Molecularly imprinted polymers (MIPs): sensing, an explosive new opportunity?

Adam McCluskey,*[a] Clovia I. Holdsworth[a] and Michael C. Bowyer[b]

Received 7th June 2007, Accepted 21st August 2007
First published as an Advance Article on the web 10th September 2007
DOI: 10.1039/b708660a

Our group is currently developing in-field detection systems alongside the Australian Federal Police Forensic Services utilising molecularly imprinted polymers as the recognition elements. This review looks at MIP synthesis and our perceptions of future directions from an Australian and forensic perspective.

Introduction

Illicit drugs and explosives detection

Traditional detection of narcotics and explosives (Fig. 1) vapors relies on the olfactory system of dogs highly trained for such a purpose. Canine detection, however, suffers from drawbacks such as the expense associated with the handling and training of dogs, their narrow attention span and the limited amount of reliable scientific information obtained.[1,2] While some instrumental trace level detection methods are commercially available for use (gas chromatography with chemiluminescence, electron capture or surface acoustic waves detectors and ion mobility spectrometers, biosensors), and are continually improving, they generally suffer from selectivity and sensitivity problems, limited mobility/tracking ability, a high level of intrusiveness, high rate of false positive results, short shelf-lives (*e.g.* immunosensors) and high cost.[1,3,4] These existing technologies also often require expert training in their use and data analysis, and may be too complex and specialised to allow a lay jury to fully comprehend the significance of the data generated.

The development of simple, safe, non-intrusive, rapid, portable, direct, cost-effective sensing equipment that is more sensitive and selective for detecting traces of concealed explosives and narcotics will greatly enhance the 'dual-tasking' capability of law enforcers in controlling security (*i.e.* protection against threat of terrorism) and preventing drug trafficking at entrance portals and other domestic situations such as in buses, trains, buildings. Vapors emitted by drugs/explosives can still be detected even after they have been removed from the site of production and/or storage, or from one's body or clothing, hence the sensor can potentially be used for passenger screening, checked and carry-on baggage screening and, on a larger scale, containers, trucks and cargo.

In the presence of a chemical vapor, a sensing material must be able to specifically recognise the vapor and elicit a measurable response (*i.e.* change in the material's properties) to the presence of the vapor (signal transduction). Most transduction methods are already well developed but the design of a suitable chemical recognition element remains a challenge to date. Techniques that have been widely used to impart recognition to sensing devices for trace levels of explosives and narcotics are based on the interactions of the substrate with biological molecules (such as host–guest and antigen–antibody interactions), which are unstable and prone to saturation and decomposition.[4,5] Over the past 5 years, we have developed considerable expertise in the field of molecular imprinting and have applied the technology in the extraction, detection and measurement of flavour contaminants in wine and some illicit drugs.[6–11] Specific recognition sites are created, based on the interaction of a template molecule (also the target) with functional monomer(s), around a rigid, robust synthetic polymer matrix, that are retained upon removal of the template such that the target can be recognized.

Molecularly imprinted polymers (MIPs)

Simply put, molecularly imprinted polymers (MIPs) are speciality polymers generated *via* the interaction of functional monomers, a target molecule (template) and a cross-linking agent. With thousands to millions of highly specific template binding pockets, MIPs, like their biological receptor counterparts, possess the ability to recognize and bind specific target molecules.[12] Unlike biological receptors, MIPs are incredibly robust, insoluble in most media and in most cases lack the natural homogeneity of active sites associated with biological receptors. The population of binding sites in MIPs, especially those imprinted using non-covalent monomer–template interactions, is heterogeneous because of the influence of the equilibria that govern the monomer–template complex formation and the dynamic of the growing polymer chains prior to copolymerization.[13]

The nature and distribution of binding sites are influenced by the method of MIP synthesis (see below), of which there are ostensibly two approaches; covalent and non-covalent, which are shown schematically in Fig. 2.[13] With the former, the functional monomer is covalently attached to the target molecule *via* a removable covalent linkage and upon addition of a suitable cross-linking agent (see below) and an initiator, the MIP is generated *via* a variety of polymerization approaches.[13] Post-polymerization template removal requires destruction of the covalent linker generating a cavity (binding site) that complements

[a]*Centre for Organic Electronics, Chemistry Building, School of Environment and Life Sciences, The University of Newcastle, Callaghan, NSW 2308287, Australia. E-mail: Adam.McCluskey@newcastle.edu.au; Fax: +612 49215472; Tel: +612 9215481*
[b]*Discipline of Applied Sciences, Central Coast Campus, The University of Newcastle, Ourimbah, NSW 2258, Australia. E-mail: Michael.Bowyer@Newcastle.edu.au; Fax: +61 243 484145; Tel: +61 243 484119*

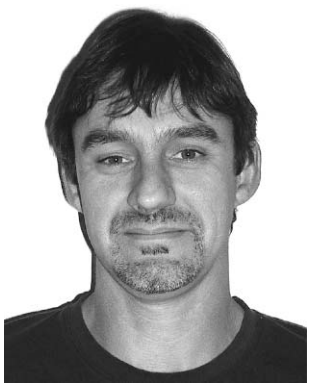
Adam McCluskey obtained his BSc(Hons) (1985) and PhD (1988) from Strathclyde University, Glasgow under the supervision of Dr Ian Dunkin. He joined Professor Curt Wentrup's reactive intermediate group at the University of Queensland at the end of 1988 and later moved to Professor Ronald Quinn's medicinal chemistry group at Griffith University. It was here that he developed an interest in medicinal chemistry before taking up a lecturing position at the University of Newcastle at the end of 1995. He is now Associate Professor and Head of the discipline of chemistry, and co-director of the Centre for Organic Electronics. Current research projects include the design and synthesis of novel inhibitors of protein phosphatases (1 and 2A) as novel anticancer agents; of dynamin GTPases as potential antiepileptic agents; and a major interest in the use of molecularly imprinted polymers as sensing devices.

Clovia Holdsworth has been involved in molecular imprinting research at the University of Newcastle, Australia, since 2002, first as a research associate, and later (2006) as an academic appointment. After obtaining her PhD from Griffith University, Australia in 1996, she returned to the Philippines and worked as an academic member of staff at De La Salle University-Manila until 2001. Aside from molecular imprinting, her research interests include polymer functionalisation and synthesis by controlled radical polymerisation.

Mike Bowyer obtained his B.Sc. in chemistry at the University of New South Wales (Australia). He then pursued doctoral studies (1988–92) at the same institution under Professor David St. C. Black working on the preparation of new indole-containing macrocyclic ligands. Upon graduation he moved to Boston College (USA) where he worked with Professor Ross Kelly on a range of synthetic projects including the preparation of the first working example of an operational 'molecular brake'. He returned to Australia in 1995 to take up a lectureship in chemistry at the University of Newcastle. He is currently a senior lecturer in the School of Environmental & Life Sciences and is the Head of the Discipline of Applied Sciences at the University of Newcastle's Ourimbah campus. Current research interests span a range of areas of chemistry and include the preparation of new MIP-based sensory devices, the catalytic properties of new chiral ligand systems and investigations into the role of nitric oxide in extending the postharvest life of selected fruit and vegetables.

Adam McCluskey

Clovia I. Holdsworth



Michael C. Bowyer

the size, shape and electronic properties of the template. With the non-covalent, self-assembly approach, the template, functional monomer and cross-linking agents are equilibrated to generate a pre-polymerisation cluster utilizing hydrogen bond interactions, electrostatic attraction and associated weak interactions. The mix is then polymerized to generate cavities and the template is subsequently removed *via* exhaustive extraction. Methods of template extraction are not covered herein and the reader is directed to an excellent work in this area by Sellergren *et al*.[14]

MIP technology has been applied in a myriad areas including, but not limited to, separation and isolation,[12] antibody and receptor mimics,[15,16] and biosensor style devices.[15] Their shelf stability, robustness and reusability mean that they are highly usable and flexible. The variety of molecules 'imprinted' is impressive in both breadth of template and also diversity, highlighting the utility of MIPs. The general MIP area has been extensively reviewed over the past decade,[12,15–17] and in this emerging area article we will focus on new developments, in particular those that we anticipate will have an impact on the forensic community as this is our primary interest.

