MOLECULARLY IMPRINTED POLYMER TECHNOLOGY: A POWERFUL, GENERIC, FACILE AND COST EFFECTIVE ALTERNATIVE FOR ENANTIO-RECOGNITION AND SEPARATION: A GLANCE AT ADVANCES AND APPLICATIONS

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ABSTRACT

The emerging Molecularly Imprinted Polymer (MIP) technology has yielded proof of concept, harnessing nature's fundamentals to yield recognition receptor mimics from the miniaturized basics, borrowing on the ground rules, but conveniently avoiding the complexity, fragility, instability, costs and ethics of animal based bio-affinity matrices. Thus, the impact of bio-sciences on the future of technology is not via direct use of the molecules, but rather the lessons learned. Molecular imprinting is a powerful specialty representing the generic, cost effective and facile alternative for preparation of synthetic custom-tailored receptors. It achieves this via creation of specific selective recognition sites for a pre-determined analyte (called template) in a polymeric matrix. The template directs the molecular positioning and orientation of the material's functional monomers. Cross-linking ensures polymer rigidity that "freezes" the 3-D molecular architecture of the binding cavity when the template is subsequently extracted. The immense potential of the emerging MIP technology is typified by enantio-separation. Beyond separation science, the MIP sorbents' potential continues to impact and revolutionize sensor development, catalysis, toxin sequestration and in environmental metal-ion de-contamination problems.

INTRODUCTION

The intriguing and exacting guest-host chemistry that drives the biological recognition machinery such as the enzyme-to-substrate and antibody-to-antigen specificities has inspired the search for the molecular level science essentials and ground rules driving such systems (Fisher 1894, Litchenhaler 1994, Pauling 1940). The goal for this being replication in synthetic mimics for science and technological applications. The eventual fabrication of Molecularly Imprinted Polymers (MIPs) capable of this recognitive function, about three decades ago, furnished proof of the concept, translating into new, exciting molecular technology applications whose potential is still unfolding (Wuff and Sarhan 1972, Wuff 1995, Mosbach and Ramström 1996). These synthetic receptor mimics comprise the miniaturized essentials from nature's ground rules, but avoid the protein based complexity of the bio-affinity equivalents sourced from life forms. The concerns include fragility, operational instability, costs as well as the ethics agenda relating to animal experiments as their production does not involve animal immunizations. As typified by the imprinting of the drug morphine (Ramström and Mosbach 1999), molecular imprinting involves the creation of template recognitive cavities in polymer matrices which leaves chemical memory for the template molecules (Fig. 1). Thus if an enantiomer is used as a molecular template in MIP preparation, the resulting imprinted polymer will preferentially rebind that one enantiomer. This validates the imprinting effect, as chiral discrimination would not be acquired if the separation was based only on the familiar ion-exchange or size-exclusion processes. The pre-polymerization assembly involves template-to-functional monomer interaction(s). Cross-linking subsequently assures a rigid polymer framework which

leaves behind a mechanically stabilized cavity complementary in size and shape to the template upon its removal.

