Selective extraction of proteins and other macromolecules from biological samples using molecular imprinted polymers

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Background

The determination of drugs, metabolites and biomarkers in biological samples continues to present one of the most difficult challenges to analytical scientists. Matrices such as plasma, serum, blood, urine or tissues for example, usually contain the analyte(s) of interest at low concentration in the presence of many other components which may interfere directly or indirectly with the accurate determination of species and concentration. Historically, the most common methods have involved some form of extraction or isolation such as liquid-liquid extraction (LLE), solid phase extraction (SPE) or protein precipitation. For a recent review of sample preparation methods for bioanalysis, see [1]. This includes comments on costs, automation, and miniaturisation with an overall focus on productivity.

Accurate quantitative measurement over the last 40 years has traditionally been carried out by chromatography, mainly high performance liquid chromatography (HPLC) and occasionally gas chromatography (GC). Although a range of detectors has been available for both, most typically, HPLC used ultraviolet (UV) and GC used flame ionisation and then both have used mass spectrometry (MS). Sample preparation has been usually by a variant of LLE, SPE or protein precipitation [2]. As the need for greater sensitivity has been a constant challenge, sophisticated and more selective methods of sample preparation have been explored. One of the most attractive of these has been the use of immobilised antibodies [3] to selectively extract drugs and metabolites in a typical

SPE format. Many examples have been published but the approach has been limited by a number of factors such as cost and uncertainty of antibody production as well as stability of the antibodies. Significant developments overcoming the coupling of MS to HPLC and its subsequent widespread use has meant that the demands on sample preparation have been reduced. As drugs and metabolites are typically small molecular mass organic compounds greater selectivity and sensitivity could be achieved by the end step measuring technique, and there has indeed been wide uptake of this technology especially in the pharmaceutical industry.

Molecularly imprinted polymers

Nevertheless, within the bioanalytical community the interest in the advantages offered by selective extraction have remained. Molecularly imprinted polymers (MIPs) have been suggested as an alternative to immobilised antibodies in a number of areas including bioanalysis [4] as these are potentially much cheaper to synthesise and more stable than biological antibodies. MIPs have been the subject of numerous reviews [5, 6] so the basic principles will only be summarised here. Briefly, the preparation involves a reaction mixture containing the analyte (the so-called template molecule), a functional monomer, a cross-linking agent and an initiator in a suitable solvent. The MIP is formed around the template. The template is subsequently removed leaving cavities that can selectively rebind the template. The aim is to create a reagent (MIP) that can selectively bind the analyte, in a similar way to an antibody. Immobilized

antibodies can be very specific but they are inherently quite fragile molecules, particularly when exposed to organic solvents, pH values of more than 2-3 units from neutral and/or heat. They can also be quite time-consuming to produce, in many cases requiring repeated dosing to animals, with no certainty that useful antibodies will eventually be obtained. In contrast, MIPs are produced rapidly in the chemistry laboratory and use well-established synthetic routes which lead to comparatively lower production costs. They are more stable over a wider pH range and can be used with a broader range of solvents. This potentially also offers the advantage that they could be re-usable, further lowering the costs.

Many papers and reviews have been written on the optimisation of conditions, different methods of characterisation and different uses of MIPs [7-9]. In many cases, the MIPs will only perform their selective capture if they are in the solvent used for their preparation. The importance of buffer conditioning has been emphasised [9]. For example, MIPs have been proposed as offering advantages as columns for HPLC, SPE [2, 3, 10, 11], capillary electrophoresis [12] and electrochromatography, replacing antibodies in enzyme-linked immunosorbent assay (ELISA) tests [13], artificial enzymes or receptors, recognition elements within sensors [14], selective drug delivery, catalysts and to aid crystallization [15]. The area of SPE has attracted most attention and new approaches are still being reported in this area. The development of nanoparticles has led to molecular imprinting onto the surface of magnetic nanoparticles [16] followed by solid phase microextraction (SPME) or ultrasonic assisted SPME [17-19] and