Synthetic approaches to MIPs

MIPs are required to be highly flexible polymers to facilitate a fast equilibrium between release and uptake of the template; however, they also should be rigid enough to maintain the integrity of the original cavity after covalent linker cleavage or template extraction, as well as possessing high thermal and mechanical stability.[17] These two properties are somewhat contradictory, hence the optimisation of MIP structures and properties has the propensity to become quite complex. Previously, a pseudo-haphazard or shotgun approach, had developed into a highly refined rationale process. The application of molecular modelling and NMR titration (MM-NMR) approaches dominates and vastly simplifies the design of MIP generation. It is the physical process of MIP synthesis that imparts key properties associated with the physical characteristics of MIPs, with control of particle size, porosity, swelling capability, *etc.*, all determined by the appropriate methodology choice. Synthetic approaches to MIPs fall into four main categories.[13]

Bulk—a simple, rapid approach with low solvent (porogen) requirements and an associated low template concentration during

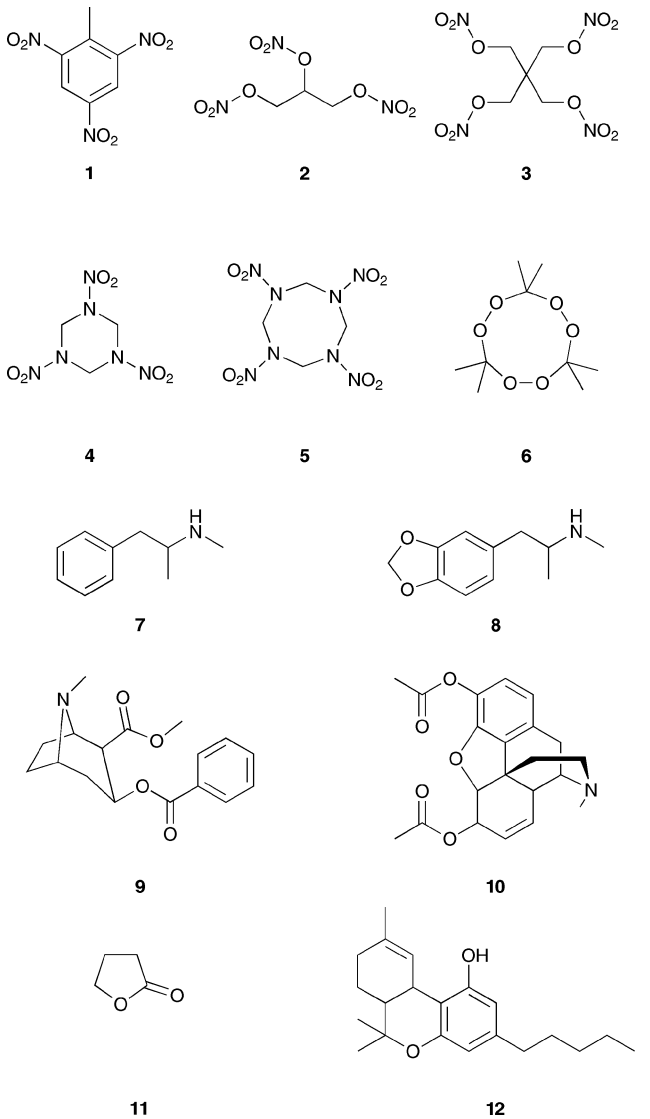


Fig. 1 Chemical structures of selected illicit drugs and explosives. (**1**) Trinitrotoluene (TNT); (**2**) nitroglycerine; (**3**) pentaerythritol tetranitrate (PETN); (**4**) cyclotrimethylene triamine (RDX); (**5**) cyclotetramethylene tetranitramine (HMX); (**6**) triacetone triperoxide (TATP); (**7**) methamphetamine; (**8**) methylenedioxmethamphetamine (MDMA, ecstasy); (**9**) cocaine; (**10**) heroin; (**11**) γ-butyrolactone; and (**12**) Δ9-tetrahydrocannabinol.

the polymerisation process. Typically, a free radical approach with no attempt to control the particle size or polymer morphology. The major disadvantage is the synthesis of polymer monoliths that require considerable post-synthesis manipulation (grinding and sieving) with the potential to remove valuable cavities, and ultimately results in lower yields of usable MIP.[13]

Precipitation/emulsion—a simple but more time consuming approach utilising higher porogen volumes in which the MIP grows slowly until it precipitates from the reaction solution. This approach typically affords MIPs with higher selectivities, higher yields of usable material and more defined and controllable particle size. The major disadvantage is the need for increased porogen volume, an increased amount of template and longer reaction times.[13] However, we have recently shown that selected examples of room temperature ionic liquids (RTILs) facilitate the rapid development of well-defined particles from low porogen volumes and reaction times. Thus RTILs may offset some, if not all, the disadvantages associated with this approach.[10]

Electrodeposition—is directly related to the generation of MIP films. Electrodeposition yields high surface area, uniform coverage of the chosen surface and overcomes problems such as back pressure build-up encountered in chromatography through the use of smaller particles (<10 μm) generated by bulk or precipitation methods. The use of conductive species such as polypyrroles makes electrodeposition a particularly attractive proposition for biosensing applications.[18]

Molecularly imprinted polymer films (MIP$_f$s)—a post-polymerisation imprinting technique involving the rapid solidification of a polymer from its solution after imprinting.[15] MIP$_f$s do not require a cross-linker, but rather a co-monomer to impart rigidity and display fairly rough morphologies, which have been reported to provide some of the best binding results. MIP$_f$s allow direct and rapid target detection using simple and portable instrumentation such as a FTIR.[19]

A typical MIP recipe comprises the following four ingredients: (*1*) the template (Fig. 1); (*2*) the functional monomer(s) (Fig. 3); (*3*) the cross-linking agent (Fig. 4), and (*4*) the porogen (polymerisation solvent); in principle a simple cocktail, but in reality a myriad possible combinations may be generated with a variety of possible outcomes in terms of MIP specificity and efficiency. The MM-NMR approach has introduced a degree of rationale synthesis to the field. However, even with application of a MM-NMR strategy, there are still multiple issues that require evaluation with the interplay of (*1*)–(*4*) pivotal in determining the outcome of the MIP synthesis.[13]

Effect of cross-linker

Conventional MIP recipes have the cross-linking agent as the major component of the pre-polymerisation mixture with 1 : 4 : 20 (template–functional monomer–cross-linker) a typical arrangement. Importantly, selectivity rises significantly if the cross-linker comprises >50% of the pre-polymerisation mixture. Traditional cross-linkers in this field include EGDMA (**CL5**, ethylene glycoldimethylacrylate), TRIM (**CL22**, trimethylolpropane trimethacrylate) and divinylbenzene (**CL4**), with the latter generating non-hydrolysable linkages, but with reduced selectivity.[20] Recent work, by Spivak *et al.* in particular, has explored the development of novel bismethacryloylethanolamine (so-called NOBE) cross-linking agents. Capable of hydrogen bonding to the template, NOBE cross-linkers eliminate the need for a functional monomer to be included in the pre-polymer mix.[21,22]

The binding site interaction between the template and the functional groups in the formed polymer must also be considered, as the template makes defined interactions with both the functional monomer and the cross-linker at the pre-polymerisation stage of MIP synthesis. It has recently been shown that the template can have a profound effect on MIP morphology, and consequently impacts on sensitivity and selectivity.[10,11] MIP selectivity depends both on the orientation of the functional groups inside the cavities and the shape of the cavities, with the former being the dominant factor.[7,8,23] Greater selectivity is achieved if the template binds in a ≥2 point binding mode, *i.e.* the higher the number of

