



Selective Extractions by Molecularly Imprinted Polymers (MIPs)

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Sample preparation is often the rate-limiting step in analytical methods and its effectiveness has a critical impact on accuracy, quantification and, ultimately, the precision of any measurement. Traditionally, liquid–liquid extraction methods are frequently used for the extraction of organic analytes from aqueous samples. To overcome some of the disadvantages of liquid–liquid extraction (e.g., its tediousness) solid-phase extraction (SPE) was developed. SPE techniques consume much less solvent, are less labour intensive, reduce exposure to organic solvents and can provide more reproducible results.

Traditionally, SPE materials are inorganic (e.g., silica) or organic resin (e.g., styrene-divinylbenzene) based solid sorbents. Silica sorbents can be varied in adsorption character by bonding a wide variety of functional groups to the silica surface. The functionalities can be non-polar, polar, ionic or mixed-mode (e.g., non-polar and ion exchange groups). The surfaces of polymeric resins can also be modified, most commonly by introducing hydrophobic, polar, acidic or basic functional groups. The resulting phases can then operate via both hydrophobic and ion exchange retention mechanisms, as mixed-mode sorbents.

Even though hydrophobic, ionic and polar sorbents are widely used for the extraction of trace levels of analytes from complex matrices, the selectivity of such traditional SPE sorbents is often not high, requiring extensive and time-consuming sample preparation procedures with limited throughput. One solution to the selectivity problem is provided by “immunosorbents” (antibodies or other binding proteins such as “affibodies” immobilized onto a support material), which are widely used in many laboratories for chromatographic separations and analysis of trace compounds in food or biological samples. The limitation in the use of protein-based adsorbents for many small molecule extraction or separation problems is their limited stability in different

solvent (e.g., organic) systems and their (often) high cost.

The transition to stable, low-cost “selective phases” will be, we believe, the choice of the future for demanding separations. One such group of selective phases is based on the principle of molecular imprinting.

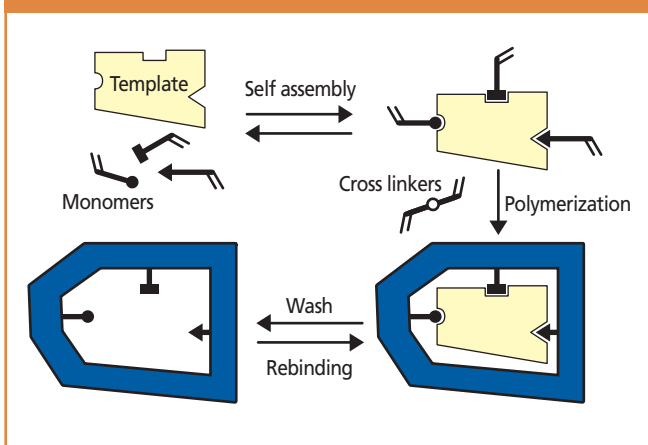
Molecularly Imprinted Polymers (MIPs)

Use of SPE sorbents based on molecularly imprinted polymers (MIPs) introduces ‘tailor-made selectivity’ into the sample preparation procedure. MIPs are highly cross-linked polymeric phases with predetermined selectivity for a single molecule or a group of structurally related molecules (Figure 1).

Selectivity is introduced during preparation of the MIP by first dissolving a template molecule in a solvent, together with one or more functional monomers. Spontaneous complex formation then occurs, the strength of which will depend on such factors as the degree of complementarity of the chemical functionalities in the template with those in the functional monomers and the properties (e.g., dielectric constant) of the solvent in which they are dissolved. After addition of cross-linking monomers, whose hydrophilic or hydrophobic character can also be varied significantly, followed by polymerization in a suitable solvent (or ‘porogen’ because the solvent also plays a pore forming role) the template is removed by extensive washing. The resulting MIP will contain specific cavities or “imprints” that are sterically and chemically complementary to the template molecule and theoretically (if the system was designed properly) to the analyte or group of analytes of interest.

