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Review

Molecular imprinting for drug bioanalysis
A review on the application of imprinted polymers to solid-phase extraction and binding assay

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Abstract

Molecularly imprinted polymers have been applied as selective sorbents in several analytical techniques, including liquid chromatography, capillary electrophoresis and capillary electrochromatography, solid-phase extraction, and 'immunoassay'. An advantage of this type of sorbent is the possibility to synthesize polymers with selectivity pre-determined for a particular analyte. This review critically discusses the use of imprinted polymers for analysis of drugs and other compounds in biological samples, with emphasis on their use as highly selective solid-phase extraction sorbents for sample pre-concentration and alternative binding entities in immunoassay type protocols.

Keywords: Reviews; Pre-concentration; Molecular recognition; Polymers

Contents

1. Introduction

Recent years have seen an increasing interest in the potential application of highly selective molecularly imprinted polymers, MIPs, in the analysis of drugs and other compounds in biological and environmental samples. The use of imprinted polymers [1–5] in several analytical techniques, including liquid chromatography [6,7], capillary electrophoresis and capillary electrochromatography [8], solid-phase extraction [9], and 'immunoassay' [10], have been investigated. These studies have included imprinting of several types of drug compounds and related substances, such as antibiotics of various

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types, beta-blocking agents, benzodiazepines, immunosuppressants, local anaestethics, opiates, steroids and xanthines, as well as triazine type herbicides. An advantage inherent to molecular imprinting, which has repeatedly been testified by the many examples above, is the possibility to synthesize sorbents with selectivity pre-determined for a particular analyte. The key step of the technique is the polymerisation of functional and cross-linking monomers in the presence of a templating ligand, or imprint species (Fig. 1). Subsequent removal of the imprint molecules leaves behind 'memory sites', or imprints, in the solid, highly cross-linked polymer network. It is believed that the functional monomers become spatially fixed in the polymer via their interaction with the imprint species during the polymerisation reaction. The result is the formation in the polymer of imprints, which are complementary, both sterically and chemically, to the templating ligand. These imprints enable the polymer selectively to rebind the imprint molecule from a mixture of closely related compounds. In some instances, binding affinities and selectivities approaching those demonstrated by antigen–antibody systems have been achieved. This review discusses the potential of using MIPs in bioanalysis, with special emphasis on solid-phase extraction and ligand binding assay, and highlight some of the problems that need to be addressed.

2. Imprint preparation – critical issues

For guidance on the preparation of molecular imprints, the reader is referred to the many excellent reviews published in recent years [1,3,4,7,11–13]. Suffice here to discuss some critical issues, namely stabilisation of monomer–template complexes in the pre-polymerisation mixture, removal of template molecules, choice of template, format of polymer and the fact that, whilst often for bioanalytical applications they are preferrably used under aqueous conditions, most MIPs are made using organic solvents.

The synthesis of a MIP entails polymerisation of monomers in the presence of a templating ligand. Depending on the experimental design, the monomers interact with the template molecules by either non-covalent interactions [2,3], reversible covalent interactions [1], or metal ion mediated interactions [5]. Of these approaches, the non-covalent strategy is the one being most widely employed, in particular for the types of applications discussed in this review. Non-covalent molecular imprinting is straight-forward, the imprint molecule is simply mixed with monomers and cross-linkers in a suitable solvent prior to initiation of the polymerisation. Frequently used functional monomers include methacrylic acid (MAA), 2- and 4-vinylpyridines (2- and 4-VPy), trifluoromethylacrylic acid (TFMAA), acrylamide

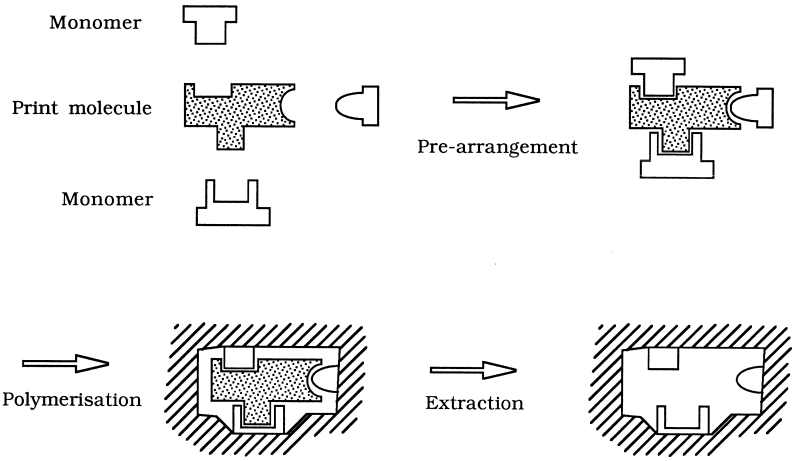

Fig. 1. Schematic depiction of the preparation of molecular imprints.