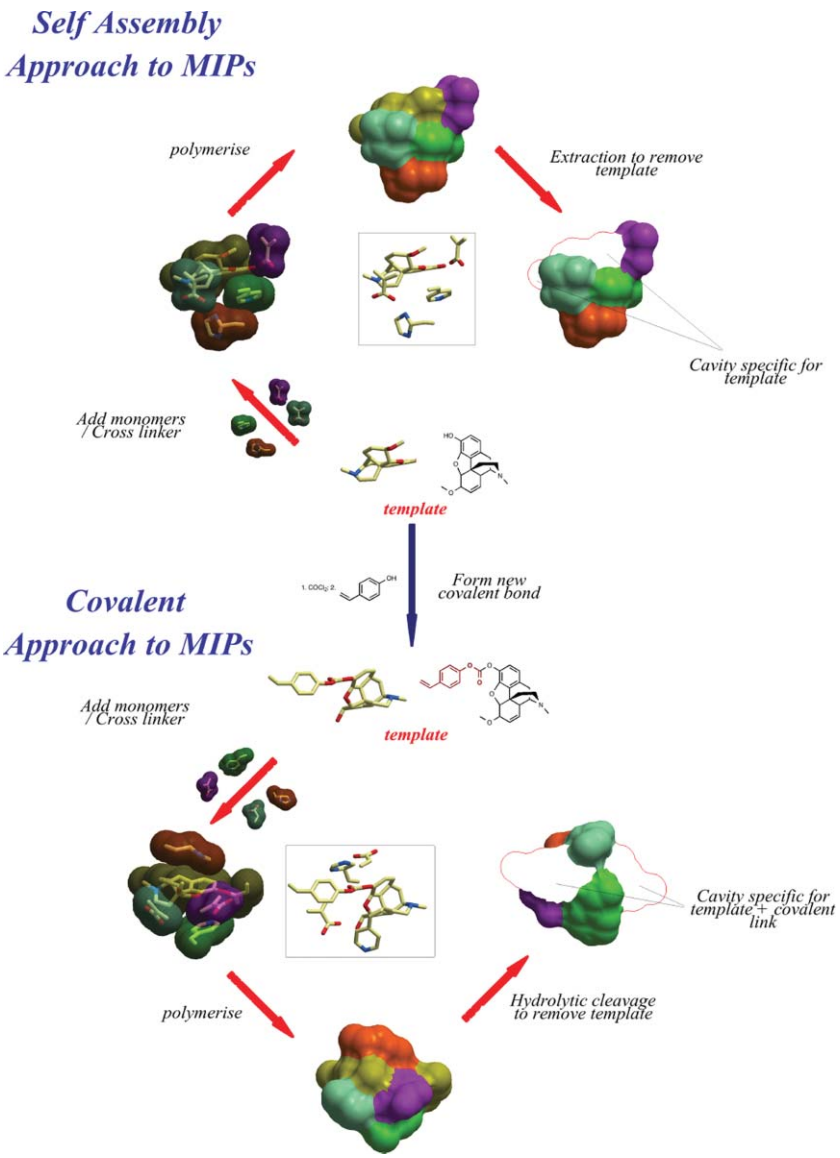


Fig. 2 Self-assembly and covalent approaches to the synthesis of MIPs.

binding interactions within a cavity that are complementary to the template, the greater the observed selectivity. With single point binding, association constants are low (~10), but rise rapidly on accessing additional binding points (>100). Removal of the template from the cavity results in solvation and the cavity swells. This facilitates easy access for the template to re-bind, at which point the cavity shrinks, giving an induced fit.[12] This does not hold in all instances. Porogenic RTILs can give rise to MIPs with no observable swelling, but with high sensitivity and selectivity.[10,11]

Computational approaches to MIP design

Recent MIP development has been primarily due to the application of computational approaches. The general acceptance of the MM-NMR approach allows for a logical filtering of the number of systems physically synthesised and evaluated. Numerous groups have their own approach, from the reductionistic low level AM1-NMR approach favoured by our laboratory to the more complex high levels of theory calculations that deliver more accurate complex geometries, but not necessarily more selective MIPs.[6–11,13] Nevertheless, all such approaches have their place in the MIP community, and have allowed an explosion in the synthesis of specific MIPs.[24] However, we are aware of no approach allowing prediction of polymer properties, or, of arguably greater interest, the impact of the template on polymer morphology. We believe that resolution of this will have an immense impact on this field, especially in the design of propriety materials for sensing applications. This and the impact of designer RTILs have the potential to change the MIP landscape.

We are now in the position of being able to conduct a series of *in silico* studies to determine the best possible interaction between the template and the functional monomer. Given the advent of computational approaches, it is somewhat surprising that few in the MIP community, our group included, have embraced the full potential of the computational approach by *de novo* design of novel functional monomers.[13] While the use of commercially available

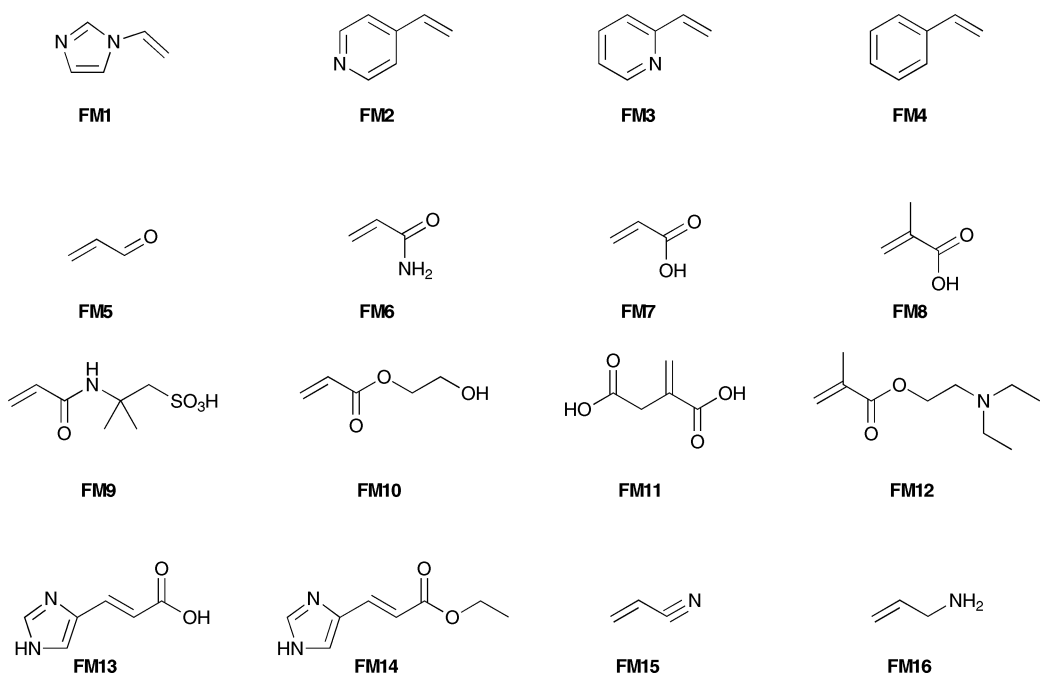


Fig. 3 Some common functional monomer units used in MIP synthesis. **FM1** 1-vinylimidazole; **FM2** 4-vinylpyridine; **FM3** 2-vinylpyridine; **FM4** styrene; **FM5** acrolein; **FM6** acrylamide; **FM7** acrylic acid; **FM8** methacrylic acid; **FM9** acrylamido-2-methyl-1-propanesulfonic acid; **FM10** 2-hydroxyethylmethacrylate; **FM11** itaconic acid; **FM12** *N*,*N*-diethylaminoethyl methacrylate; **FM13** urocanic acid; **FM14** urocanic acid ethyl ester; **FM15** acrylonitrile; **FM16** allylamine.

monomers is normally adequate, sometimes it is seen as advantageous to prepare monomers that specifically target structural features of the template. In particular, multiple hydrogen bonding regimes can enhance association constants and hopefully lead to more selective recognition sites. Tanabe *et al.*[25] prepared 2,6-bis-acrylamidopyridine as a complement to the cyclic imide functionality of the barbiturates. The related 2-(meth)acrylamidopyridines have also been prepared as complements to carboxylic acids.[21,26] Spivak and Shea also prepared a monomer based on the nucleotide base adenine as a complement to carboxylic acids.[21] In a recent example, Hall *et al.* designed and synthesised a polymerizable bis-urea monomer.[27] One could argue that it is only Spivak's group that has shown any significant progress in this area with the development of OMNI MIPs. Spivak's development of NOBE based OMNI MIPs, where the role of functional monomer and cross-linker are integrated (see *the way forward*), represents one of the few attempts to take MIP design in radically new directions.[21,22]

Synthesis of MIP films

The combination of MIPs and FTIR spectrometry might allow analytical problems to be addressed where the selectivity of the MIP alone is not sufficient, *e.g.*, when samples with complex matrices are to be investigated or when structurally very similar analytes are present in the sample.[19]

MIP films are essentially two-dimensional MIPs, holding considerable promise in forensic applications,[24,28] synthesised from specifically designed monomer–solvent combinations,[29] or by imprinting protocols based on pre-formed polymer chains, *e.g.* poly(methacrylic acid-*co*-ethylene glycol dimethylacrylate), poly(methacrylic acid-*co*-acrylonitrile).[30] Interestingly, there have been recent reports of MIP films exhibiting recognition properties under aqueous conditions, an area where MIPs are deficient.[30] The composition of the co-polymer is a compromise between imprinting (methacrylic acid) and an ability to maintain a rigid structure (ethylene glycol dimethylacrylate and acrylonitrile), but a significant advantage is the preparation of reproducible thin films (assumes solvents are compatible with spin casting approaches). Alternatively, it is possible to develop quality films *via* immersion in a non-solvent (a coagulation approach).[15,31] We have utilized this approach in the synthesis of MIP films exhibiting selectivity for ephedrine, heroin, cocaine and other substances of interest to the forensic community.[19] Selectivity is easily determined by FTIR.[15]

MIP films can be modified post imprinting. Additional binding sites can be introduced by covalent imprinting using disulfide linkages and subsequent cleavage and oxidation to a non-covalent sulfoxide/sulfonic acid recognition groups.[13] In this manner, not only molecular recognition but also catalytic and signaling functionality can be easily introduced into the synthetic polymers, using appropriately designed template molecules. It is a small step to envisage post-imprinting modifications incorporating improved functionality in aqueous environments and subsequent integration of reporter devices either *via* the generation of a colour change or an electrical signal allowing *in situ* signal generation. However, we are not yet aware of such reports. Importantly, the thin film facilitates mass transfer, critical to rapid signal generation and analysis.