matrix dispersant SPME [20, 21]. MIPs which are integrated with magnetic nanoparticles offers the added advantage of a simple separation using a magnet following the selective template (analyte) binding/extraction step. Ding *et al.* 2014 [22] has written a recent review on surface imprinting technologies for nano-MIPs. This described both small and large molecule templates in two different sections. Examples of biomacromolecules that have been imprinted include lysozyme, bovine haemoglobin, human haemoglobin, amylase and bovine serum albumin (BSA) as well as virus particles.

The preponderance of reviews on the use of MIPs for separation science has led to a review of reviews [23]. Nonetheless the use of commercially available MIPs using validated methods for bioanalysis is not considered commonplace. Li *et al.* 2014 [24] has written an extensive review on macromolecules concentrating on proteins, carbohydrates, DNA, viruses and cells. The review contrasts the development of small molecular mass versus macromolecule templates. Progress with the latter has been slower and unremarkable. Several commercial companies are producing MIPs for SPE mainly for small molecular mass analytes such as drugs and pesticides. These however are not commonplace.

Many of the applications published in scientific literature consider only the comparison of a MIP with a non-imprinted polymer (NIP), along with comparisons of a very small number of other related compounds as evidence of a MIP effect. Studies looking at the rebinding of the analyte to the MIP compared to a NIP are

commonplace. In many applications the MIPs will often only work satisfactorily when the rebinding is carried out in the solvent in which the MIP was synthesised, typically organic solvents. This is a considerable drawback when the need is to extract from aqueous biological fluids such as plasma, serum, urine, tissue extracts and faeces. It is also unsuitable for most macromolecules of biological interest as they are not stable in organic solvents. Biologicals (greater than 1000 Da) are metastable and can undergo intra-molecularly-induced changes in conformation depending on their chemical environment. They therefore need to be exposed to less harsh polymerisation conditions compared with the imprinting of small and robust molecules (less than 700 Da) the latter inherently possessing less degrees of freedom in molecular arrangement. MIP preparations for biologicals have therefore focused on the use of water-compatible polymers, namely hydrogels based on using acrylamide (AAm) as a functional monomer [25-27] and the repertoire extended more recently using a combination of acrylo-based functional monomers to polymerise in the presence of a second (more biocompatible) polymer including polyethylene glycol (PEG) and chitosan [28]. Chitosan is a derivative of chitin (extracted from crustacean species), and is produced by deacetylation of chitin under alkaline conditions. At around physiological pH and below, chitosan is positively charged. Thus in addition to the generally accepted hydrogen bonding interactions and cavity fit offered by MIPs, the presence of positive charge offers an additional (electrostatic) anchor for the imprinting of proteins.

MIPs for extraction/enrichment of macromolecules

One area of growing interest in bioanalysis has been in the preparation of MIPs to peptides, proteins or other large biomolecules [29, 30]. The changing nature of drug development suggests that macromolecules are increasingly being proposed as new therapeutic agents or indeed as biomarkers for a range of diseases. Novel approaches for their reliable accurate measurement is thus of growing interest. In many cases the macromolecules will be present in biological fluids at low concentrations so the application of MIPs for selective extraction to allow pre-concentration and clean-up is a very attractive approach. The development of such MIPs using protein templates was reviewed [31]. The latter review was focused on sensors but the methods used to prepare the MIPs should be a useful guide for their eventual application in selective enrichment or other applications. The review discussed template selection, bulk compared with surface imprinting, the use of whole protein or epitopes, solvent conditions used for imprinting, the choice of monomers and cross-linkers, procedures for template removal as well as the sensor development aspects, Many of the examples of MIPs for proteins use a low degree of cross-linking to give soft hydrogels rather than the highly cross-linked rigid gels used for small molecule imprinting. The advantages of using surface imprinting when preparing protein MIPs has been described in ref [32]. This review included sections on SPE, mainly of small molecular mass analytes. The use of carbon nanofibres, nanodiamonds, fullerenes, carbon nanotubes, graphene and graphene oxide were evaluated by

ref [33] as possible materials for isolation and pre-concentration of proteins and where MIPs can improve selectivity.