(AA) and hydroxyethylmethacrylate (HEMA), and cross-linkers include ethylene glycol dimethacrylate (EGDMA), divinylbenzene (DVB) and trimethylolpropane trimethacrylate (TRIM). One of the key success factors is the stabilisation of complexes between template molecule and monomers formed in the pre-polymerisation mixture. For the above monomers, these complexes are based mainly on polar type non-covalent interactions, such as hydrogen bonding, ionic interactions, etc. Maximal efficiency of imprint formation occurs when the polymerisation reaction is performed using an aprotic solvent as apolar as possible without compromising solubility of the imprint species [2]. This ensures maximal strength of the non-covalent interations employed, which are strongly dependent on the polarity of the solvent. Recently, a 4-VPy-*co*-EGDMA based polymer system, which instead relied on the combination of hydrophobic and ionic interactions, was used for imprinting of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) [14]. In this instance, since hydrophobic interactions are strong under aqueous conditions, a solvent of polymerisation comprising of a mixture of methanol–water was used.

Following addition of an azobis-nitrile initiator, the polymerisation can be conducted either by elevation of the temperature or irradiation by UV-light. It has been demonstrated repeatedly that MIPs prepared at lower temperatures (below 0°C), using photoinitiation, exhibit higher molecular recognition capabilities [1]. This is attributed to the complexes of monomers and imprint molecule in the pre-polymerisation mixture are more stable at low temperatures due to a more favourable entropy term, leading to more well-defined imprints in the resultant polymer.

While being less important in many applications, near-quantitative removal of the imprint species is crucial for a sensitive SPE or MIA application (see below). A more thorough extraction yields a MIP where more of the high-avidity sites are free, leading to a material better equipped to adsorb analyte from highly diluted samples, and less prone to leak reamining template molecules at use. Complete extraction requires extensive washing using solvents with strong elution power, such as aqueous ethanol containing acid or base. Alternating acid and base washings may be beneficial. In some instances, e.g. for chromatographic type applications, the extraction can be made on line. In this context, the use of an alternative imprint molecule may be considered. Ideally, this should be a structurally similar compound, one that gives rise to imprints that have the ability to bind the target analyte but do not interfere with the intended use. This strategy was illustrated by SPE of sameridine using a MIP made against a close structural analogue [15] (see below). Another example is the molecular imprinting of phenylalanine anilide and the use of the resultant MIP for capillary electrochromatographic separation of the enantiomers of phenylalanine [16]. Due to their low solubility in the pre-polymerisation mixture, amino acids are normally not amenable to non-covalent imprinting. The availability at reasonable cost of a suitable analogue has to be evaluated on a case-by-case basis.

To date, bulk polymerisation to create a block of macroporous polymer, followed by grinding and particle sizing, has been the most often used technique for imprinted polymer preparation [1–3]. The grinding process produces irregular particles as well as a considerable quantity of fine particles which have to be removed, for instance by sedimentation. Typically, about 40–60% of the ground polymer is recovered as useable particles. Although being simple, requiring no speciality knowledge or equipment, bulk polymerisation is suitable for the lab scale only. Attempts to improve particle shape have included imprinting in the pores of preformed beaded silica [17] and TRIM [18], and by a dispersion polymerisation procedure using a polar solvent based continuous phase [19]. Monodisperse imprinted particles can be made by a two-step swelling technique, using water as the suspension medium [20]. Recently, a suspension polymerization technique which is compatible with the non-covalent imprinting approach have been developed [21]. Small droplets of imprinting mixture are polymerised in a continuous phase composed of a liquid perfluorocarbon, which is inert, and do not interfere with the interactions between monomers and template required for the efficient formation of imprints. A modification of this technique made available composite beads of methacrylic acid–TRIM copolymers containing magnetic iron oxide [22]. These beads could easily be collected from a solution by an applied external magnetic field. Furthermore, imprinted continuous

polymers can be made in situ in LC-columns [23] and as superporous monoliths with good flow-through properties in capillaries [24].

Most MIPs are made using an organic solvent as the porogen, however, for bioanalytical applications, it is desirable that the resultant MIPs can be efficiently used under aqueous conditions (see below). One issue that needs attention is the different balance between hydrophobic and polar interactions in organic solvents and water. Preparation of imprints is done in the presence of an organic solvent where polar interactions, such as hydrogen bonds, are strong. The opposite is true for aqueous media where polar interactions are weak and hydrophobic interactions are strong. These facts leads to strong, non-specific binding in water due to adsorption to the hydrophobic polymer surface. Furthermore, upon change from organic solvent to aqueous based incubation, the selectivity is changed such that in organic solvents the imprints recognise subtle differences in polar functionalities of the molecule, and in aqueous media recognition of hydrophobic parts of the molecule is efficient. A typical example is binding of β-blockers to an S-propranolol MIP [25]. The aqueous buffer based assay showed high substrate-selectivity for propranolol in the presence of structurally similar β-blockers. The corresponding assay using toluene as the incubation medium showed excellent enantio-selectivity, the cross-reactivity of the R-enantiomer being only 1%. Whereas the different β-blocking drugs differ by their hydrophobic aromatic ring system, which for propranolol is a naphthyl ring, enantio-recognition requires recognition through hydrogen bonding of the configuration of the polar functionalities around the chiral carbon.