To permit real-time analysis, it is imperative that the analyte can rapidly diffuse to the MIP cavity; given the two-dimensional nature of MIP films, this is an area in which they excel. Films have been allied with surface plasmon resonance as an alternative approach to signal generation with the incorporation of Au,

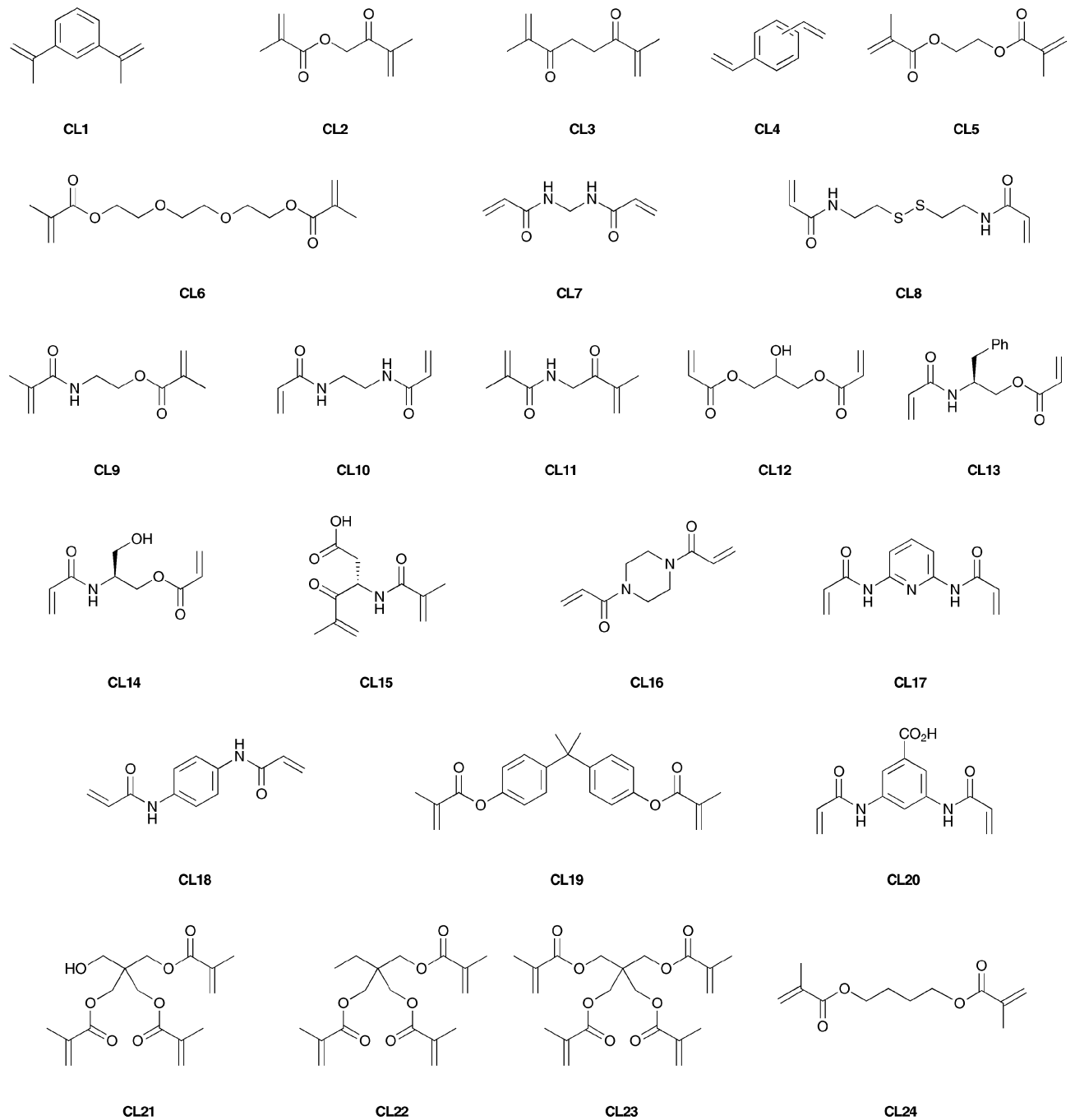


Fig. 4 A variety of cross-linking agents used in MIP synthesis. **CL1** 1,3-diisopropenylbenzene; **CL2** 2-methacrylic acid 3-methyl-2-oxobut-3-enyl ester; **CL3** 2,7-dimethylocta-1,7-diene-3,6-dione; **CL4** (1,2; 1,3 and 1,4)-divinylbenzenes; **CL5** ethylene glycol dimethylacrylate (EGDMA); **CL6** tri(ethyleneglycol) dimethylacrylate; **CL7** *N*,*N*′-methylenebisacrylamide; **CL8** *N*,*N*′-bis(acryloyl)cystamine; **CL9** 2-(methacryloylamino)ethyl 2-methyl acrylate; **CL10** *N*,*N*′-methylenebisacrylamide; **CL11** 2-methyl-*N*-(3-methyl-2-oxo-3-butenyl)-2-propenamide ; **CL12** glycerol diacetate; **CL13** *N*,*O*-bis-acryloyl-L-phenylalaninol; **CL14** *N*,*O*-bis-methacryloyl-L-serine; **CL15** *N*,*α*-bis-methacryloyl-L-aspartic acid; **CL16** diacryloyl piperazine; **CL17** 2,6-bis-acrylamidopyridine; **CL18** 1,4-phenylene-bis-acrylamide; **CL19** bisphenol A dimethylacrylate; **CL20** 3,5-bis-acrylamidobenzoic acid; **CL21** pentaerythritol triacrylate (PETRA); **CL22** trimethylolpropane trimethacrylate (TRIM); **CL23** pentaerythritol tetraacrylate; **CL24** 1,4-butanediol dimethylacrylate.

Ag or Pt nanoparticles.[32] The intensity and position of the surface plasmon resonance absorption bands are characteristic of the types of metal, nanoparticle size, shape and distribution as well as being highly sensitive to changes in local environments. Sensitivity can be further enhanced by attaching gold nanoparticles onto the terminal end of the attached molecules.[33] Immobilised gold nanoparticles have the potential to become a novel chemosensor, promoting a specific chemical reaction and converting the chemical event to useful signals such as electronic or spectroscopic signals. Au–nanoparticle–MIP composites have

demonstrated selective colorimetric detection of templates. Ideally, Au nanoparticles should be ~5–20 nm in diameter with a narrow size distribution allowing for a sharp response. In these circumstances, sensors can exhibit rapid response (6–10 min) and low detection limits ~5 nM.[34]

Miscellaneous methods

Also noteworthy, and of relevance for sensor technology, is the templating of self-assembled monolayers (SAM),[12,15,28] which can be regarded as two-dimensional molecular imprinting. For example, when a SAM of alkane thiols is formed on a gold surface, the presence of a foreign molecule results in a hole in the SAM, which is complementary in size with the guest molecule, thus forming a binding site. The main drawback of this type of imprinting is the lack of stability of a non-cross-linked film, with a high probability of recognition site collapse on template removal.

The combination of multiple synthesis approaches and the MM-NMR design stratagem allow for a rapid response to emerging targets and threats, *e.g.* new illicit drugs and chemical warfare agents.[35] The approaches thus far do not account for device integration.

Approaches for integration of MIPs into signalling devices

General detection principles

The first reported *integrated* MIP-based sensor was a capacitance sensor comprising a field effect capacitor containing a thin phenylalanine anilide-imprinted polymer membrane.[12,15] Other early MIP-based sensing developments include ellipsometric, changes in the electrical streaming potential over an HPLC column packed with a MIP, or permeability studies of imprinted polymer membranes, which have been reviewed previously and are beyond the scope of this article, with our focus on the development of simple devices with forensic applications.[12,13,15]

Generally, electrical devices offer potential advantages of low cost, small size, the possibility of achieving low detection limits and ease of automation. As with any sensor, the integration of the recognition element with the transduction element is crucial. Table 1 summarises the methodologies that have been used in the development of MIP-based electrochemical sensors to detect materials that may be, or are, of forensic interest.[18]

Voltammetric/electrochemical MIP sensors

Voltammetric/electrochemical MIP sensors come in various configurations that offer the possibility of controlling electrode characteristics such as hydrophobic/hydrophilic character, permeability and film thickness, all of which are essential for obtaining (and maintaining) good sensor performance.