There have been several reviews of the use of MIPs for SPE. For example, Augusto *et al.* 2013 [34] considered the merits of immunoaffinity, MIPs, aptamers, carbon nanotubes and other nanomaterials. These give numerous examples of the use of MIPs to extract small molecular mass compounds but generally give few examples of macromolecule extraction. SPE can be carried out in several formats. Examples include a conventional small syringe packed with the MIP, coated fibres, capillaries, surface coated particles, coated stir bars, membranes, magnetic beads and nanoparticles [35]. All have advantages and disadvantages and these were evaluated. Hu *et al.* 2013 also emphasised that the major obstacles include the difficulty of finding optimised conditions for selective extraction, compatibility with aqueous solutions and the low number of binding sites obtained [35].

Schirhagl *et al.* 2014 [36] reviewed the particular approaches to imprinting large biomolecules and highlighted the advantages of using more flexible polymers than the rigid polymers used for small molecules. The review covered methods of synthesis, template removal, applications using various methods (optical, electrical and mass sensitive) of signal production in sensors, separation science and possibilities in drug discovery. The article concluded that selectivities

obtained for large biomolecules are still not as good as those for small drug like molecules.

One interesting approach recently reported was the use of a surface imprinted polymer using myoglobin as the template [37]. The MIP allowed selective capture and release of the target using temperature, rather than the much more widespread use of a change of solvent or pH.

The basic principle of using a selective extraction followed by desorption into a chromatograph with an MS detector or other instrumental technique is attractive, as accurate measurement and a high degree of specificity or identification can be achieved. Again the evidence quoted in scientific literature for a MIP effect is often that the macromolecule is extracted with greater recovery from the MIP than the NIP and selectivity to similar molecules in terms of molecular mass, function or isoelectric potential. Conclusive evidence of a molecular imprinting effect has been questioned [38]. Although comparison of MIP to NIP is some evidence of a MIP effect the non-specific binding to the NIP does suggest that further studies such as structural characterization would be helpful. Non-specific binding will prove to be a particular obstacle to widespread acceptance when complex samples such as biofluids are processed. Ultimately, the crucial point is not whether the selective capture is an effect requiring specific interactions at specific points on the polymer; rather, it is whether or not MIP-based selective

extraction provides improvement in the analytical methods developed. This would then need widespread uptake to become completely convincing.

This article will review recent examples in the development of the use MIPs for selective extraction or enrichment of proteins and other large biomolecules appropriate to biological samples. A very extensive collection of articles describing the preparation or use of MIPs in all their applications is listed online [39]. The majority of applications of MIPs are in the area of separation science or sensors. The reality that there are few examples of methods based on MIPs for selective extraction of macromolecules suggests something of an unmet need here.

Examples of extraction/enrichment of macromolecules using MIPs (see also Table 1)

Qadar *et al.* 2014 [40] developed MIPs to the nonapeptide progastrin releasing peptide (ProGRP), a possible biomarker for small cell lung cancer. A range of acrylamide monomers were evaluated in the SPE format with fractions analysed by HPLC-UV. Selectivity was checked against 4 other peptides. In a follow up paper [41] this group applied the optimised protocol to enrich the peptide from fortified serum. The limit of detection from the optimised protocol was reported to be about 600 pM. The elution protocol used 80% acetonitrile as elution solvent. The MIP retained the targeted peptide more than the NIP, which nonetheless does show non-specific binding. Importantly an example showed a much cleaner chromatogram for the MIP compared with the NIP. Although a nonapeptide rather

than a protein, this paper illustrates the potential of a method based on selective SPE with a MIP followed by LC-MS for an important low abundance biomarker. There are several other examples of polypeptide MIPs [30, 40, 42, 43]. Shinde *et al.* 2012 [44] described how an SPE MIP format could distinguish between sulpho- and phosphorylated peptides. Fractions were analysed by HPLC and matrix assisted laser desorption ionisation (MALDI) to confirm the elution fraction contents.