3. Sample pre-concentration

Most biological and environmental analyses require a prior sample preparation step. The extent of sample pre-treatment depends on the complexity of the sample, and is especially important when analysing drugs and endogenous compounds in biological matrices, such as plasma, urine or tissue homogenates. Solid phase extraction (Fig. 2) is continuously growing in importance, and is currently a routine

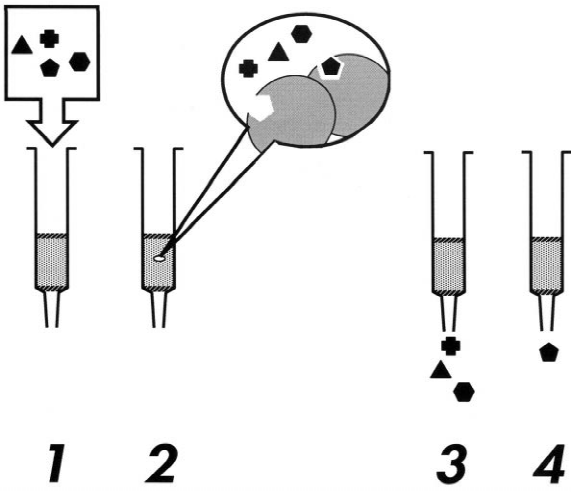

Fig. 2. General principle of a solid-phase extraction procedure. The sample is applied onto a solid-phase extraction cartridge, in this instance containing a MIP (1), the analyte is specifically bound to the imprints of the polymer (2), and after washing off matrix components (3), the analyte is eluted (4).

sample preparation technique employed in numerous bioanalytical applications. Depending on its physico-chemical properties, the analyte is extracted by adsorption to a reversed-phase sorbent such as C_8 or C_{18}, a straight phase such as silica or diol, or an anion or cation-exchanger. The adsorption conditions are tuned such that the SPE-column traps the analyte, or a group of structurally related compounds, whereas matrix components are not retained. After washing these off the SPE-column, the compound of interest is eluted for further analysis. To eliminate matrices and other interferences in the subsequent assay, the sorbent employed must enable selective extractions. Often a considerable amount of method development work is spent on optimising the SPE and new strategies are called for. One approach is to implement MIPs as chromatographic material in solid-phase extraction (referred to as MIP–SPE for Molecularly Imprinted Polymer based Solid Phase Extraction), potentially allowing a higher degree of selectivity to be achieved.

The first reported study on MIP–SPE employed a pentamidine selective MIP for demonstration of on-line sample enrichment of a spiked urine sample [26]. Later, this was followed by a number of groups who have presented SPE applications for various

Table 1
Summary of studies where MIPs have been applied to biosamples

Analyte	Sample	Reference
SPE[a]		
Atrazine	Choroform extract of beef liver homogenate	[28]
Darifenacin	Plasma–acetonitrile (1:1; v/v)	[33]
Hydroxycoumarin	Urine	[31]
Propranolol	Dog plasma, rat bile and human urine	[27,34]
Pentamidine	Diluted urine	[25]
Sameridine	Heptane–ethanol (9:1; v/v) after extraction of human plasma	[14]
Tamoxifen	Human plasma and urine	[30]
Theophylline	Chloroform extract of human serum	[26]
MIA[a]		
Cyclosporin	Diisopropyl extract of human whole blood	[43]
Diazepam	Toluene–heptane (3:1; v/v) after extraction of human serum	[42]
s-Propranolol	Human plasma and urine	[47]
Theophylline	Acetonitrile–acetic acid (99:1; v/v) after extraction of human serum	[42]
Competitive displacement sensor		
Chloramphenicol	Acetonitrile after extraction of bovine serum	[51]
Sensor		
Glucose	Porcine plasma	[52]

[a] Abbreviations used: SPE, solid-phase extraction; MIA, molecularly imprinted sorbent assay.

types of analytes (Table 1) (for a review see [9]). Different modes of MIP based SPE have been tried, including various modes of on-line SPE [26,27], conventional SPE where the MIP is packed into columns or cartridges [28–35], and batch mode SPE where the MIP is incubated with the sample [15]. The sample matrices have been various biological fluids in undiluted form or diluted with buffer or water [26,28,31,32,35], plasma which have been protein-precipitated with acetonitrile [34], organic solvent extracts of biological tissues and biofluids, such as chloroform extracts of beef liver [29] and human serum [27], and heptane extracts of human plasma [15]. Another example is an ethyl acetate extract of nicotine chewing gum [33]. The selectivity of the MIP can be pre-determined by the choice of template employed for its preparation. Another major benefit of MIP based SPE relates to the high selectivity of the sorbent, leading to efficient sample clean up. The versatility of MIP based SPE is here exemplified by a model batch-wise pre-concentration of sameridine prior to gas chromatography (Fig. 3). The selectivity of the extraction leads to distinctly cleaner chromatographic traces and ability to improve sensitivity by extracting sameridine from larger sample volumes. The analytical performance of the MIP–SPE based method was found to be equivalent to or better than that of the standard method based on the use of liquid–liquid extraction for sample cleanup.