A key technical challenge in the development of widespread cheap MIP-based sensors is in achieving an appropriate interface between the recognition element (MIP) and the reporter circuit (transducer). In most cases, the MIP has to be brought into close contact with the transducer surface. An obvious approach would be to generate the MIP *in situ* at or on the transducer surface or actually incorporate the MIP into the surface itself (a self assembled monolayer).[12,15,18] *In situ* approaches exist with electropolymerisation on conducting surfaces such as gold, but this demands specialized polymer recipes. These are less well understood than the traditional MIP recipes comprising acrylic and vinylic monomers.

At their simplest, films have been generated *via* electropolymerisation at the electrode surface; casting of polymeric membranes by drop casting of a solution of pre-formed polymer (*e.g.* polyphosphazene) and template in a low boiling point solvent at the electrode surface;[18] preparation of a composite membrane containing conductive materials (graphite or carbon black), typically in an acrylate based MIP of small particle size and PVC as a binder; and, by *in situ* polymerisation of a thin layer of acrylate MIP deposited on the electrode surface by spin coating. Film thickness can be varied by the addition of varying concentrations of the monomer added or the concentration of the pre-formed polymer solution. The porosity and permeability of the film can be controlled by adjusting the concentration of polymer and template in the casting solution. Similar films have been used ~25 times with no loss of signal or sensitivity.[18] A great advantage of these procedures is that the preparation of modified electrodes, for example, from a polyphosphazene solution is very simple and similar design has been used to develop a voltammetric sensor by means of single-use screen printed electrodes, although fraught with poor response times. Overoxidised polypyrrole grown on glassy carbon electrodes provides excellent outcomes and electropolymerisation of polyprotoporphyrin yields nitrobenzene sensors with obvious forensic applications.[18] Capacitive detection has been employed in conjunction with imprinted electropolymerized polyphenol layers on gold electrodes.

Future directions—the way forward

The challenge for the next generation MIPs will be in the delivery of robust materials with low detection limits and rapid response rates. The ideal MIP will be low-cost and easily incorporated into a signalling device and, with the current focus on intelligence driven policing and the requirement to protect our borders, these devices will also report remotely 'back to base' through incorporation into the existing telecommunications grid. Classical MIP formulations utilising acrylate and vinyl monomers currently cope with almost all MIP requirements, and are still undergoing development albeit slowly, other materials are gaining favour. Polyphenols, polyurethanes, *etc.*, being better suited to a given application or being easier to synthesise in the desired format are of increased prominance in the MIP literature.[17] However, improved monomer–template interactions is only part of the equation for the next generation of 'smart MIPs'. In this section, we attempt to highlight some of the budding innovations that we feel will have a significant impact on the rapid development of smart MIPs, especially with regard to signalling and forensic applications. The big question is how do we achieve this?

Optical sensing MIPs

The ideal MIP is one that will specifically and quantitatively 'self-indicate' receptor site occupancy, thereby indicating the concentration of analyte present—a 'smart MIP' in a manner conducive to device integration. To this end, there has been considerable interest in introducing fluorescent or luminescent

Table 1 Electrochemical MIP recognition approaches of potential forensic interest. (Adapted from ref. 18.)

Monomers	Transduction/substrate	Forensic interest
MAA–EGDMA[a]	Amperometry/Pt	Used for the detection of morphine—adaptable to the detection of opiates in general. Detection range: 0.1–10 mg ml^{-1}.
4-VP–EGDMA	DPV/screen printed electrodes	Screen printing approach to the generation of electrode materials—rapid and cost effective method of sensor generation. Generates thin reproducible films (1–1000 μM).
Styrene–DVB (19 : 1)	CV, DPV, screen printed	Single use sensors with no potential of target carry over. Detection range: 0.1–1 mM.
MAA–EDGMA	ISFET/silicon wafer	ISFET allows for signal amplification. Incorporation of corresponding NIP would potentially allow automatic subtraction of non-specific binding.
DMF MAA + DEAEM + OUA (DMF, chloroform)	Conductometric	Trivial synthesis–monomer mixture poured between two quartz plates, MIP generated *in situ* and yields a thin (60–120 μM), flexible and stable material. Excellent reproducibility RSD < 5% and rapid signal response (6–10 min), approaching real time. Excellent detection limits: 5–100 nM.
MAA–EDMA	CV/ITO	Used in the detection of theophyline, possible adaptations to illicit drugs. The use of ITO and report of gate effects as first step in generating a field effect transistor device for signal amplification.
(1 : 4.5) DMF AMPS–MBA (1 : 2) H_2O	Capacitance/Au coated with SAM of hexadecanethiol	Ultra thin layer generation—capable of rapid target diffusion to signal transducer. Excellent response time (5 min) approaching real time. However poor detection limit for original template, creatine (1–7 mM).
AMPS–MBA (1 : 2) H_2O	Capacitance/Au coated with SAM of hexadecanethiol	Reversible, reproducible (RSD 10%) sensor response over a 6 month window. Excellent shelf life. Wide, but poor operational detection range (10–600 mM).
MAA–EDGMA	DPV/glassy carbon	Outstanding response time (2 min).
Pyrrole (overoxidised)	CV/glassy carbon	Ease at which ultra thin films (0.16 nM) can be formed and the potential wide and low concentration detection window (50 μM–0.5 M).
Monolayer (hexadecylmercaptane)	Cv/Au	Good reproducibility (RSD < 5% with 3 sensors). Excellent response time (5 min) approaching real time. Low limit of detection (15–60 μM).
Monolayer (hexanethiol, dodecanethiol)	Capacitance/Au	Simplicity of approach—mix and spread with a spreader bar approach to avoid distortion of the two-component monolayer.
Monolayer (several thiols) SiO_2 sol-gel	CV (and QCM)/Au; CV/glassy carbon	Photochemical imprint in two-component monolayer. Effect of chain length of the thiols in the template release. Selectivity study; drop cast (450 nM film thickness). Permeability studies with template in the medium. Used for the detection of dopamine—easy transition to amphetamine type substances.
TiO_2 sol-gel	ISFET/SiO_2	ISFET allows for signal amplification. Incorporation of corresponding NIP would potentially allow automatic subtraction of non-specific binding. Drop coat on the gate; 5 min equilibrium time.
TiO_2 sol-gel	ISFET/Al_2O_3	Thickness 85 ± 10 μM.
Preformed polymer (polyphosphazene–THF solution)	CV, DPV/glassy carbon	Drop coating evaporations; 25 use electrode; fast response time. Low limits of detection (0.25–6.6 μM).

[a] MAA (methacrylic acid); EGDMA (ethylene glycol dimethylacrylate); 4-VP (4-vinylpyridine); DPV (differential pulsed voltammetry); DVB (divinyl benzene); CV (cyclic voltammetry); ISFET (ion-selective field-effect transistor); DMF (dimethylformamide); DEAEM (diethylaminoethyl acrylate); OUA (oligourethane acrylate); ITO (indium tin oxide); AMPS (2-acrylamido-2-methyl-1-propane sulfonic acid); MBA (*N*,*N*′-methylenediacrylamide); THF (tetrahydrofuran).

groups into imprinted polymers. Multiple approaches are valid, including incorporation of a fluorescent monomer such as *trans*-4-[*p*-(*N*,*N* dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride, in a fluorescent cAMP sensor; a competition approach with the incorporation of an efficient fluorescence quencher (*p*-nitrobenzaldehyde) in a L-tryptophan sensor; to develop a sensor for L-tryptophan. A number of other fluorescent monomers have been prepared and successfully used to prepare fluorescent reporter MIPs.[37] The idea is that analyte binding will alter the microenvironment (polarity, pH) in the vicinity of the fluoro/luminophore, and hence alter the optical properties.[14,37–40] This may lead to quenching, enhancement, energy transfer *etc.* Although fluoresence is intrinsically more sensitive than UV–Vis, the latter has been successfully applied in the detection of μM to mM concentrations of a variety of templates.[40]

Quartz crystal microbalance/mass sensing

The combination of a quartz crystal microbalance (QCMB) and a specific MIP is a forensically compelling one, a mass change of as little as 1 ng (in a 10 MHz resonating system) will give rise to a 1 Hz frequency change. Thus, a QCMB coated with a MIP permits analyte detection with a high degree of specificity.[36] Whilst there is the issue of non-specific binding to consider, Dickert *et al.* have extensively exploited this combination, especially with cross-linked urethanes giving rise to a series of systems specific for poorly functionalized templates, *e.g.* tetrahydrofuran and chloroform. Dickert's group further extended their studies utilizing in selective polyaromatic hydrocarbon MIP layers on to QCMB surfaces.[40,41] In this latter instance, cross-linked polyurethanes were used to prepare chrysene and anthracene imprinted polymers. More recently, Bunte *et al.* have reported a QCM-based detector for TNT.[42]

Similar mass sensitive detection techniques based on the more sensitive Love wave approach have also been developed, especially in the area of therapeutically relevant templates—caffeine, epinephrine, pyrimethamine, phenobarbital and trimethoprin.[36]

Enhancing site accessibility

Poor diffusion kinetics for ingress and egress of templates presents a significant problem for most MIPs. This situation is exacerbated

if the MIP is used in a displacement type assay format where a competition is set up between the free analyte and a tagged analyte. Obviously this situation would be greatly improved if the receptor site could be localised at or near the surface of the MIP. Several approaches to achieve this have been investigated.