Qin *et al.* 2009 [45] showed the possibility of enriching lysozyme from aqueous and biological samples – in this case egg white. *N*-(4-vinyl)-benzyl iminodiacetic acid (VBIDA) was co-polymerized with *N*-isopropylacrylamide (NiPAm) and AAm in the presence of copper (Cu^{2+}) ions. Greater adsorption capacity was shown for the lysozyme template than for several other proteins (cytochrome C (CytC), ribonuclease A (RNase A), ovalbumin, bovine haemoglobin (BHb), BSA, and glucose oxidase). A gel electrophoresis figure showed enrichment of the lysozyme from diluted egg white. There is growing interest in incorporating metal ions (through complexation) to improve the binding affinity of MIP for a target protein [46]. The electron donating effect of amino groups of the protein to the metal centre offers an additional anchor point for the protein to dock within the vicinity of the cavity.

Gao *et al.* 2010 [47] prepared a surface modified MIP to lysozyme using methacrylic acid (MAA) as functional monomer and hydroxyethylmethacrylate

(HEMA)/ *N*-vinylpyrrolidone (VNP) as cross-linked microspheres. Although biological samples were not evaluated, dynamic binding curves clearly illustrated the delayed elution of the lysozyme compared to bovine haemoglobin.

Gai *et al.* 2010 and 2011 [48, 49] prepared MIPs to BHb and lysozyme. The lysozyme MIP was surface imprinted and showed greater selectivity for the lysozyme compared with BHb, myoglobin, BSA, Trypsin inhibitor (TI) and CytC. The BSA MIP similarly showed greater selectivity in adsorption experiments, potentially applicable as a sample preparation/enrichment method. Non-specific binding to NIP was also shown which could lessen the use of such a MIP for accurate measurement.

Dan *et al.* 2013 [28] reported MIPs to ovalbumin using the polysaccharide chitosan and acrylamide as monomers and described extensive optimisation of synthesis. Selectivity was ascertained by comparing MIP rebinding with the non-cognate proteins BSA, BHb and lysozyme. They also looked at surface morphology using several techniques. Gels using chitosan and acrylic acid (AA) and MAA showed the best potential but non-selective binding to NIP and selectivity to other proteins still needs addressing. Biological samples were not evaluated.

Wan *et al.* 2015 [50] showed how a polydopamine MIP surface imprinted on nanoparticles could enrich lysozyme spiked diluted egg white samples. The MIP

was compared to NIP and cross reactivity studies versus five proteins (RNase A, BHb, BSA, trypsin and CytC) demonstrated preferential binding to the target protein. Samples were analysed using MALDI-TOF.

Deng *et al.* 2011 [51] prepared a monolithic MIP to BSA using a freeze thawing polymerisation method with acrylamide as the monomer. Both HPLC and SPE demonstrated a greater retention for the BSA versus Hb. A gel electrophoresis plate showed a SPE extract enriched with the target protein compared to carbonic anhydrase, lysozyme, BSA, and trypsin. The MIP column showed the BSA, the NIP column showed none of the aforementioned proteins.

Lin et al. 2013 [52] described the selective extraction of horseradish peroxidise (HRP) from spiked human serum samples. Dopamine was the functional monomer used for MIP preparation. Although the paper was mainly concerned with a monolithic HPLC column it also described the use of the MIP approach in SPME format. It showed a gel electrophoresis plate with significantly enriched HRP.