Formation of complementary binding cavities is likely to be dependent on several factors such as the pre-complex formation event, any re-orientation of monomers around the template or template complexes during polymer formation and the partitioning influences of the porogenic solvent.^{1,2} These

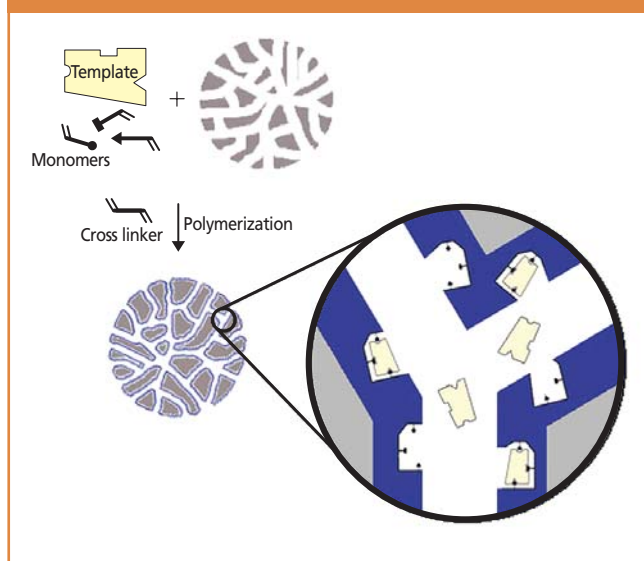
Figure 1: Outline of the molecular imprinting process. A template, which is an analogue of a desired target is allowed to interact with designed monomers, polymerized and finally extracted to yield a MIP binding pocket.



variable influences may give rise to MIPs having either homogeneous binding affinities or more typically having a distribution of affinities, rather like that of a polyclonal antibody population.³ Homogeneous affinities (Titirici et al. unpublished results) can be obtained using methods such as those in which the MIP polymer is grafted onto a solid support, exemplified by the “grafting from” approach of Sulitzky et al. (Figure 2).⁴ Such materials can be particularly useful where efficient mass transfer is important such as in chiral separation.

By careful “design” of the template-monomer constituents, either by molecular modelling, experimental design methods or screening,⁵⁻⁷ binding cavities can be engineered where several interaction points for the analyte are present leading to a stronger interaction between the sorbent and the analyte. As a consequence, harsher washing conditions can be tolerated during the SPE procedure leading to a simplified extraction procedure and to cleaner analyte extracts. Due to this improved selectivity, lower levels of chemical “noise” are present compared with conventional extraction methods, resulting in lower detection limits, reduced sample volumes, quicker analysis times and overall, a more cost-effective extraction method. A further consequence is that the method development will be less “matrix” dependent and where MS detection is used, ion suppression will be minimized because of the improved quality of the extracts.

Figure 2: Outline of the grafting process. The MIP is grafted from the surface of a support material to yield a thin layer of polymer that allows fast binding and elution and hence fast kinetics.



Although molecular imprinting was first described in the 1970s and 1980s it has only been commercially applied during recent years.^{8,9} Many of the “materials science” challenges associated with routine use of the technology have now been met and solved. However, some areas remain where further development is still ongoing. In the following section we deal with some of the advantages of MIPs over other types of SPE phases by reference to real examples. We also draw attention to examples where the apparent frailties of the technology, occasionally drawn attention to in the literature (e.g., bleeding of templates or selective binding in aqueous solution), can largely be explained by inexperience in the users or perhaps even by poor experimental design.

MIPs, Templates and Monomers

Template design and monomer selection are two of the most critical features of the molecular imprinting process. Where possible for analytical applications, the “MIP Rule of 6” should be followed:

1. Never use the analyte as a template unless there is absolutely 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 polymerization — this may sound obvious but it sometimes requires the performance of solubility tests.
5. Ensure, as far as possible, that the template–monomer mixture is stable and does not undergo side reactions under the polymerization 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, acrylic, methacrylic, acrylamide etc.) with varying chemistries are available to create the porous organic network material.

MIP Behaviour — Bulk Polymers

When preparing MIPs by bulk polymerization, certain post-polymerization processing steps can be critical and important for the final performance of the MIP. Whether the bulk method