Yilmaz *et. al.* specifically imprinted the surface of immobilised templates covalently bonded to the surface of porous silica.[43] The monomer–solvent mixture was polymerised in the silica pore network around the templates and the silica network was etched away with HF. The very harsh conditions necessitated the use of DVB as cross-linker, but the approach had the advantage that the resulting receptor sites were at the surface of the polymer and should therefore have very good accessibility. Similarly, Titirici *et al. in situ* imprinted surface confined sites on a silica support.[44] While the surface confined sites were indeed more accessible to larger templates than those of the equivalent conventionally prepared bulk MIPs, the latter exhibited a greater selectivity for the template than the surface imprints.

A much simpler approach is the grafting of a very thin layer of MIP onto a preformed support. This might be a porous polymer, a silica particle, a porous polymer membrane, a surface such as an electrode or microtitre plate well or a nanoparticle. Although there are numerous examples of such grafted MIPs in the literature, there is little in the way of demonstrations that they really deliver enhanced binding kinetics or accessibility by direct comparison with a conventional particle. This, along with the supposition that the template could be more effectively extracted, leading to reduced or eliminated template leaching, remains to be demonstrated experimentally.

Computer modelling of mixed/*de novo* functional monomer–template interactions

The use of computational methods to select a functional monomer(s) from a virtual library of compounds to maximise interactions with a given target has assumed increased prominence in MIP synthesis. Chianella and co-workers designed a MIP specifically for the cyanobacterial toxin microcystin-LR.[45] The monomers selected, acrylamidopropanesulfonic acid and imidazole-4-acrylic acid ethyl ester, were copolymerized with EGDMA as the cross-linker in the presence of the template. The MIP had an affinity 3 times higher for the template than did the MIP made from the standard methacrylic acid-co-EGDMA polymer. New monomers are constantly being added to the repertoire.

NOBE MIPs

Cross-linkers constitute ~80–90% of MIP recipes, but are essentially ignored during the initial pre-polymerisation complex design phase. The vast majority (arguably all) of studies examine the 10–20% of the recipe that comprises the functional monomer–template interaction and this is where the emphasis on development is lain. However, Wulff noted that increased enantioselectivity (for a chiral template) could be achieved by employing a smaller cross-linker (**CL5**), EDGMA *vs.* BDMA (**CL24**).[12] (Cross-linker) size matters. It is often overlooked that the template and functional monomer are of similar size. Consequently, it is the cross-linker that ultimately determines the spatial arrangement, resolution and number of specific cavities generated. Presumably, the effect noted by Wulff represents a better 'induced fit' with the smaller EDGMA. With this in mind, it is apparent that small improvements in the 80–90% has the potential to have a more significant impact on MIP performance than a major improvement in the 10–20%. We should take a page from nature's design handbook and recognise that the near-perfect active site–ligand interactions in these systems are not solely a function of active site residues, but of the overall polymer architecture.

Spivak's dual function cross-linker—the *NOBE MIP approach*—has embraced this concept. Spivak's data clearly shows that this is a highly feasible and indeed desirable approach with simpler MIP recipes, comparable, if not better, selectivities and overall better performing MIPs, although we have not seen reports relating to materials of forensic interest.[21,22] A number of key points have arisen from this work: studies revealed that the improved performance of cross-linker/monomers such as **CL5** (Fig. 4) was due to: the cross-linking nature of this monomer; control of conformational flexibility; and a strong influence of monomer chirality on enantioselectivity in MIPs. The NOBE MIPs work well with O containing targets but show no improvement for N containing systems—presumably a reflection of the lesser amounts of H bonding. The key difference in architecture *cf.* proteins is the wider array of interactions—particularly S–S.

The other factor appears to be proximity of the pendant carboxylate group with respect to the cross-linker backbone. From a design point of view, it appears that providing closer proximity of the pendant functional group to the cross-linking group improves selectivity, again due to reduction of the conformational flexibility of the pendant group. The effects of binding group flexibility have been shown to take an active role in the imprinting process by Wulff and co-workers, who examined the influence of conformational flexibility of functional monomers on selective behaviour by MIPs. In this study, it was determined that decreasing binding group flexibility resulted in greater selectivity; however, if the binding group became too rigid, specificity was diminished.[12]

It also appears that diastereomeric complexes "imprint" differently from complexes formed *via* nonstereogenic monomers—a match/mis-match pairing. Preferential imprinting of one diastereomeric complex could occur from a more distinguishable geometry (*e.g.*, twisted *versus* spherical), or by a complex having physical properties more compatible with the polymer (*e.g.*, a more hydrophobic complex for the hydrophobic polymer). This observation indicates that diastereomeric complexation effects can dominate any improvements to selectivity by the use of a cross-linking functional monomer. However, more studies will be needed to determine whether the origins of this effect are to be found in the solution-phase pre-polymer complex or the final polymeric binding site. The degree of cross-linking is maximized without imposing restrictions on functional group concentrations. Covalent tethering of the functional group to the binding site matrix reduces conformational entropy that would otherwise interfere with specific binding.

Room temperature ionic liquids

We have a long-standing interest in the synthesis and applicability of RTILs in organic chemistry.[10,11,46,47] Fascinated by their apparently tunable and unique solvating properties,[48,49] we

were the first group to report MIP generation in RTILs.[10,11] Interestingly, in our original report we noted considerable rate enhancements, a known phenomenon in RTIL-mediated free radical polymerisations, however, we also observed a greater degree of control over particle size (in specific cases) and also an increase in imprinting values.[10,48,49] Commensurate with this was a reduction in time required to achieve optimal binding. Subsequent studies have indicated the nature of imprinting template and choice of RTIL have a pronounced effect on all MIP properties, from initial synthesis to performance and usability.[11] Other interesting features include no observable swelling if the RTIL is used as the rebinding porogen, suggesting an increase in specific surface binding interactions, which partly explains the reduced time required to optimal binding. It is also possible that this is due to an increased number of surface located specific cavities.[10,11]

Given that current literature indicates that rapid mass transport/analyte diffusion is the key to fast response times and reproducible sensors, if these initial RTIL results are translatable to existing ultra rapid sensing MIPs (see Table 1), there is the tantalising possibility of further sensing enhancements to real-time measurement. However, as with all new MIP areas, there is insufficient literature to accurately predict the future. RTILs are fraught with issues: they are not amenable to spin casting, hence the synthesis of MIP films will be problematic. However, Rogers *et al.* have reported an elegantly simple methodology for synthesis of cellulose films from [bmim][Cl].[50] The factors required for a specific RTIL to deliver the required properties are unknown, largely research and development is *via* a 'suck and see', reminiscent of the MIP field a decade ago. In the RTIL field, perceived difficulties are a euphemism for the next challenge leading to a greater understanding of these remarkable species.

Sensor arrays—do we need absolute specificity?

An eight channel MIP sensor array was prepared that was able to differentiate seven different aryl amine analytes, including diastereomers with 94% accuracy (Fig. 5).[51] MIPs have been shown to be easily tailored with selectivity for a wide range of analytes and demonstrate high thermal and chemical stabilities. They are also notable for being quickly and inexpensively generated from a common polymer matrix, and thus MIPs appear to be well suited for use in a sensor array format.

Combining multiple sensors together can compensate for any of the limitations in binding of MIPs such as high levels of cross reactivity and low overall affinities. In the array, individual sensors may show high levels of cross reactivity and poor selectivity but as long as the signal from one or more sensors in the array is different then a unique pattern or 'fingerprint' will be generated for each analyte.