MIPs are made in the presence of large amounts of template molecules and small amounts of imprint molecules remaining in the resultant polymer may later leak during SPE. This has been observed in several cases [15,31,34,35]. Hence, method development must include a confirmation that leakage of remaining traces of the imprint species does not interfere with the assay, giving rise to an uncertainty in the concentration determination. This is particularly important when dealing with trace analysis. One approach to avoid this risk completely is the use of a close structural analogue of the analyte(s) of interest for the preparation of the MIP. Provided the imprint species and the analyte(s) can be separated

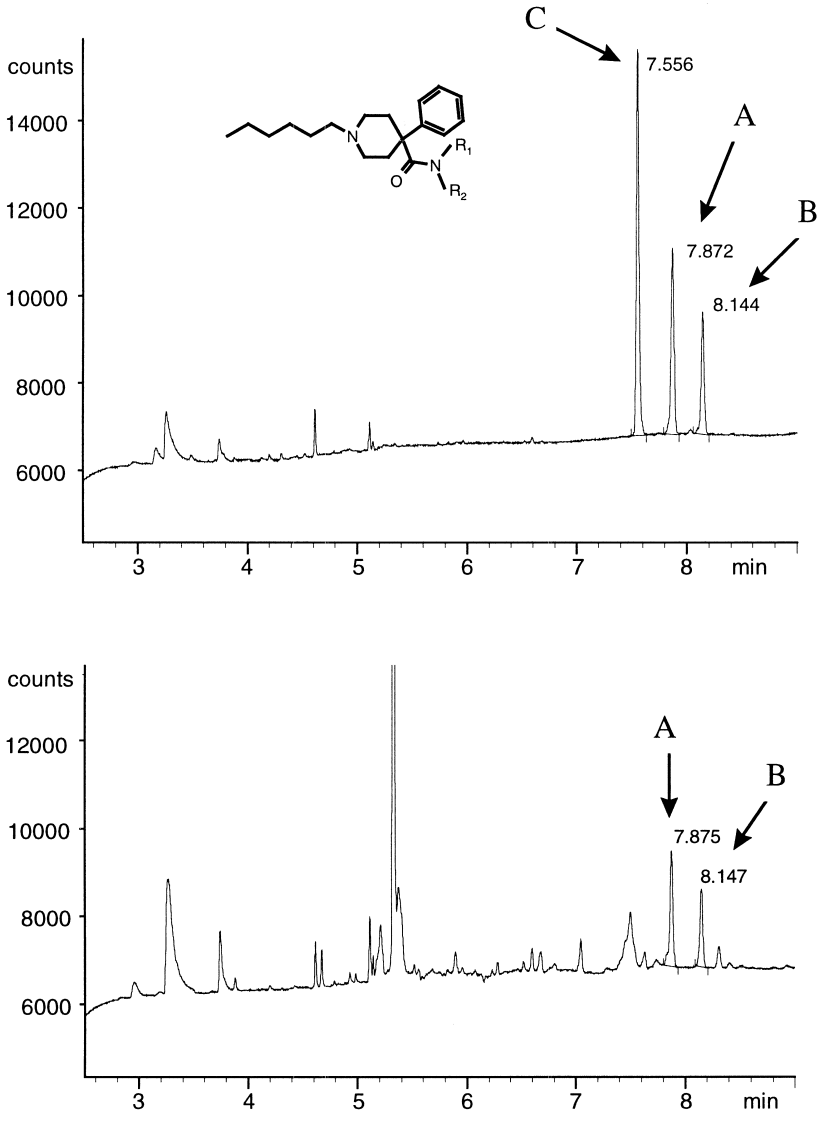

Fig. 3. Representative GC-traces of spiked human plasma samples subjected to either (top) liquid–liquid extraction followed by MIP-based solid-phase extraction or (bottom) standard liquid–liquid extraction only. Human plasma was spiked with 66.8 n*M* of sameridine and 50.2 n*M* of internal standard. The retention times are: (A) sameridine (R_1=Me, R_2=Et) 7.87 min; (B) internal standard (R_1=R_2=Et) 8.14 min; and (C) imprint species (R_1=R_2=Me) 7.56 min. Adapted from [15] with permission from the authors and publisher.

by the subsequent LC or GC, which in most instances can be made a valid assumption, the leakage appear as a separate peak and present no problem. This alternate-imprint species approach has been demonstrated for SPE of sameridine [15]. The significant peak due to leaching template molecules did not interfere neither with the analyte nor with the internal standard peaks in the subsequent GC-analysis, allowing the method to be used for accurate determinations of trace amounts of sameridine (Fig. 3). A propranolol MIP has been used for SPE of close structural analogues of propranolol [35]. However, in another study, it was claimed that bleeding of template from the MIP phase could not be detected [33]. This was attributed to heat-treatment of the polymer accompanied with excessive washes

with strong eluents. Due to the strong affinity of the MIP for the analyte, difficulties in effecting quantitative elution of the analyte have been observed in a few cases [15,28], and sometimes very harsch elution conditions are required [15].

