

Molecularly Imprinted Polymers: A New Generation of Affinity Matrices

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The increased use of pharmaceutical drugs for human and veterinary applications provides an important clinical solution to the treatment of disease; however, there are technical challenges involved in monitoring these drugs in biological and environmental matrices. Quantitation of pharmaceutically active compounds and their metabolites in blood, serum, or urine is difficult, often requiring tedious analytical procedures. A further complication is that certain pharmacologically useful compounds, such as the antibiotic chloramphenicol or the group of beta-agonists that are used as growth promoters in animals, are frequently banned in foodstuffs of animal origin to be used for human consumption. For these types of compounds, the extremely low detection limits set by various organizations (i.e., EU, FDA, and U.S. EPA) place pressure on the analytical detection methods and demand labor-intensive preparation and analysis steps.

One area of particular concern is the release of active pharmaceuticals into the environment. Their persistence in various environmental matrices is not always mitigated by the standard wastewater treatment processes. Since such compounds can persist in ground and surface waters at levels below the sensitivity limit of many existing analytical methods, they may remain undetected in sources of drinking water used for human and animal consumption, emphasizing the importance of advances in analytical measurement.¹

Pharmacologically active substances are typically active at low concentrations and normally require concentration prior to analysis. Commonly used extraction methods are liquid-liquid extraction and solid-phase extraction (SPE). The former method will not be addressed here. Conventional SPE has well-known limitations, for example, in the extraction of polar pharmaceuticals from aqueous samples in which recoveries are often low. Further, in cases in which the sample is part of a complex matrix containing many different chemical classes, conventional SPE materials usually lack the ability to selectively extract the desired analyte without simultaneous extraction of contaminants, thus compromising recovery of the desired targets.

To overcome these limitations, selective sorbents based on immunoaffinity materials or molecularly imprinted polymers

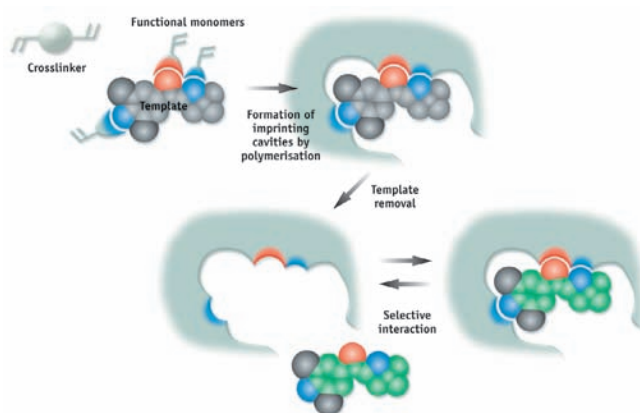


Figure 1 Basic principle of noncovalent molecular imprinting.

(MIPs) have been developed. It is not the purpose of this article to compare and contrast immunoaffinity methods with MIPs; recent reviews on the use of MIPs as SPE materials can be found in Refs. 2 and 3.

This application note illustrates the power of SPE using MIPs with two examples: 1) the extraction of beta-blockers from environmental water samples⁴ (collaboration with the Chemical and Environment Research Institute [IIQAB] of Barcelona, Spain) and 2) the development of an MIP for SPE that allows rapid, simple, and near-quantitative analysis of chloramphenicol in food matrices.

Technical description of MIPs

MIPs are highly cross-linked polymeric phases with predetermined selectivity for a single molecule or a group of structurally related molecules (see Figure 1). In noncovalent imprinting, 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

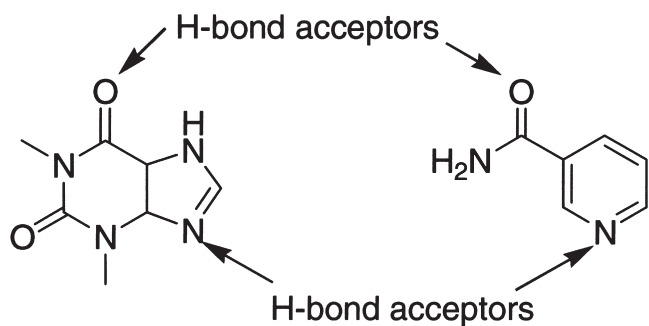


Figure 2 Good hydrogen-bond acceptors in theophylline (left) closely match the hydrogen-bond acceptors in nicotinamide (right). As a result, nicotinamide-imprinted materials show good selectivity also for theophylline.