Namatozola *et al.* 2014 [53] used AAm to prepare MIPs for human serum albumin (HSA) and IgG. Part of their article described the evaluation using SPE. Comparison of MIP and NIP shows a slightly increased recovery in the elution fraction for the imprinted protein particularly for the IgG. For both MIPs much of

284 the protein was eluted in load and wash fractions suggesting very low selective
285 binding capacities within the MIP.
286
287 Solemani *et al.* 2012 [54] described the preparation of a BSA MIP under the
288 conditions normally used for small molecule analytes. They evaluated the MIP in
289 SPE format, optimising the flow rate, the effect of pH, ionic strength, sample
290 volume and different ratios of methanol/acetonitrile on elution. After optimisation
291 with standard solutions, more challenging solutions such as serum, urine, whey
292 and milk were applied. MIPs were compared with NIPs for recovery. It should be
293 noted that elution fractions from the SPE columns were evaluated by UV-Vis
294 spectrophotometry not by chromatography or MS. The possibility of denaturation
295 of the BSA during MIP synthesis or the analytical protocol cannot be discounted
296 and could be evaluated by, for example, using circular dichroism spectroscopy to
297 assess the nature of the protein during and following the MIP production process
298 [55, 56].
299
300 Liu *et al.* 2014 [57] prepared MIPs for extraction of HSA using porcine serum
301 albumin as a dummy template with methacrylate monomers. The aim of this work
302 was to selectively extract high abundance protein that was not the analyte of
303 interest, thereby enhancing the detection limits of low abundance proteins of
304 interest. Much higher binding affinity for the desired protein was obtained
305 compared with β-lactoglobulin, CytC or ribonuclease B. The use of a dummy
306 template was common with small molecule SPE. It involved the use of a

structural analogue of the target analyte to form the MIP. To date it is much less common with macromolecules.

An example of virus imprinting was shown by Sykora *et al.* 2015 [58] where preliminary results indicated the synthesis of surface MIPs to a *Human Norovirus* strain. They pointed out some of the difficulties of this type of work. Quite apart from the problem of biomolecule stability, the need to use large amounts of pathogenic virus in the MIP synthesis stage restricts this type of work. This issue was overcome by using a genetically modified virus-like particle as the template. The paper showed a much larger binding to the MIP compared with the NIP. Field emission scanning electron microscopy pictures were also shown as evidence of MIP structure.

Comments

Sample preparation includes trying to isolate the analyte to improve detection limits, especially if the analyte is at very low concentration when there is plenty of sample. It can also include trying to remove matrix components that interfere even if they do not give a direct signal to the detector, for example ion suppression in MS.

In contrast to MIPs, antibodies are extensively used commercially especially in clinical (bio) chemistry laboratories. There are examples where MIPs have been shown to replace antibodies in clinical tests [13, 59]. So their increasing use for

selective extraction of macromolecules is anticipated. Whether it will be for special applications or widespread depends on the reality of commercially developing suitable products. The virtues of combining immunoaffinity sample preparation with MS detection have been highlighted in a special issue of Bioanalysis especially in the overview given by Ackerman [60]. The advantages offered by biological antibodies will be potentially superseded if suitable MIPs can be reliably produced. The attraction of specific analyte capture, trace enrichment from a large volume and then release into a small volume of liquid compatible with injection into an LC-MS is clear. The use of antibodies for this is increasing. If this type of procedure could be achieved with MIPs this would be an even more attractive approach.

With proteins and other large biomolecules analyte stability is a problem, so aqueous based SPE protocols are essential. Several papers look at morphology or cavity size, but to be of use to bioanalysts with real measurements to make and defend this ultimately depends on how clean the samples are and the reproducibility of results that is demanded by the end user. One of the drawbacks with the use of MIPs has been the reality that they are not yet as specific as biologically developed antibodies. Whereas K_d values for antibody-antigen interactions are of the order of 10^{-9} M, the majority of MIP-antigen interactions are still at the 10^{-6}-10^{-7} M range, However, recently Piletsky's group has developed a technique for the mass production of nanosized MIPs (plastic antibodies) reporting K_d values matching biological antibodies [61]. When used

as reagents for SPE followed by a specific and sensitive end-step such as LC-MS the lack of high affinity MIPs is less of a drawback. Potentially they can offer enough selectivity in extraction to provide a clean enough sample for the chromatography or other measurement. The reality that there are currently few examples of this approach suggests it is worthy of more effort.

