

Analysis of analytes

The use of MIPs in solid-phase extraction increases efficiency and improves detection limits

Analysis of individual analytes in a complex sample environment often requires several pretreatment steps during which interfering compounds are removed. Solid-phase extraction (SPE) has become a widely used technique for this purpose, due to its ease of automation and flexibility. Furthermore, it is environmentally friendly with less organic solvent required compared to liquid/liquid extraction. Conventional SPE sorbents are based on reversed phase, ion exchange or a combination of these (mixed-phase SPE). In general these sorbents are non-selective and compounds having similar physicochemical characteristics will co-extract. Where such mixtures are not separable in the subsequent chromatographic analysis step this can lead to significant analytical problems. An advantage with selective sorbents, such as molecularly imprinted polymers (MIPs) or immunoaffinity materials, is that the extraction step results in a much cleaner analyte sample for subsequent analysis. The disadvantages with immunoaffinity sorbents are their limited stability in different solvents, their short shelf-life and their (often) high cost. By contrast, MIPs share none of these disadvantages.

Molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, is dissolved in a solvent together with one or more functional and cross-linking monomers. Spontaneous complex formation between functional monomers and template occurs, the strength of which will be reflected in the selectivity of the MIP polymer. After polymerisation the template molecule is removed leaving behind vacant cavities or imprints that are sterically and chemically complementary to the template. These cavities are then capable of binding a single target analyte or a class of chemically similar analytes present within a complex sample.

It can be seen that the design of the appropriate template-

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monomer composition is crucial. This part of the process can be carried out by molecular modeling, experimental design or by screening methods¹⁻³. The binding cavities are preferably engineered in such a way that several interaction points for the analyte are present, leading to a strong interaction between the sorbent and the analyte and thus a high selectivity.

The most critical parameters in the development of a MIP for SPE are the choice of the template molecule and the selection of monomers. We have recently introduced the MIP Rule of 6 as a guideline for successful MIP development in analytical applications⁴:

1. Never use the analyte as a template unless there is no alternative.
2. Make rational choices about which regions of an analyte are likely to command the best types of interaction in a low dielectric medium (organic solvent) and then incorporate these elements in an analogue of the analyte molecule.
3. Select monomers that are likely to form strong interactions in the chosen solvent (e.g. Brønsted acids or bases/H-donors or acceptors/non-polar groups etc) – this will increase capacity and influence homogeneity of the binding cavities.
4. Choose templates and monomers that will be soluble in the porogenic solvent to be used in the polymerisation – this may

sound obvious but carrying out solubility tests in advance can avoid wasted time and efforts.

5. Ensure as far as possible that the template-monomer mixture is stable and does not undergo side reactions under the polymerisation conditions.

6. Consider the nature of the matrix from which the analyte will be eventually extracted when selecting the cross-linking monomer – a range of di- or tri-unsaturated cross-linking monomers (e.g. vinylic, and acrylic) with varying chemistries are available to create the porous organic network material.

MIPs as solid-phase extraction products

While the molecularly imprinting concept was introduced more than 20 years ago^{5,6} MIPs have only recently become commercially available. The reason for this lengthy commercialisation path is that many of the demanding materials chemistry challenges associated with the properties and behaviour of the MIP material took time and considerable effort to solve.

Since the introduction of the first commercially available MIP phase for extraction of Clenbuterol in 2002, the use of MIPs as selective sorbents in SPE is fast becoming the method of choice for clean-up of single compounds or compound classes from complex matrices. MIPs have been reported in the literature for a large number of analytes of environmental and pharmaceutical interest⁷. The list of commercially available MIPs is also growing fast and includes such applications as analysis of banned pharmacological substances^{8,9}, analysis of bioactive compounds for health control monitoring¹⁰, and monitoring of water contamination in the environment¹¹ – for further details see www.miptechnologies.com.

Class selective MIPs can also be made, in which case the design of a common template is required. An example of this is a MIP selective for many different beta-agonists which, since its launch as a commercial SPE, has been investigated by several independent research groups. This MIP can selectively extract a wide range of beta-agonists from matrices as diverse as urine, liver and muscle tissue and has been shown to give minimised ion suppression where MS analysis is used.^{8,9,12}

Literature reports often describe MIPs where the analyte itself is used as template molecule. For SPE applications where low levels of analyte are present in the sample matrix, this can (but is not always a problem) lead to sensitivity issues arising from template (analyte) leaching from the MIP during SPE. This problem can be circumvented to some extent during the MIP preparation using washing protocols (e.g. in Soxhlet extraction) where solvents such as methanol containing acid or base are used¹³. However, quantitative removal of the template molecule is not always possible. Where a structural analogue of the analyte is used as template¹⁴ the bleeding problem, if it exists, can be avoided (see the MIP Rule of Six above). Where structural analogs of the analyte are not available or difficult to synthesise, MIP preparation methods using the analyte as tem-

plate, such as immobilisation of the template on a solid support¹⁵ or grafting¹⁶, will avoid the problem. The latter methods are, however, best performed by molecular imprinting experts.