A limitation of MIP based SPE relates to the relative lack of knowledge of using MIPs for biological samples. MIP preparation entails the use of organic solvents (see above) and, in consequence, most studies on rebinding to imprints have been conducted using organic solvents as the incubation medium. A key success factor to aqueous rebinding is the ability to balance specific binding to the imprints and non-specific binding, in aqueous media mainly of hydrophobic nature, to the polymer. For each compound, analyte as well as all other components of the sample, the observed retention is due to the sum of specific and nonspecific binding. Hence, if the non-specific element dominates, any selectivity shown by the imprints will be obscured. Problems with non-specific adsorption to the polymer can be reduced by the use of small amounts of MIP, thereby reducing the polymer surface area available for non-specific adsorption. For SPE in the column or cartridge mode, polymer amounts ranging from 500 mg down to 50 mg have been used [28–35]. In the batch mode, 5 mg were used for SPE of the organic layer after liquid–liquid extraction of 0.6 ml of plasma [15]. Binding capacity does not seem to be a problem, at least not for trace analysis, and samples with up to several μM concentrations can be extracted using 5–20 mg of MIP. In this context, an attempt to use MIPs for removal of cholesterol from an 'intestinal mimicking medium' has been presented recently [36]. A second means of reducing problems with non-specific adsorption is the use of proper washing schemes prior to elution. An interesting proposal is the use of protocols that include wash steps with organic solvent [30]. The rationale is that the selective imprint-analyte binding, which is due to hydrogen bonding etc., increases in strength and non-specific adsorption of hydrophobic nature is weakened. This leads to redistribution of non-specifically bound analyte to imprint sites and washing off of non-related structures.

MIP–SPE can be described as being analogous to immunoaffinity extraction, which relies on the highly selective interaction between antibody and antigen [37,38]. For this purpose antibodies are covalently bound to a suitable support, such as silica or controlled pore glass, which can then be packed into an SPE cartridge or a pre-column. Such immunosorbents have been used in trace analysis for selective and efficient extraction of drugs and pesticides from biological and environmental samples in both on-line and off-line modes prior to LC and GC [37–41]. Depending on the extent of cross-reactivity expressed by the antibody used, the procedure can be optimised to obtain purification of a single analyte or a group of structurally similar analytes. Immunosorbents provide simple clean-up procedures with high degree of purification leading to sensitive methods. Whereas these methods involve the use of aqueous-based environmentally friendly solvents, they are also restricted to those.

In common with all antibody-based techniques, immunoaffinity extraction relies on the availability of a suitable antibody. Since a preparation of antibodies against low-molecular weight compounds requires conjugation of the compound to a carrier protein [42], thereby changing the structural properties of the antigen exposed to the immune system of the animal, the antibodies elicited may be directed against a structure subtly different to the intended one. This may, however, be beneficial for class-specific immunosorbents. For MIP synthesis, provided the solubility of the analyte or a structural analogue (see above) is sufficient, the polymerisation mixture is prepared by simply mixing monomers and template in a suitable solvent. Furthermore, MIP preparation can be reproduced with each batch having properties close to identical to the previous one. Monoclonal antibody technology, however, offers scale up possibilities and long-term production of antibodies of consistent quality [37].

4. Binding assay

MIPs have been employed as non-biological mimics of antibodies in competitive radioligand binding assays for determinations of drug compounds (Table 1). The imprint based assay was referred to as MIA for Molecularly Imprinted sorbent Assay. In the first study of this type, theophylline and diazepam MIPs were used in the development of

radiolabelled assays for the determination of these drugs in human serum [43]. Following liquid–liquid extraction of the biological sample, the actual assays were performed using organic solvents as the incubation medium. Both drugs could be determined in clinically significant concentrations with an accuracy comparable to that obtained using a traditional immunoassay technique. Specifically, a comparison of the results obtained using a commercial immunoassay technique and the MIA competitive binding assay for the determination of theophylline in patient samples, showed good correlation between the two methods [43]. In a later study, organic solvent based incubation conditions was exploited in the development of a MIP based assay for cyclosporin [44]. Solubilisation of cyclosporin from the whole blood sample is often done by addition of an organic solvent and, in this instance, the organic layer could be transferred to an incubation tube containing cyclosporin MIP and directly assayed. Several fundamental studies, which include radiolabelled ligand binding to corticosterone and cortisol [45], methyl-α-D-glucoside [46], morphine and Leu–enkephalin [47], and propranolol [25] MIPs, have been presented. Whereas in all studies organic solvent based binding conditions were used, the two latter studies, in addition, explored the possibilities to perform selective, high-affinity binding under aqueous conditions. These studies paved the way for the development of a MIP based model assay for direct determination of propranolol in plasma samples [48].

