Analysis of analytes

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

A

alysis of individual analytes in a complex sample environment is necessary for many analytical methods. During this process, interfering compounds are removed. Solid-phase extraction (SPE) has become a widely used method 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. Chromatographic SPE products 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 they create a specific cavity designed to bind the target analyte.

MIPs as solid-phase extraction products

MIPs have recently become commercial products for solid-phase extraction (SPE). They are prepared using molecular imprinting technology, which has been shown to give minimised interference elution and analyte elution. MIPs improve SPE selectivity, allowing a greater degree of analyte recovery. MIPs are made by incorporating a template molecule into a polymer matrix, which is then made highly cross-linked with a di- or tri-unsaturated cross-linking monomer. After polymerisation the template molecule is removed leaving behind vacant cavities or imprints that are sterically complementary to the template molecule. 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 monomer composition is crucial. This part of the process can be carried out by molecular modeling, experimental design or by screening methods\textsuperscript{4-5}. The binding cavities are preferentially 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 Six as a guideline for successful MIP development in analytical applications\textsuperscript{6-7}.

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 bind to the template molecule and the medium (organic solvent) and then incorporate theses elements in an analogue of the analyte molecule.
3. Select monomers that are likely to form strong interactions in the chosen solvent (e.g. Bronsted 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. vinyl, and acryl) with varying chemistry are available to create the porous organic network material.

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\textsuperscript{8,9,10}.

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 Soldeet extraction) where solvents such as organic containing acid or base are used\textsuperscript{11}. However, quantitation of the template molecule is not always possible. Where a structural analogue of the analyte is used as template\textsuperscript{10} the bleeding problem, if it exists, can be avoided (see the MIP Rule of Six above). Where structural analogues 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 support\textsuperscript{12} or grafting\textsuperscript{13}, 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:

1. 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).
2. 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, ion, van der Waals) interactions. Therefore, a step to a different organic solvent is made and the analyte is eluted from the MIP.

Figure 1: The basic principle of molecular imprinting. A template, which is an analogue of a desired target is allowed to interact with designed functional and crosslinking monomers, polymerized and finally extracted to yield a MIP binding pocket, which selectively interacts with the analyte(s).

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Figure 2: The figure shows a typical SPE protocol including the following steps: 1) sorbent conditioning, 2) sample application, 3) interference elution and 4) analyte elution.
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 scene14,15 that will revolutionise their use in areas as diverse as SPE, chromatographic separations and sensors.

References
1. Piletsky, S.A., K. Karim, E.V. Piletska, C.J. Day, K.W. Freebairn, C. Legge and A.P.F. Turner. 2001. Molecularly imprinted 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 scene14,15 that will revolutionise their use in areas as diverse as SPE, chromatographic separations and sensors.

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