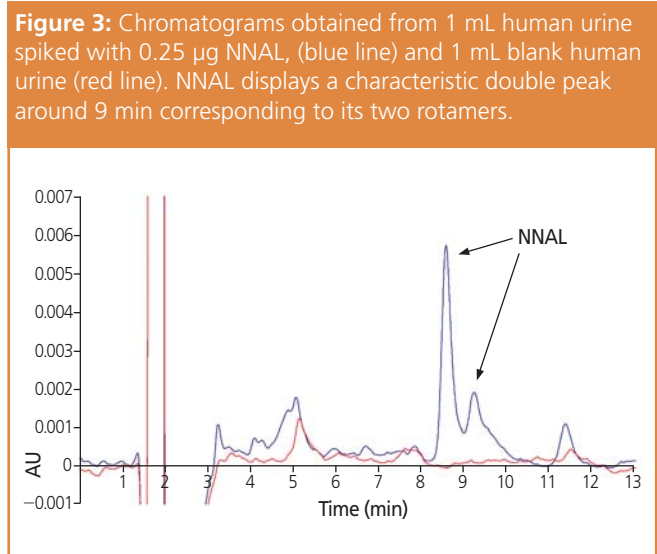
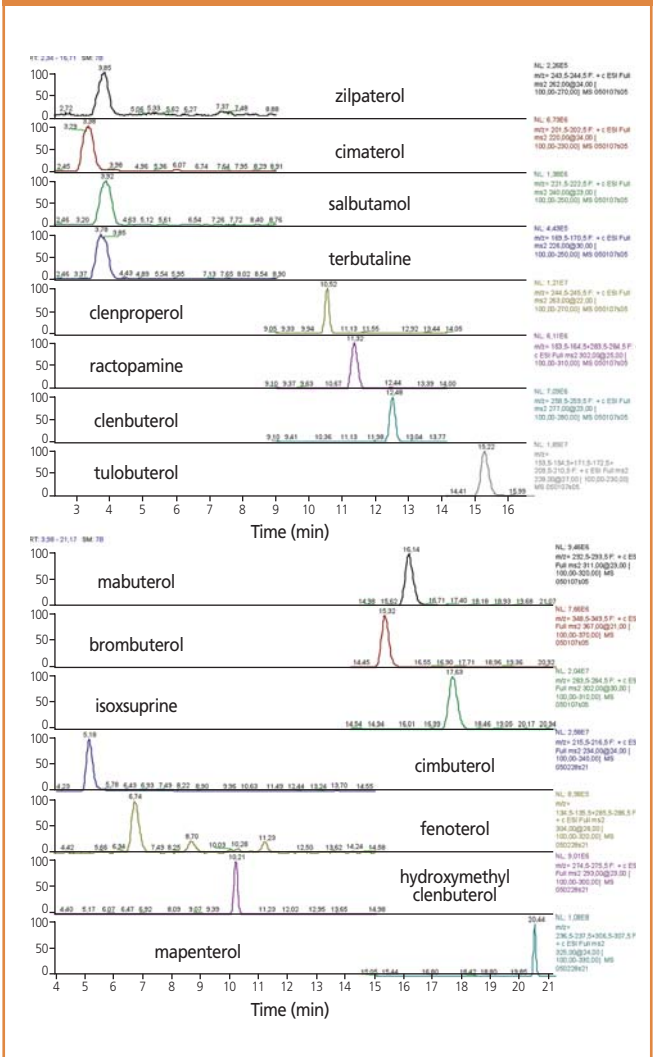


Figure 4: Extracted ion chromatograms for the different beta-agonists at their MRPL concentrations in calf urine using MIP clean-up (no glucuronidase treatment). * *(Reproduced from “Multi-residue liquid chromatography/tandem mass spectrometric analysis of beta-agonists in urine using molecularly imprinted polymers” by Nathalie Van Hoof et al., published in *Rapid Commun. Mass Spectrom.*, **19**, 2801–2808 (2005). Copyright year 2005. Copyright John Wiley & Sons Limited. Reproduced with permission.)



has used block or suspension polymerization methods the particles or beads obtained must be thoroughly washed and various extraction methods can be employed. This washing procedure is often performed at elevated temperature and employs a range of organic solvents and even solvent-organic acid mixtures. Where incomplete washing has taken place there may be residual template in the polymer particles which could complicate any subsequent analysis if the template and analyte have similar elution behaviours. To illustrate the potential problems that can arise, we refer to a study where a MIP sorbent in SPE format was compared with either liquid-liquid extraction or standard SPE using C18-bonded and immobilized phenyl boronic acid.¹⁰ While the MIP-based sorbent performed best in the removal of endogenous interferences, the authors concluded that this advantage was lost because the template-derived material co-chromatographed with the analyte. Inspection of the chromatogram in Figures 3–4 reveals that the peak originating from the template is considerably larger (perhaps >100x) than the analyte peak.¹⁰