can also be varied significantly, followed by polymerization (where the solvent acts as a porogen and 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. (Note: covalent or semicovalent imprinting can also be employed.⁵)

Molecular imprinting process

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

- Never use the analyte as a template unless there is absolutely no alternative
- 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 analog of the analyte molecule
- Select monomers that are likely to form strong interactions in the chosen solvent (e.g., Brønsted acids or bases/H-donors or acceptors/nonpolar groups, etc.)—this will increase capacity and influence homogeneity of the binding cavities
- Choose templates and monomers that will be soluble in the porogenic solvent to be used in the polymerization—this may seem obvious but it sometimes requires carrying out solubility tests
- Ensure as far as possible that the template–monomer mixture is stable and does not undergo side reactions under the polymerization conditions
- Consider the nature of the matrix from which the analyte will eventually be 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.

MIPs in pharmaceutical analysis

Several MIPs have been described in the literature for applications in pharmaceutical analysis. Early examples described in the 1990s suffered from interferences with template bleeding due to use of the analytes themselves as templates.^{6–10} To overcome this problem, Andersson et al. described an MIP for sameridine (a morphine-like anesthetic) that used a structural analog as the template.¹¹ In this example, template bleeding was not an analytical issue since the analyte and template could be chromatographically separated. Other examples using structural analogs followed.^{12,13} Despite this improvement, it is still extremely important to remove the template molecule during production of the polymer down to ppb levels if the MIP is to be used to clean up trace-level compounds. The authors have previously commented³ on an example in which an analyte could not be measured at the required (low) level due to inefficient removal of the template molecule. In this case, the peak originating from the template in the chromatographic separation step was considerably larger than the analyte peak (>100×).

While use of a structural analog that is very similar to the analyte itself can often solve the problem of bleeding, other issues with this approach remain. For example, the analog can be more costly to synthesize than the analyte; it can exhibit different solubility characteristics in the polymerization mixture than the parent analyte; and it can be toxic.

Addressing the template problem

The concept of molecular cross-reactivity led to the development¹⁴ of ExploraSep™ (MIP Technologies AB, Lund, Sweden). The idea is that an MIP prepared with one template molecule will not only be selective for that molecule but also for other target molecules with a similar

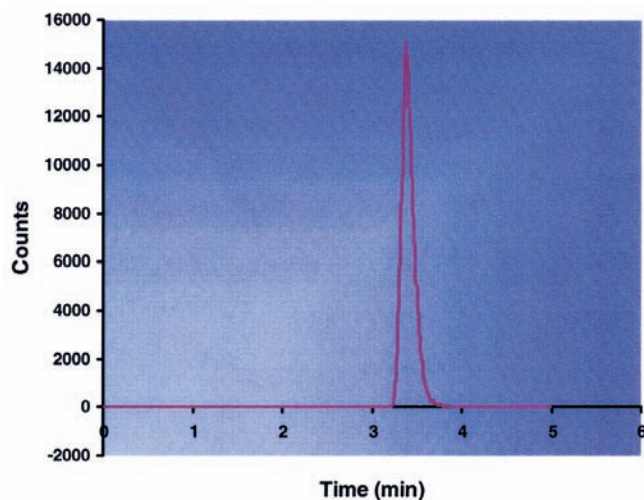


Figure 3 LC-MS-MS chromatogram of extract from MIP4SPE^{Chloramphenicol} (MIP Technologies AB) for an incurred bovine milk sample contaminated with 1.7 ng/mL of chloramphenicol.

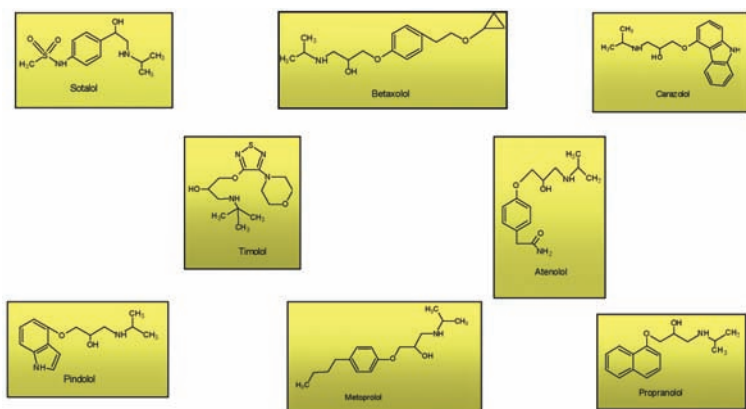


Figure 4 Molecular structures of the beta-blockers selected for investigation in wastewater.