Peptides are not as challenging because they are more stable than proteins and also less expensive in terms of requiring a relatively large amount of template. Other similar approaches for selective extraction have also been developed. The use of aptamers (short single stranded DNA or RNA molecules) has been reviewed by [62, 63] including their use in SPE format. The importance of measuring new therapeutic agents or small abundance protein biomarkers means that the quest for improved methods of selective enrichment/clean-up will continue. Other areas where MIPs may show promise include virus imprinting [64-66] where preliminary experiments showed that tobacco mosaic virus could be imprinted using polyallylamine.

Difficulties such as the need for a large amount of template for MIP synthesis, reliable and complete template removal, minimisation of non-specific binding, a reasonable shelf-life and commercial availability of quality controlled products that are suitable for rebinding in aqueous solutions still need to be overcome. Nonetheless the approach of selective (enough) extraction followed by HPLC-MS

is an attractive proposition in bioanalysis. Hence, the development and validation to regulatory authority guidelines of macromolecule MIPs is tentatively awaited.

Conclusions

Molecularly imprinted polymers offer an alternative approach to biological antibodies for selective capture reagents in bioanalytical chemistry. Most of the developments in MIPs have involved small molecules particularly drugs and metabolites. Although several different applications have been proposed, none have come into widespread routine use in laboratories. Use as selective sorbents for SPE have been the most promising area. Even in this area, uptake has been slow. This is in part due to the advent of techniques such as LC-MS seemingly requiring less rigorous sample preparation requirements. It is also, in part, caused by the nature of the technique. If you develop a product that is specific to only one drug or class of drug – it is not going to attract a big market. However generic protocols would be helpful here.

There is growing interest in accurate measurement of proteins and other macromolecules or biological entities such as viruses. These are being introduced as new drugs or being validated as biomarkers both for drug efficacy and diagnostics. Not surprisingly, MIPs are being produced to macromolecules and are now being evaluated for use in sensors and for sample preparation. Selective extraction both for analytical and preparative purposes is worthy of more research as there are few examples of macromolecule determination in

biological samples. Methods proposed will need to be subject to the rigorous validation protocols required by regulatory authorities, not just publication in academic journals.

Future Perspectives

The determination of large molecules in biological fluids will continue to be an area of growing importance. Problems with determining intact macromolecules will present greater challenges than for small molecules not least due to their lack of stability. Improvements in the preparation of macromolecular MIPs are needed. This will facilitate investigations into the use of such selective reagents for improved methods of sample preparation. These could then be utilised along with methods such as LC-MS to provide accurate quantification at low concentrations in biological fluids.

Keywords

Proteins, Macromolecules, Selective extraction, Molecular imprinted polymers, Antibody mimics, Bioanalysis

Table 1 – Example of analytes imprinted within a varied mix of matrices and monomer/cross-linker combinations.