This suggests to us (although we cannot be sure) that template removal in this particular MIP preparation was incomplete. It appears that the wash procedure employed was not efficient for exhaustive template removal. In our considerable experience, template bleeding is usually much lower than a few ppb and often as low as 0.1 ppb.¹¹ Commercially available MIP sorbents typically have a negligible level of template bleeding and when present it is at a level where no interference with the analyte determination will be seen.¹²

As we indicated in our set of simple rules above, it is a simple process to ensure that a prospective template analogue and analyte exhibit non-overlapping separation behavior (see for example the use of the template analogue brombuterol in the preparation of a clenbuterol MIP).¹³

Aqueous Samples

A commonly held misconception is that because MIPs are usually prepared in organic solvents they are ineffectual in extraction from aqueous solution or that selectivity will be poor. The logic presented is that in organic solvents H-bonding and ionic interactions are optimal while in water they will be considerably weakened. The misconception is not so much that interactions will be “weakened” which may be correct but that this is directly connected to “loss of selectivity”. The assumption then is that high affinity (tight binding) is required

to obtain high selectivity. An interesting biological example that tests this assumption is the glucose transporter system present in most cells of the human body. In aqueous solution this transporter has an affinity for D-glucose of a few mM (a pretty poor affinity by any standards) but is absolutely specific for D-glucose and does not transport L-glucose.

What should be made clear is that where a MIP is being used in an SPE “extraction” mode, the loading of sample is typically performed under aqueous conditions while the selectivity is gradually “revealed” by propitious use of solvents or solvent mixtures in multiple wash steps.^{12,14} However, selectivity can also be achieved in a totally aqueous process, as exemplified in the following publications to which the reader is referred.^{15,16} We have shown that, for example, oligosaccharides can be effectively separated by MIPs operating under purely aqueous conditions (data not shown).

MIP Sorbents and Their Applications

MIP sorbents can be used for a variety of analytes or analyte “classes”. Our own work, alone and in combination with academic colleagues, has led to a range of MIPs for SPE such as Clenbuterol,¹³ Beta-Agonists,¹⁴ Triazines (internal project), the tobacco-specific nitrosamine NNAL,¹² Riboflavin¹⁵ and more recently Chloramphenicol (internal project). In the wider scientific literature many hundreds of MIPs have been described.¹⁷ Below, we describe two examples that illustrate the utility of the “MIP for SPE” approach.

Example 1: In collaboration with Bernert et al. we have developed an extraction method for the carcinogenic tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) from urine samples.¹² Total urinary NNAL is a valuable biomarker for monitoring exposure to carcinogenic TSNAs in smokers and non-smokers exposed to second-hand smoke. However, measuring the low concentrations of NNAL present in urine is challenging and typically involves multiple extraction steps that can take more than a day to perform.¹⁸ Using a MIP selective for NNAL, even in the presence of nicotine, quantification limits were lowered and reproducibility increased. In Figure 3 we show a typical chromatogram obtained after SPE sample pretreatment which takes no more than 30 minutes starting from a urine sample diluted 1:1 with buffer.



Example 2: In a second application we have developed a MIP that is class selective for a family of hormone agonists, the beta (for beta adrenergic) agonists. This illustrates another principle, that where “group or class” selectivity is desired, design of a “common template” will be a required step. The MIP beta agonist behaviour has been studied by various external groups — who investigated and compared the MIP beta agonist phase with other sample clean-up methods.^{19–21} In general the MIP phase behaves very well and is able to extract up to 10 different beta agonists from matrices as diverse as urine, liver and muscle tissues. We show in Figure 4 an example of the extraction behaviour taken from Van Hoof et al.²⁰

Conclusions

In this short review we have attempted to explain and clarify the advantages of MIP phases in SPE. We believe a number of “myths” still remain out in the public domain about the strengths and weaknesses of this technology, driven largely by the fact that many users are perhaps employing old methods which have now been superseded. One such myth is that MIP phases cannot be “scaled-up”. We ourselves can produce MIPs at the kilogram level and are developing methods that can drive production towards the multi-ton level. Where selectivity is required MIP phases have distinct advantages over standard SPE phases. The ability to “dial-up” a separation phase is a further advantage that will become particularly important for applications where removal of intransigent contaminants from complex matrices or separation of highly similar molecules, for example, enantiomers is required.

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