic representation of the synthesis of MIPs.

Molecularly Imprinted Solid-Phase Extraction

Several modes of on-line SPE, conventional off-line SPE (where the MIP is packed into cartridges) and batch SPE (where the MIP is incubated with the sample) have been assayed during the past few years. Although the later has been displaced by the conventional off-line SPE format, it is important to highlight the work by Andersson at al. (11) for the determination of sameridine in human plasma samples, because it was one of the first examples demonstrating the great potential of MIPs for the selective extraction of target analytes.

Off-line protocols

Off-line MISPE protocols do not differ from other SPE procedures. Typically, an amount of 15–500 mg of imprinted polymer is packed into polyethylene cartridges. Then, after the conditioning, loading, and washing steps, analytes are eluted, ideally free of co-extractives, and the elution extract is further analyzed by liquid chromatography, gas chromatography, or capillary electrophoresis.

The last few years have seen a huge development in off-line MISPE methods for the determination of a great variety of analytes in environmental samples (river water, groundwater, wastewater, sea water, and soil extracts), biofluids (urine, plasma, serum and blood), and food samples. In general, a sample is loaded onto the MIP cartridge in a low-polarity solvent, because in such media, specific interactions are maximized, and after a washing step for the removal of compounds non-specifically bound to the polymeric matrix, analytes are eluted with a solvent able to disrupt the typical non-covalent interactions that take place between the analyte and the imprinted polymer.

Aqueous samples can also be directly loaded onto MIP cartridges. However, in this case, MIPs behave like a reverse-phase sorbent and thus both target analytes and matrix components are retained through non-specific interactions. Then, a washing solvent able to remove matrix components and to re-distribute non-specifically bound analytes to the selective imprints is introduced. Typically, the washing solvent is immiscible with water and thus an exhaustive drying step has to be included.

On-line protocols

In this format, a small pre-column packed with the imprinted polymer (typically about 50 mg) is placed in the loop of a six-port injection valve. After loading the sample and washing out interfering compounds, the analytes are eluted by the mobile phase and then separated in the analytical column. The first application of an on-line MISPE procedure coupled to HPLC was described by Masqué et al. (12) for the selective extraction of 4-nitrophenol from a mixture of phenolic compounds in river water samples.

In spite of the clear advantages of on-line protocols, only ~ 20 papers have been published on on-line MISPE studies. This low activity might be attributed to the usual lack of compatibility of the elution solvent necessary to desorb analytes from the MIP pre-column and the mobile-phase required to perform the separation on the analytical column. This problem can be overcome by directing the eluent from the MIP pre-column to the injection loop and subsequently injected on the chromatographic system (13), or by mixing the organic elution solvent with an aqueous-rich solvent before reaching the analytical column (14). However, although both methods were successfully applied, extra instrumentation (i.e., additional pumps, multiposition valves) for the automation of the whole system is required, increasing the complexity and costs of the analysis.

In-line protocols

Thanks to the high selectivity provided by MIPs, it is possible the direct coupling of an MIP column in-line with the detection system. In this manner, extraction, enrichment, separation, and determination of target analytes can be achieved in one single step. MISPE with direct in-line UV detection was first described by Sellergren for the determination of pentamidine in urine (15), and has been further exploited recently in the determination of pesticides in food sample extracts. In this regard, Turiel et al. (16) developed an analytical method for the determination of the fungicide thiabendazol (TBZ) in fruit sample extracts in acetonitrile. Organic sample extracts (50 µL) were injected onto a TBZ-imprinted polymer column using a 100% methanol mobile phase. Then, after 2.4 min, the mobile phase was switched to methanol–acetic acid (80:20, v/v) in 0.1 min, keeping these conditions constant for 5 min before returning to the initial conditions. Following this methodology, the high selectivity of the MIP column permitted the target analyte to be retained on the column while the interferences were rapidly eluted and, thus, TBZ was unambiguously detected and quantified in less than 15 min.

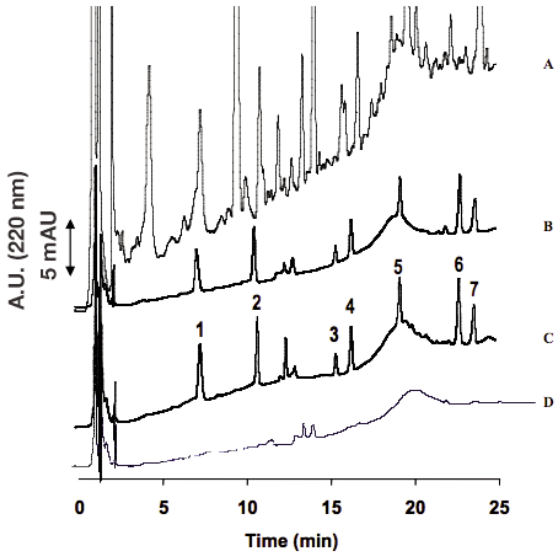

Figure 2. LC–UV chromatograms obtained at 220 nm for a soil sample extract directly injected without any previous cleanup (A); a soil sample extract enriched with triazines at 0.1 mg/L concentration level after MI-SPME (B); a 0.1 mg/L standard solution of triazines after MI-SPME (C); and a nonspiked soil sample extract after MI-SPME (D). Peak numbers: Desisopropylatrazine (1); Desethylatrazine (2); Simazine (3); Cyanazine (4); Atrazine (5); Propazine (6); Terbutylazine (7). Reprinted from the Literature (21) with permission of American Chemical Society.