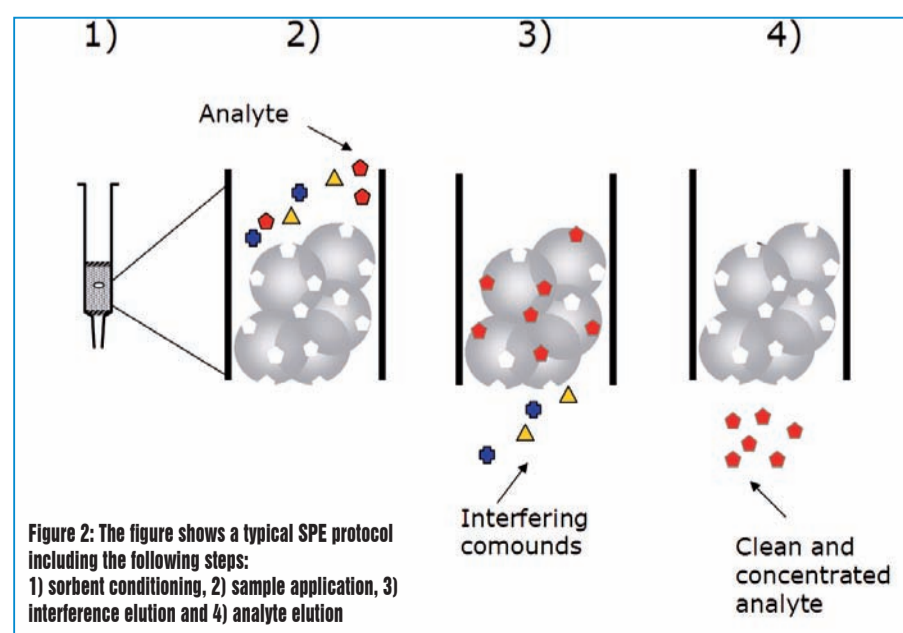
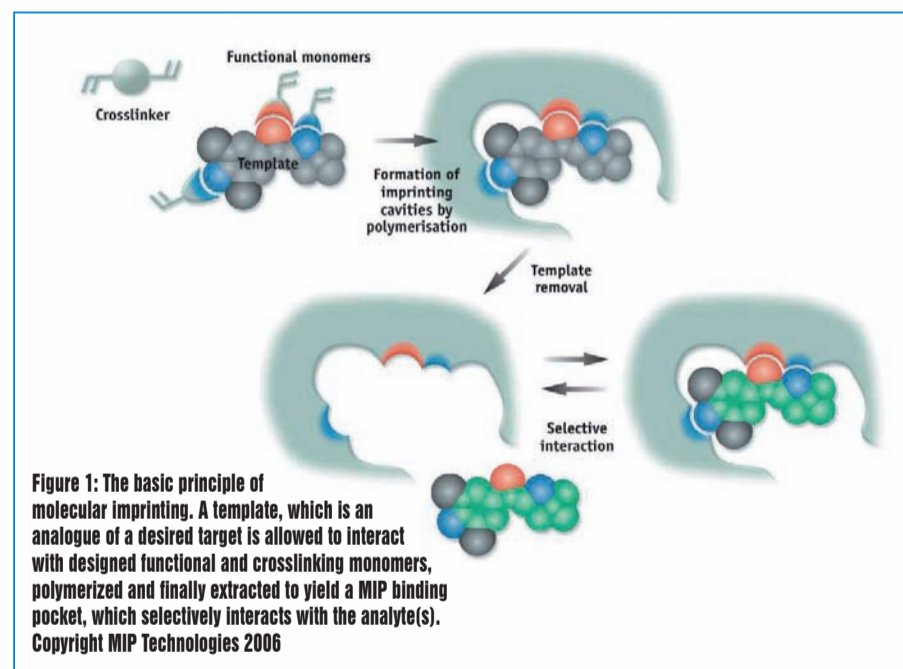
SPE protocol

A typical SPE protocol includes the following steps:

Sorbent Conditioning: Initially, the sorbent is typically first conditioned in an organic solvent that allows the MIP to swell properly followed by application of the sample in organic solvent (usually obtained from a liquid-liquid extraction step). More often the analyte is present in an aqueous sample, the sorbent is then first conditioned with an organic solvent and then with an aqueous buffer.

Sample application: Usually, samples can be applied directly onto the column with only minor pretreatment if necessary, such as dilution, centrifugation etc, depending on the sample matrix.