Clearly a sensor array such as the above is feasible for the detection of a variety of simple amphetamines, and the corresponding sensor array could be developed for the more insidious methylenedioxamphetamine-type substance. Such sensor arrays will not be limited to the simple detection of amphetamine-type substances, or opiates, but rather have a direct application in intelligence-driven policing on drug detection. Incorporation of multiple sensing elements also offers the potential to identify the synthetic route in the case of ATS and geographic region of origin with opiates based on the known route specific/geographical origin markers. For example, *cis*- and *trans*-1,2-dimethyl-3-phenylaziridines (**20**) (Fig. 6) can be considered marker compounds as their formation during methamphetamine synthesis is specifically related to ephedrine/pseudoephedrine. It is proposed that during the synthetic reaction the intermediate haloephedrine (iodoephedrine or chloroephedrine) undergoes a ring closure to produce both the *cis* and *trans* aziridines.[52] We have shown that the presence of oripavine-derived products **21–24** (Fig. 6) are characteristic of Tasmanian derived heroin.[53] Hence the identification of these compounds in a sensor array indicates a synthetic route in the former and geographic origin in the latter case. Both these outcomes are of significance to law enforcement agencies and can, in principle, be simply achieved by the use of sensing MIPs.

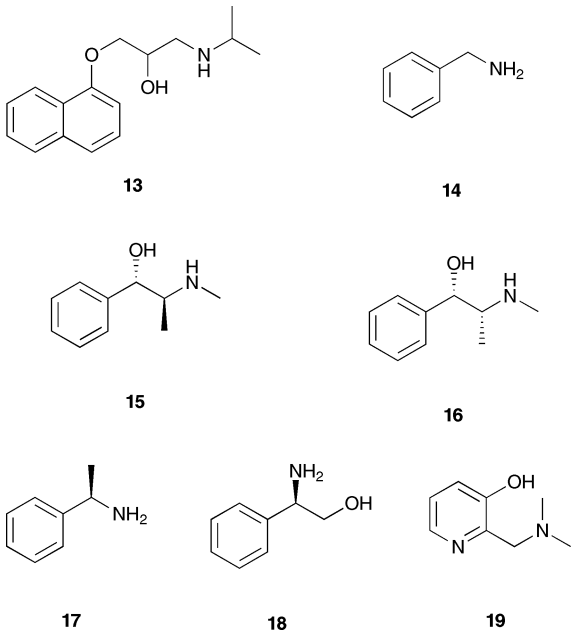


Fig. 5 **13** (±)-Propanolol, **14** benzylamine, **15** (+)-pseudoephedrine, **16** (−)-ephedrine, **17** α-methylbenzylamine, **18** *R*-(−)-2-phenylglycinol and **19** 2-(dimethylaminoethyl)-3-hydroxypyridine.

The strength of this approach is not relying on a single signal, but rather a MIP fingerprint for illicit drugs; again we believe that this is also applicable to remote sensing of both illicit drugs and explosives. Transducers based on, for example, electrochemical, capacitance, quartz crystal microbalance, or optical detection allow for the preparation of array structures containing several MIPs with different specificities. Consequently, the appearance of microprocessor-controlled multisensing devices that detect multiple analytes simultaneously and that allow for pattern recognition are no longer in the realm of science fiction and we foresee the development of multimodal systems detecting substances of interest, such as those highlighted in Fig. 1, as a logical development of this field.

Conclusions

We have sought to address a number of developing issues in this review, from Spivak's NOBE MIPs to RTILs, to sensor arrays and beyond. We have deliberately not discussed direct incorporation of MIPs into devices as this is beyond our experience and capability,

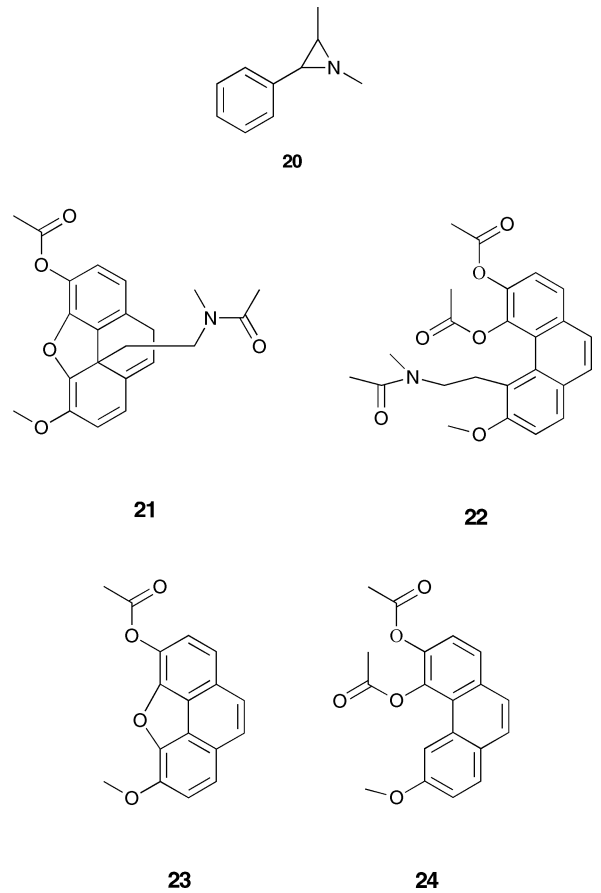


Fig. 6 Potential target templates for intelligence-driven policing in the fight against drugs: **20** *cis*- and *trans*-1,2-dimethyl-3-phenylaziridine; **21** 3-acetyl-*N*-acetyldesthebaine; **22** 3-acetyl-6-methoxy-4,5-epoxyphenanthrene; **23** 3,4-diacetyl-6-methoxyphenathrene; **24** 3,4-diacetyl-6-methoxy-5-[2-(*N*-methylacetamido)]ethylphenanthrene.

but do highlight some potential approaches. In the ideal world such incorporation would be trivial, however, there are complex fabrication issues remaining unresolved—how do we link MIPs and NIPs electronically to generate a real-time signal and how is this interfaced into a chemometric signature relevant to the forensic community? Whilst we have made significant advances in ease of generation, design and control of particle size, the seemingly simpler issues of ensuring batch-to-batch reproducibility of MIPs, still eludes us. Hence in a forensic arena, low-cost single use MIP-based sensors is the logical way forward. Forensically, the ultimate 'smart MIP' will be a hybrid system targeting multiple molecules determined as 'of interest' by the relevant authorities, be this explosives, illicit drugs or chemical warfare agents. We have made significant progress towards incorporation of MIPs into existing forensic capabilities within Australia, *e.g.* MIP_ts and field-portable FTIR instrumentation.

The linchpin for the development of MIP-based sensors will be the mass production of low-cost single or multiple use disposable transducers. In general, electrochemical devices have advantages such as low cost, small size, possibility of achieving low detection limits, and easy automation. One of the most selective types of electrochemical transduction is voltammetry, because the signal (current intensity) is generated by the analyte at a characteristic potential. A critical aspect of the development of a sensor is integration of the recognition element with the transducer. If the sensor is to be re-used, a procedure for surface renewal such as mechanical polishing or thorough washing should be provided. This is especially relevant for environmental and biomedical analysis. As an example, for electrochemical sensors, screen printed electrodes fulfil this need. The ease of preparation and low cost of MIPs make them attractive as recognition elements for such devices. Such systems are now being reported in the primary literature.[54]

We believe that, because of the potential low production costs, the combination of screen-printed electrodes and MIPs is particularly well suited for the design of disposable sensing elements. An elegant way of designing the MIP/transducer couple is to have the signal generated by the polymer itself. This approach appears promising since it does not depend on a special property of the analyte.

Are we there yet? The recent report of a europium-MIP based sensor for a Soman hydrolysis product suggests that we are approaching our destination or we are at least at a turning point in the use of MIPs as sensors.[55] The complex of europium ligated by divinylmethyl benzoate (ligating monomer) and by the analyte pinacoyl methylphosphonate was co-polymerized with styrene, whereafter the analyte molecule was removed by washing. Rebinding of the analyte was quantified from laser-excited luminescence spectra. Although it is not clear whether imprinting has contributed to the selectivity of the sensor, this detection principle appears exceptionally promising, taking into account the very low detection limits that can be obtained (7 ppt in this particular case).

Forensically, MIPs offer unparalleled possibilities for in-field/roadside drug testing. Their integration into electronic sensing devices, remote monitoring systems or even a 'lab on a chip' device appears a foregone conclusion.[56] Our ability to rapidly design and synthesise MIPs facilitates swift development of new sensors against emerging threats.

Acknowledgements

We acknowledge the financial support provided by the Australian Research Council (ARC)-Linkage Project grants and the Australian Federal Police Forensic Service (AFP FS). A special thanks to our AFP FS liasons Drs James Robertson and Chris Lennard. This work would not have been possible without the endeavours of our research team—the students: Carrie Brisbane, Katherine Booker, Timothy Kirkman, Ryan Shaw, Natalie Holmes, and Kathleen Wright.