The MIA protocol is analogous to that of a competitive immunoassay (also referred to a limited reagent assay), in which the analyte ligand and a fixed concentration of labelled ligand are incubated with a limited number of antibody binding sites (Fig. 4). The two ligands compete for binding to the same sites and, hence, the amount of labelled ligand bound the the antibodies, as well as the amount free in solution, is quantitatively related to the amount of analyte added to the incubation mixture. The label can be a radioactive isotope, an enzyme or a fluorescent structure. The unique antibody complementarity to the antigen may enable the selective binding of an antigen in a complex biological matrix such as whole blood, plasma, serum or urine. Therefore, characteristic for many immunoassays is their ability to detect minute amounts of analyte in small

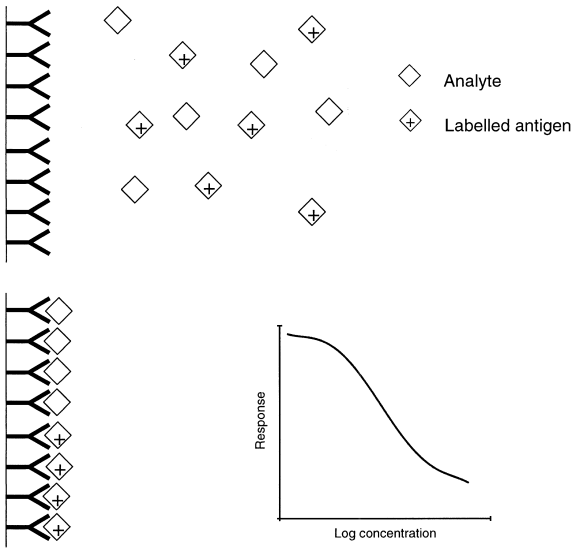

Fig. 4. General principle of a competitive immunoassay. Analyte and labelled antigen compete for binding to a limited number of binding sites, resulting in a sigmoidal dose-response curve, where the signal is inversely related to the concentration of analyte in the sample.

sample volumes without prior extensive sample pretreatment. The main driving forces for using MIPs as alternatives to antibodies in immunoassay are the high affinities and selectivities often achievable, combined with other attractive properties like high thermal, chemical and stress tolerance, and extremely long shelflife in ambient temperatures and humidities. Most MIAs have been based on the use of radiolabelled reporter ligands. However, the successful introduction of usable alternatives to radioactive tracers is crucial to a wider acceptance of MIA. This is in line with the general trend towards increased use of non-radioactive techniques of immunoassay. A 2,4-dichloro-phenoxyacetic acid (2,4-D) MIP have been synthesised [14] and used in the development of a fluorescent ligand displacement assay for this herbicide [49]. The assay used a nonrelated fluorescent coumarin derivative, although having some structural elements in common with 2,4-D, as the tracer. The fluorescent assay was claimed to be useful both in aqueous buffer and in organic solvents. Whilst in pure acetonitrile the displacement curve was near-identical to that obtained using a radiolabelled 2,4-D tracer, in aqueous buffer the displacement curve for the fluorescent

tracer was shifted to higher concentrations of analyte relative to that for the radioligand.

For MIA to become a true alternative to conventional immunoassay techniques, the assay have to be able to be performed directly on biological samples. Liquid–liquid extraction pre-treatments and organic solvent based assays are only acceptable in exceptional cases. Again, in aqueous media the problem is to reduce the non-specific adsorption while maintaining a strong specific binding of analyte to the imprints. It is advantageous to use very low concentrations of tracer and polymer, one reason being the hydrophobic polymer surface area exposed to the sample is minimized. Furthermore, the competitive assay format helps reduce the problems with non-specific binding, since the displacement events occur predominantly at the saturated high-affinity sites. Empirically, it has been found beneficial to use a polymer concentration of less than 1 mg/ml, the optimal concentration being dependent on affinity and number of the imprints. Polymer concentrations as low as 50 μg/ml have been used [25]. Other means of reducing non-specific binding is addition of an organic modifier, such as ethanol, or a detergent. The upper limit of ethanol content, however, is in part dependent on the type of sample, as protein precipitation may occur. Detergents tested and found useful include Tween 20 and Triton X-100, but others may work as well. Furthermore, in two studies [22,48], it was found that at a fixed pH, different buffers gave different levels of specific and non-specific binding. Although less pronounced, increased concentrations of both buffer and added salts reduce ionic interaction type non-specific adsorption to randomnly incorporated methacrylate residues on the polymer surface.