During sample application the analyte may interact with the MIP through both non-selective (e.g. hydrophobic) and selective (H-bonding, ionic, van der Waals) interactions. In the interference elution step a switch to an organic solvent is made and the



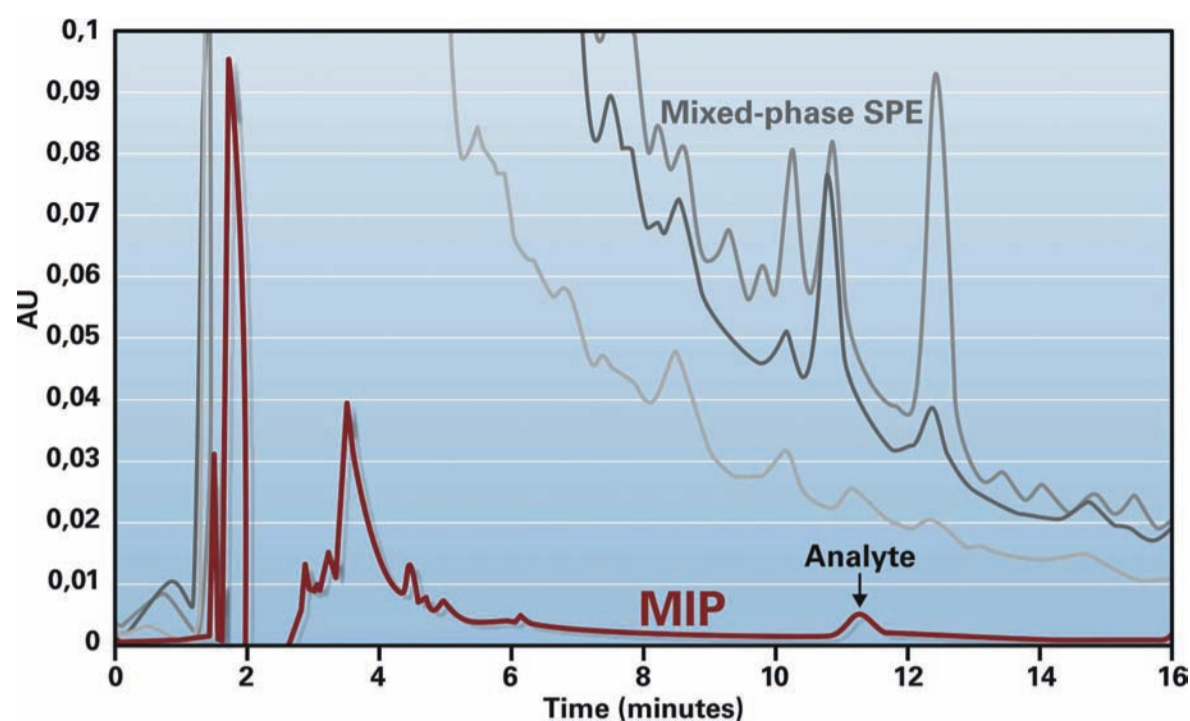


Figure 3: The chromatograms show the extraction clean-up of a typical analyte from a 5ml urine sample. Superior clean-up with the MIP sorbent is shown in red, whereas the other chromatograms show clean-up with a much higher level of chemical noise, using mixed-phase SPE sorbents

selective interaction between the analyte and the sorbent will be optimised, whereas interfering compounds binding essentially via non-specific interactions are removed. The conditions need careful optimisation in terms of pH, ionic strength and solvent composition in order to fully exploit the MIP's ability to selectively recognise the analyte(s).

Interference elution: Since MIP sorbents typically possess more interaction points with the analyte than conventional SPE sorbents, stronger interactions are observed. As a result, harsher conditions for interference elution can be tolerated during the SPE procedure leading to lower levels of chemical noise. This leads in turn to lower detection limits, reduced sample volumes, quicker analysis times and overall, a more cost-effective extraction method.

Analyte elution: In the final step the analyte is eluted in a solvent or solvent mixture that breaks the selective bonds. An example, with superior clean-up using a MIP sorbent selective for clenbuterol compared with mixed-mode SPE, is shown in Figure 3.¹⁷

Where selectivity is required MIP sorbents have distinct advantages over conventional SPE sorbents - less chemical noise, lower detection limits, minimised ion suppression and a broader selectivity range

Molecularly imprinted polymers and their use as selective sorbents in solid-phase extraction have been briefly discussed in this review. Where selectivity is required MIP sorbents have distinct advantages over conventional SPE sorbents - less chemical noise, lower detection limits, minimised ion suppression where MS is used and a broader selectivity range. Newer methods of preparing MIPs are now emerging onto the commercial scene^{14, 15} that will revolutionise their use in areas as diverse as SPE, chromatographic separations and sensors.

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