three-dimensional arrangement of interacting functional groups. This is analogous to the “pharmacophore” concept in drug design, and for separations the authors refer to this as a “selectophore.” For example, during the development of ExploraSep, it was found that an acidic MIP imprinted with nicotinamide also shows selectivity for theophylline. In this case, the result can be easily explained. In the imprinting process, the strongest interactions are expected to be with the pyridine nitrogen and the amide oxygen. If theophylline is superimposed on nicotinamide, good hydrogen-bond acceptors in theophylline almost perfectly match the best hydrogen-bond acceptors in nicotinamide (Figure 2). In other words, nicotinamide can act as a template analog for theophylline.

For more complex templates and target molecules, templates have been designed using *in silico* methods.¹⁵ The authors created a database of interactions fueled by results from screening many MIPs already made; this can be likened to high-throughput screening for MIPs. The database can then be used to develop selectophore models for new analyte targets using an empirical design process. One key advantage of this approach is that template analogs found by screening will typically be different from the target molecules and thus unlikely to interfere with the analysis.

Screening procedure for chloramphenicol

Chloramphenicol (CAP) is a veterinary antibiotic frequently employed in animal and fish production. Due to the suspicion that CAP is carcinogenic and can cause aplastic anemia in humans, it was totally banned in the EU in 1994. Today, a zero tolerance level in food exists since maximum tolerance levels cannot be established for compounds with this level of toxicity. Consequently, a sensitive, accurate, and selective analytical procedure was required. Several complicated methods have been developed for the analysis of CAP.¹⁶ However, none of the published methods has the required

specificity to confirm unequivocally a positive result. The MIP SPE method described below fulfills these requirements.

The screening procedure using ExploraSep identified several “hits,” one of which was selected for development. Its original polar template is unrelated to CAP, although its selectophore was similar. The MIP for CAP exhibits detection limits well below the minimum required performance limit (MRPL) for CAP (0.3 µg/kg or 0.3 ppb) using LC-MS-MS (see Figure 3), enhancing the reliability of the detection. The performance of the method is equal to or better than immunoassays, with none of the drawbacks such as false positives or specialized handling. The selectivity of the MIP also ensures the required performance over a range of sample matrices such as milk, urine, shrimp, and honey.

Screening procedure for trace-level determination of beta-blockers in natural water samples

Beta-blockers are an important class of antihypertensive drugs. With their growing use, the contamination of environmental waters has increased, making a highly selective and sensitive method for the simultaneous determination of this class of drugs in water an urgent requirement. An SPE method based on a class-selective MIP extraction was developed by the authors in collaboration with Pizzolato et al.⁴ The method, based on the use of MIPs designed for the extraction of a different class of compound but predicted to match the selectophore for beta-blockers, offers both good efficiency and selectivity in the extraction of atenolol, carazolol, pindolol, propranolol, sotalol, timolol, and metoprolol (see Figure 4) from environmental water samples. Recoveries achieved were higher than 80%, and limits of detection (LODs) obtained for the beta-blockers investigated were in all instances lower than 1 ng/L (1 ppt) in water. Tests performed with complex wastewater samples indicate that the MIP material efficiently avoids matrix interferences, in addition to being a simple and rapid analytical method.

Conclusion

The advantages of MIP phases in solid-phase extraction have been described. In instances in which selectivity is required, MIP phases have distinct advantages over standard SPE phases. A number of myths abound in the analytical chemistry community regarding the strengths and weaknesses of this technology, driven largely by the fact that many users are perhaps employing old methods that have now been superseded. One such issue is analyte bleeding from the polymer. Different ways to solve this problem have been described, such as the use of a template analog or exploitation of the fact that an MIP prepared with one template molecule will also show selectivity for other target

molecules with a similar three-dimensional arrangement of interacting functional groups.

Screening of MIP libraries—a type of high-throughput screening for MIPs—will lead to the discovery of template analogs that may be very different from the target molecules and thus less likely to interfere with the analysis. Ultimately, this screening approach, combined with empirical design, will speed up the development process of new MIPs and allow the introduction of novel separation and extraction phases.

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