The poor limits of detection, in the μ*M* range, reported initially have been lowered to the n*M* range through refinements of the MIP preparation and optimisation of the rebinding conditions. Like polyclonal antibodies, MIPs contain a heterogeneous population of binding sites with a range of affinities, from high to low, for the imprint molecule. Apparent K_D values down to 10^{-9} *M* have been recorded. Further lowering of the limit of detection relies on the appropriate optimisation for each MIP–analyte system of preparation and washing protocols. Near-quantitative removal of the imprint species is crucial to a sensitive MIA, since a more thorough extraction yields a MIP where more of the high-avidity sites are free. The objective is to obtain imprints with a precise complementarity to the imprint molecule, and make these imprints available for re-binding.

5. Conclusions and future outlook

MIPs provide a combination of polymer mechanical and chemical robustness with highly selective molecular recognition comparable to biological systems. Their simple and rapid preparation have drawn interest to MIPs as alternatives to biological antibodies in immunoassay development (Table 2). MIP synthesis is particularly suited for low-molecular weight compounds, so-called haptens, whereas antibody preparation requires conjugation of the hapten to a carrier protein before injection into the animal

Table 2
Some characteristics of MIP based MIA

Benefits	Limitations
Permits assay development based both on organic solvent and aqueous buffer.	Limited experience with biosamples: further research focusing on the analytical performance is warranted.
Simple preparation of MIPs.	Poor sensitivity.
High tolerance to mechanical and thermal stress.	Other detection modes than measurement of radioactivity is warranted.
Excellent storage stability: ambient temperature and humidity is not problematic.	
Non-biological origin of binding species.	

Table 3
Some characteristics of MIP based SPE

Benefits	Limitations
Selectivity pre-determined by the template used for preparation of the MIP.	Limited experience with biosamples: further research focusing on the analytical performance is warranted.
Highly selective clean-up of sample.	Leakage of imprint molecules.
Simple preparation of MIPs.	

[42]. For biomacromolecules, antibody technology is, and will remain so in the foreseeable future, the obvious alternative. The ability to be used in both organic solvents and aqueous buffers adds flexibility to assay development, for instance in environmental analysis where poorly water soluble analytes can be assayed. To extend the applicability of MIP-based assays to bioanalysis generally, further investigations into the direct assay of biosamples are warranted. Also, the relative lack of sensitivity compared with that of conventional immunoassays, in part due to leakage of imprint molecules into the incubation medium, needs to be addressed.

Provided the problems with leakage of imprint molecules during elution can be solved or circumvented, the application of molecular imprinting in drug bioanalysis most close to practical realisation is probably that of solid-phase extraction (Table 3). Drawbacks generally associated with the use of MIPs in chromatography, such as peak broadening and tailing peaks, may be a less of a problem in SPE. The on-off type chromatography employed is less sensitive to the poor chromatographic performance of the sorbent. However, recent demonstrations of the use of imprinted polymers in CEC [16,24] and open tubular LC [50] show promise of greatly improving column efficiency of MIP-based separations. Again, better knowledge about experimental conditions for efficient use of MIPs on biofluids have to gained. Problems with non-specific adsorption is present but can be reduced by the use of small amounts of MIP and appropriate washing schemes prior to elution. Already, several groups have applied MIP-based solid-phase extraction to biological samples and MIP–SPE may well be established generally in the not-to-distant future.

References

[1] G. Wulff, Angew. Chem. Int. Ed. Engl. 34 (1995) 1812–1832.
[2] K. Mosbach, O. Ramström, Bio/Technology 14 (1996) 163–170.
[3] R.J. Ansell, O. Ramström, K. Mosbach, Clin. Chem. 42 (1996) 1506–1512.
[4] J. Steinke, D.C. Sherrington, I.R. Dunkin, Adv. Polym. Sci. 123 (1995) 81–125.
[5] S. Mallik, S.D. Plunkett, P.K. Dhal, R.D. Johnson, D. Pack, D. Schnek, F.H. Arnold, New J. Chem. 18 (1994) 299–304.
[6] M. Kempe, K. Mosbach, J. Chromatogr. A 694 (1995) 3–13.
[7] O. Ramström, R.J. Ansell, Chirality 10 (1998) 195–209.
[8] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 817 (1998) 5–13.
[9] J. Olsen, P. Martin, I.D. Wilson, Anal. Commun. 35 (1998) 13H–14H.
[10] L.I. Andersson, K. Mosbach, in: C.P. Price, D.J. Newman (Eds.), Principles and Practice of Immunoassay, 2nd Ed, Macmillan Reference Ltd, London, UK, 1997, pp. 139–152.
[11] A.G. Mayes, K. Mosbach, Trends Anal. Chem. 16 (1997) 321–332.
[12] B. Sellergren, in: R.A. Bartsch, M. Maeda (Eds.), Molecular and Ionic Recognition with Imprinted Polymers, ACS Symposium Series, Vol. 703, 1998, pp. 49–80.
[13] T. Takeuchi, J. Matsui, Acta Polymer. 47 (1996) 471–480.
[14] K. Haupt, A. Dzgoev, K. Mosbach, Anal. Chem. 70 (1998) 628–631.
[15] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatographia 46 (1997) 57–62.
[16] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Chromatographia 43 (1996) 585–591.
[17] S. Vidyasankar, M. Ru, F.H. Arnold, J. Chromatogr. A 775 (1997) 51–63.
[18] M. Glad, P. Reinholdsson, K. Mosbach, React. Polym. 25 (1995) 47–54.
[19] B. Sellergren, J. Chromatogr. A 673 (1994) 133–141.
[20] K. Hosoya, K. Yoshizako, Y. Shirasu, K. Kimata, T. Araki, N. Tanaka, J. Haginaka, J. Chromatrogr. A 728 (1996) 139–147.
[21] A.G. Mayes, K. Mosbach, Anal. Chem. 68 (1996) 3769–3774.