References

1 K. G. Furton and L. J. Myers, *Talanta*, 2001, **54**, 487.
2 D. D. Stubbs, S.-H. Lee and W. D. Hunt, *Anal. Chem.*, 2003, **75**, 6231.
3 M. A. T. I. Q. Nambayah, *Talanta*, 2004, **63**, 461.
4 X. Yang, X.-X. Du, J. Shi and B. Swanson, *Talanta*, 2001, **54**, 439.
5 E. H. L. Lan, B. Dunn and J. L. Zink, *Chem. Mater.*, 2000, **12**, 1874.
6 C. Brisbane, *The formation of molecularly imprinted polymeric films for illicit drug detection*, Honours Thesis, The University of Newcastle, NSW, Australia, 2005.
7 L. Schwarz, M. C. Bowyer, C. I. Holdsworth and A. McCluskey, *Grape & Wine Growers Journal. Technical Issue.*, 2004, 1151.
8 L. Schwarz, C. I. Holdsworth, A. McCluskey and M. C. Bowyer, *Aust. J. Chem.*, 2004, **57**, 759.

9 C. I. Holdsworth, M. C. Bowyer, C. Lennard and A. McCluskey, *Aust. J. Chem.*, 2005, **58**, 315.
10 K. Booker, M. C. Bowyer, C. I. Holdsworth and A. McCluskey, *Chem. Commun.*, 2006, 1970.
11 K. Booker, M. C. Bowyer, C. Lennard, C. I. Holdsworth and A. McCluskey, *Aust. J. Chem.*, 2006, **60**, 51.
12 G. Wulff, *Chem. Rev.*, 2002, **102**, 1.
13 A. G. Mayes and M. J. Whitcombe, *Adv. Drug Delivery Rev.*, 2005, **57**, 1742.
14 A. Ellwanger, L. Karlsson, P. K. Owens, C. Berggren, C. Crecenzi, K. Ensing, S. Bayoudh, P. Cormack, D. Sherrington and B. Sellergren, *Analyst*, 2001, **126**, 784.
15 K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495.
16 L. Ye and K. Haupt, *Anal. Bioanal. Chem.*, 2004, **378**, 1887.
17 K. Haupt, *Ann. Chem.*, 2003, **75**, 377A.
18 M. C. Blanco-López, M. J. Lobo-Castañón, A. J. Miranda-Oridieres and P. Tuñón-Blanco, *TrAC, Trends Anal. Chem.*, 2004, **23**, 36.
19 C. Brisbane, C. I. Holdsworth, M. C. Bowyer, C. L. Lennard and A. McCluskey, unpublished data.
20 G. Wulff, J. Vietmeier and H. G. Poll, *Makromol. Chem.*, 1987, **188**, 731.
21 D. A. Spivak and K. J. Shea, *J. Org. Chem.*, 1999, **64**, 4627.
22 M. Sibrian-Vazquez and D. A. Spivak, *J. Org. Chem.*, 2003, **68**, 9604.
23 B. Sellergren, *Molecularly Imprinted Polymers—Man-Made Mimics of Antibodies and their Applications in Analytical Chemistry* in Analytical Chemistry, Elsevier Science, B. V., Amsterdam, 2001, p. 113; P. K. Dhal, *Molecularly Imprinted Polymers—Man-Made Mimics of Antibodies and their Applications in Analytical Chemistry* in Analytical Chemistry, Elsevier Science, B. V., Amsterdam, 2001, p. 185; M. J. Whitcombe and E. N. Vulfson, *Molecularly Imprinted Polymers—Man-Made Mimics of Antibodies and their Applications in Analytical Chemistry* in Analytical Chemistry, Elsevier Science, B. V., Amsterdam, 2001, p. 203.
24 S. Fireman-Shoresh, I. Turyan, D. Mandler, D. Aviner and S. Marx, *Langmuir*, 2005, **21**, 7842.
25 K. Tanabe, T. Takeuchi, J. Matsui, K. Ikebukuro, K. Yano and I. Karube, *J. Chem. Soc., Chem. Commun.*, 1995, 2303.
26 J. H. G. Steinke, I. R. Dunkin and D. C. Sherrington, *TrAC, Trends Anal. Chem.*, 1999, **18**, 159.
27 A. J. Hall, L. Achilli, P. Manesiotis, M. Quaglia, E. De Lorenzi and B. Sellergren, *J. Org. Chem.*, 2003, **68**, 9132.
28 M. Lahav, E. Katz, A. Doron, F. Patolsky and I. Willner, *J. Am. Chem. Soc.*, 1999, **121**, 862.
29 C. Lübke, M. Lübke, M. J. Whitcombe and E. N. Vulfson, *Macromolecules*, 2000, **33**, 5098.
30 B. Dirion, Z. Cobb, E. Schillinger, L. I. Andersson and B. Sellergren, *J. Am. Chem. Soc.*, 2003, **125**, 15101.
31 P. S. Reddy, T. Kobayashi, M. Abe and N. Fuji, *Eur. Polym. J.*, 2002, **38**, 521.
32 I. Tokareva, I. Tokarev, S. Minko, E. Hutter and J. H. Fendler, *Chem. Commun.*, 2006, 3343.
33 I. Tokareva, S. Minko, J. H. Fendler and E. Hutter, *J. Am. Chem. Soc.*, 2004, **126**, 15950.
34 *Clusters and Colloids, from Theory to Applications*, ed. G. Schmid, VCH, New York, 1994.
35 D. Pavel, J. Lagowski and C. J. Lepage, *Polymer*, 2006, **47**, 8389.
36 A. L. Hillberg, K. R. Brain and C. J. Allender, *Adv. Drug Delivery Rev.*, 2005, **57**, 1875.
37 A. Salinas-Castillo, I. Sánchez-Barragan, J. M. Costa-Fernández, R. Pereiro, A. Ballesteros, J. M. González, A. Segura-Carretero, A. Fernández-Gutiérrez and A. Sanz-Medel, *Chem. Commun.*, 2005, 3224.
38 S. Al-Kindy, R. Badia, J. L. Suarez-Rodriguez and M. E. Diaz-Garcia, *Crit. Rev. Anal. Chem.*, 2000, **30**, 291.
39 F. L. Dickert and O. Hayden, *TrAC, Trends Anal. Chem.*, 1999, **18**, 192.
40 F. L. Dickert, M. Tortschanoff, W. E. Bulst and G. Fischerauer, *Anal. Chem.*, 1999, **71**, 4559.
41 F. L. Dickert and S. Thierer, *Adv. Mater.*, 1996, **8**, 987.
42 G. Bunte, J. Hurtttlen, H. Pontus, K. Hartlieb and H. Krause, *Ann. Chim. Acta*, 2007, **591**, 49.
43 E. Yilmaz, O. Ramström, P. Möller, D. Sanchez and K. Mosbach, *J. Mater. Chem.*, 2002, **12**, 1577.
44 M. M. Titirici, A. J. Hall and B. Sellergren, *Chem. Mater.*, 2002, **14**, 21.
45 I. Chianella, S. A. Piletsky, I. E. Tothill, B. Chen and A. P. F. Turner, *Biosens. Bioelectron.*, 2003, **18**, 119.
46 C. M. Gordon and A. McCluskey, *Chem. Commun.*, 1999, 1431.
47 J. A. Whitehead, G. A. Lawrance and A. McCluskey, *Green Chem.*, 2004, **6**, 313–315.
48 T. Welton, *Chem. Rev.*, 1999, **99**, 2071.
49 P. Wasserscheid and W. Keim, *Angew. Chem., Int. Ed.*, 2000, **39**, 3772.
50 M. B. Turner, S. K. Spear, J. D. Holbrey and R. D. Rogers, *Biomacromolecules*, 2004, **5**, 1379.
51 N. T. Greene, S. L. Morgan and K. D. Shimizu, *Chem. Commun.*, 2004, 1172.
52 A. McCluskey, I. D. Evans, Y. Qi, *Forensic Sci. Int.*, 2007, 10.1016/j.forsciint.2006.08.016.
53 L. R. Odell, J. Skopec and A. McCluskey, *Forensic Sci. Int.*, 2007 accepted for publication.
54 B.-J. de Gans, S. Hoeppener and U. S. Schubert, *J. Mater. Chem.*, 2007, **17**, DOI: 10.1039/b701947e.
55 A. L. Jenkins, O. M. Uy and G. M. Murray, *Ann. Chem.*, 1999, **71**, 373.
56 M. Zourob, S. Mohr, A. G. Mayes, A. Macaskill, N. Pérez-Moral, P. R. Fielden and N. J. Goddard, *Lab Chip*, 2006, **6**, 296.