MISPE with pulsed elution (MISPE-PE), proposed by Mullet and Lai (17), represents an alternative in which a small volume of elution solvent, instead of a steed solvent-switch, is used. In this first work, theophylline in chloroform-diluted serum samples was injected onto a theophylline-imprinted polymer packed into a stainless steel column using chloroform as mobile phase. Subsequently, after interfering compounds had passed through the column, 20 µL methanol was injected, allowing the elution of theophylline eluted free of co-extractives and determined directly spectrophotometrically at 270 nm. MISPE-PE has been subsequently improved by the application of successive 20 µL pulses of different solvents [MISPE with differential pulsed elution (MISPE-DPE)] (18). This approach is more efficient in removing both remaining interfering compounds and the analyte fraction non-specifically retained. Both MISPE-PE and MISPE-DPE allow analyte enrichment through injection of large volumes of sample and provide a high analytical sensitivity, thanks to the narrow band obtained by pulsed elution.

Molecularly Imprinted Solid-Phase Microextraction

Since its introduction by Pawliszyn and co-worker (19) in the early 1990s, solid-phase microextraction (SPME) has been more and more used for sample preparation in analytical laboratories thanks to its simplicity of operation, solventless nature, and the availability of commercial fibers. However, the variety of commercially available fibers is rather limited and just roughly covers the scale of polarity, which leads to a lack of selectivity during the extraction process. The required selectivity in SPME can be provided through molecular imprinting as was nicely demonstrated by Koester et al. (20) by preparing a fiber coated with a clenbuterol-imprinted polymer. In this work, silica fibers were activated by silylation and subsequently immersed in the polymerization solution composed by clenbuterol, methacrylic acid, ethylene glycol dimethacrylate and azo(bis)-isobutyronitrile dissolved in acetonitrile. Then, polymerization was performed during 12 h at 4°C under irradiation at 350 nm. According to the authors, fibers with a polymeric film thickness of ~ 75 µm were obtained in a reproducible manner and were successfully used for the selective extraction of brombuterol from urine samples.

A completely different and much simpler approach for the preparation of imprinted fibers has been proposed independently by the groups of Martin-Esteban et al. (21) and Djoand and co-worker (22) and consists of the direct synthesis of molecularly

imprinted polymeric fibers (monoliths) using silica capillaries as molds, being silica etched away after polymerization. Figure 2 shows the chromatograms obtained for a soil sample extract directly injected without previous cleanup (Figure 2A), a soil sample extract spiked with triazines at 0.1 mg/L concentration level subjected to MI-SPME using a propazine-imprinted fiber (Figure 2B), a standard solution of triazines after MI-SPME (Figure 2C), and a non-spiked soil sample extract subjected to MI-SPME (Figure 2D). It is clear that a high degree of selectivity is obtained by MI-SPME procedure, allowing the detection of target analytes at very low concentration levels, which would be extremely difficult without performing any cleanup. Besides, the baseline obtained for the analysis of soil extracts after MI-SPME is as clean as that obtained for the standard solution, demonstrating even more clearly the high selectivity provided by the imprinted fiber. Although still some aspects need some improvements (i.e., low capacity of the fibers), it seems clear that the combination of molecular imprinting and SPME is possible and thus it opens new areas of research.

Conclusion

The use of MIPs in sample preparation has undergone wide development during the past few years; although, in parallel, some drawbacks have been observed. In this sense, the necessity of using a high amount of functional monomer leads to the formation of non-selective binding sites. Besides, template bleeding, overuse of certain "standard" formulations, and tedious synthesis procedures are other weak points associated to this area that need to be improved. However, it is also true that there are already several ways to circumvent, minimize or even suppress such mentioned drawbacks, making MISPE a powerful analytical tool. In fact, there are already MISPE cartridges commercially available for the extraction of certain analytes, which will ease the implementation of MISPE in analytical laboratories.

The direct coupling of a MIP column in-line with the detection system, although still in a rather preliminary stage, will lead to very simple analytical methods facilitating their incorporation to routine laboratories. Moreover, a further development of some other strategies, such as the preparation of imprinted fibers for SPME or the development of micro-MISPE devices, is expected in the near future.

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