[22] R.J. Ansell, K. Mosbach, Analyst 123 (1998) 1611–1616.
[23] J. Matsui, T. Kato, T. Takeuchi, M. Suzuki, K. Yokoyama, E. Tamiya, I. Karube, Anal. Chem. 65 (1993) 2223–2224.
[24] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179–1183.
[25] L.I. Andersson, Anal. Chem. 68 (1996) 111–117.
[26] B. Sellergren, Anal. Chem. 66 (1994) 1578–1582.
[27] W.M. Mullett, E.P.C. Lai, Anal. Chem. 70 (1998) 3636–3641.
[28] P. Martin, I.D. Wilson, D.E. Morgan, G.R. Jones, K. Jones, Anal. Commun. 34 (1997) 45–47.
[29] M.T. Muldoon, L.H. Stanker, Anal. Chem. 69 (1997) 803–808.
[30] J. Matsui, M. Okada, M. Tsuruoka, T. Takeuchi, Anal. Commun. 34 (1997) 85–87.
[31] B.A. Rashid, R.J. Briggs, J.N. Hay, D. Stevenson, Anal. Comm. 34 (1997) 303–305.
[32] M. Walshe, J. Howarth, M.T. Kelly, R. O'Kennedy, M.R. Smyth, J. Pharm. Biomed. Anal. 16 (1997) 319–325.
[33] Å. Zander, P. Findlay, T. Renner, B. Sellergren, A. Swietlow, Anal. Chem. 70 (1998) 3304–3314.
[34] R.F. Venn, R.J. Goody, Drug Development Assay Approaches Including Molecular Imprinting and Biomarkers, in: E. Reid, H.M. Hill, I.D. Wilson (Eds.), Methodological Surveys in Bioanalysis of Drugs, Vol. 25, 1998, pp. 13–20.
[35] P. Martin, I.D. Wilson, G.R. Jones, K. Jones, Drug Development Assay Approaches Including Molecular Imprinting and Biomarkers, in: E. Reid, H.M. Hill, I.D. Wilson (Eds.), Methodological Surveys in Bioanalysis of Drugs, Vol. 25, 1998, pp. 21–27.
[36] B. Sellergren, J. Wieschemeyer, K.-S. Boos, D. Seidel, Chem. Mater. 10 (1998) 4037–4046.
[37] V. Pichon, M. Bouzige, C. Miege, M.C. Hennion, Trends Anal. Chem. 18 (1999) 219–235.
[38] D. Stevenson, Trends Anal. Chem. 18 (1999) 154–158.
[39] J. Dallüge, T. Hankemeier, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 830 (1999) 377–386.
[40] J. Cai, J. Hennion, Anal. Chem. 68 (1996) 72–78.
[41] V. Pichon, M. Bouzige, C. Miege, M.C. Hennion, Anal. Chim. Acta 376 (1998) 21–35.
[42] B. Law (Ed.), Immunoassay, A Practical Guide, Taylor and Francis Ltd, London, 1996.
[43] G. Vlatakis, L.I. Andersson, R. Müller, K. Mosbach, Nature 361 (1993) 645–647.
[44] M. Senholdt, M. Siemann, K. Mosbach, L.I. Andersson, Anal. Lett. 30 (1997) 1809–1821.
[45] O. Ramström, L. Ye, K. Mosbach, Chem. Biol. 3 (1996) 471–477.
[46] A.G. Mayes, L.I. Andersson, K. Mosbach, Anal. Biochem. 222 (1994) 483–488.
[47] L.I. Andersson, R. Müller, G. Vlatakis, K. Mosbach, Proc. Natl. Acad. Sci. USA 92 (1995) 4788–4792.
[48] H. Bengtsson, U. Roos, L.I. Andersson, Anal. Commun. 34 (1997) 233–235.
[49] K. Haupt, A.G. Mayes, K. Mosbach, Anal. Chem. 70 (1998) 3936–3939.
[50] Z.J. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055–2060.
[51] R. Levi, S. McNiven, S.A. Piletsky, S.-H. Cheong, K. Yano, I. Karube, Anal. Chem. 69 (1997) 2017–2021.
[52] G. Chen, Z. Guan, C.-T. Chen, L. Fu, V. Sundaresan, F.H. Arnold, Nature/Biotechnology 15 (1997) 354–357.