Analyte	Matrix	Monomer	Cross-linker	Validation	Ref
BHb	Aqueous buffers	AAm	MBAA	MIP vs NIP Selectivity vs BSA	[49]
Lysozyme	Aqueous and diluted egg white	NiPAm/AAm	MBAA	MIP vs NIP Selectivity vs BSA, Mb, BHb, TI, CytC	[48]
Ovalbumin	Aqueous non-biological	Chitosan/AA,AAm, MAA	MBAA	MIP vs NIP Selectivity vs BSA, BHb, lysozyme	[28]
ProGRP	Aqueous non-biological	EAMA	DVB	MIP vs NIP Selectivity vs 3 other poly peptides	[40, 41]
Lysozyme	Aqueous buffers	VBDIA/ NiPAm/AAm Plus Cu ions	MBAA	MIP vs NIP Selectivity vs CytC, RNasaA, OB, BSA, Hb, GOx	[45]
Lysozyme	Aqueous and diluted egg white	Dopamine	Not reported	MIP vs NIP and selectivity vs CytC, RNase A, BHb, BSA, CytC	[50]
HPR	Spiked human serum	Dopamine	PETA	HSA, IgG, Trf and other serum proteins	[52]
HSA, IgG	Aqueous buffers	AAm	MBAA	MIP vs NIP	[53]
BSA	Aqueous buffers	AAm	MBAA	MIP vs NIP and selectivity vs CA, lysozyme, BSA, and trypsin	[51]
BSA	Aqueous buffers, serum, urine	2VP	EGDMA	MIP vs NIP	[54]

Acrylamide (AAm); Acrylic acid (AA); Methylacrylic acid (MAA); N-(2-Aminoethyl methacrylamide hydrochloride (EAMA); N-isopropylacrylamide (NiPAm); Divinyl benzene (DVB); N,N-methylenebisacrylamide (MBAA); N-(4-vinyl)-benzyl iminodiacetic acid (VBIDA); 2-vinylpyridine (2VP); Cytochrome C (CytC); Bovine haemoglobin (BHb); Bovine serum albumin (BSA); Myoglobin (Mb); Trypsin inhibitor (TI); Glucose oxidase (GOx); Carbonic Anhydrase (CA); Ovalbumin (OB); Pentaerythritol triacrylate (PETA); Horseradish peroxidase (HPR); Transferrin (Trf); Ribonuclease A (RNase A); Ethylene glycol dimethylacrylate (EGDMA).

Executive Summary

Background

- The measurement of drugs, metabolites and endogenous compounds is a very challenging area for Analytical Chemists. The most common methods involve some form of extraction to give sample clean up and pre-concentration. This is then followed by injection into a gas or liquid chromatograph and measurement using a variety of detectors but most commonly nowadays mass spectrometry.
- As demands for better sensitivity are a challenge methods of selective extraction have been explored. One of the most attractive of these has been the use of immobilised antibodies to selectively extract drugs and metabolites using solid phase extraction.

Molecularly Imprinted Polymers

- MIPs are synthetic polymers formed around a template molecule (the analyte). These are then used as reagents to selectively rebind the analyte during sample preparation. They are much cheaper than biological antibodies and are more stable.
- There are many literature applications using MIPs to extract small molecular mass drugs and metabolites but they are not in common use in industrial laboratories.

MIPs for extraction/enrichment of macromolecules

- With the development of macromolecules as candidate drugs and biomarkers there has been increased interest in developing selective extraction to large molecules.
- The use of soft gels, where the MIPs are formed in aqueous solutions is much more applicable to biomolecules which are generally not stable in other solvents.

Examples of extraction/enrichment of macromolecules using MIPs

- Examples of selective binding of a number of macromolecules are given. These include peptides and polypeptides, lysozyme, bovine haemoglobin, bovine serum albumin, ovalbumin, horseradish peroxidise, human serum albumin, and viruses.

Comments

- The combination of selective extraction along with HPLC-MS to measure macromolecules is very attractive.
- However there are as yet few examples where this has been achieved with MIPs as opposed to biological antibodies.
- There are some questions as to whether or not a MIP effect is as selective as desired.

Conclusions

- Use of selective extraction is an area likely to grow as more macromolecular drug candidates and biomarkers are developed.

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Highlights

41 This paper shows a peptide MIP spiked into serum

51 This paper shows an enriched SPE trace on gel electrophoresis

58 This paper shows the possibility for virus imprinting.