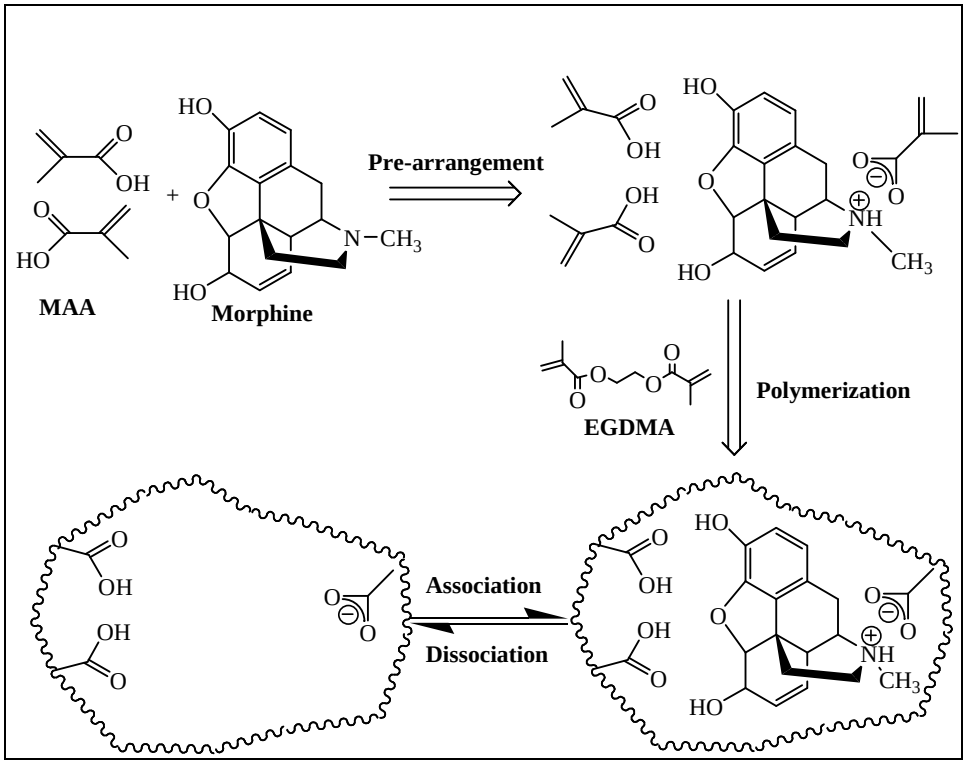


Figure 1: An imprinting protocol for morphine on a MAA/EGDMA type polymer. MAA = methacrylic acid (functional monomer), EGDMA = ethyleneglycoldimethacrylate (crosslinking monomer) (Ramström and Mosbach 1999)

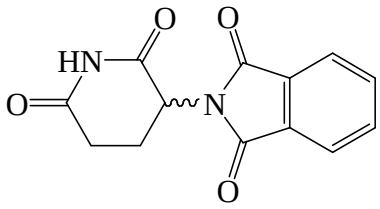
ADVANCES IN MIP TECHNOLOGY

MIPs in enantio-purity compliance in drug development

Enantiomeric forms may possess altered properties, effectiveness, side effects and may even be toxic. With the increasing structural complexity of new drugs and their differing effects, the importance of enantiomerically pure compounds is growing. The discoveries of many optically active drugs in the market worldwide support the need for enantio-purity as a routine specification (Ali *et al*. 2007). The thalidomide saga is a stark illustration of what can go wrong with enantiomers of the same drug. Table 1 lists a selection of known enantiomeric drugs presenting a range of different enantiomer/activity profiles (Kempe and Mosbach 1995, Nicholls *et al*. 1995, Mosha 2006).

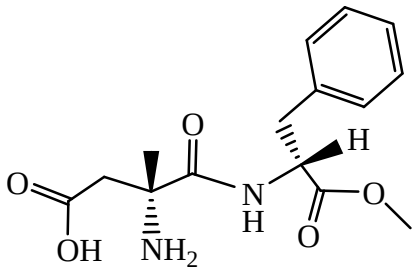
Table 1: The effect of chirality on drug activity for selected drugs

Thalidomide (2-(2,6-dioxo-3-piperidyl)isoindole-1,3-dione)

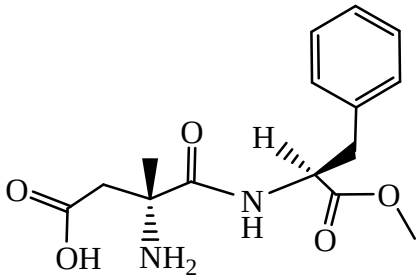


An example of a racemic drug, producing a desirable antiemetic effect with one enantiomer, whereas the other is toxic and teratogenic. However, the enantiomers interconvert in vivo, so chemical processes cannot mitigate the toxicity.

Aspartame (*L*-Aspartyl-*L*-phenylalanine methyl ester) and its stereoisomer



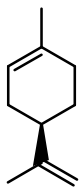
***L*-Aspartyl-*L*-phenylalanine methyl ester**



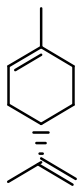
***L*-Aspartyl-*D*-phenylalanine methyl ester**

While *L*-Aspartyl-*L*-phenylalanine methyl ester is sweet, its stereoisomer, *L*-Aspartyl-*D*-phenylalanine methyl ester, is bitter.

Limonene



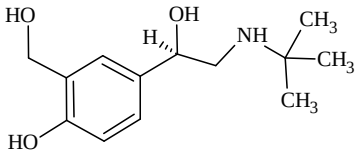
(*R*)-Limonene



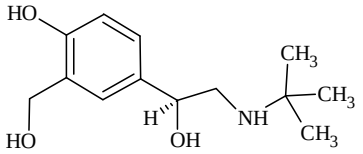
(*S*)-Limonene

Limonene (methyl-4-prop-1-en-2-yl-cyclohexene) is a colourless liquid terpene hydrocarbon. The *R*-isomer has a strong smell of oranges; the *S*-isomer that of lemons.

Salbutamol (2-(hydroxymethyl)-4-[1-hydroxy-2-(*tert*-butylamino)ethyl]phenol)



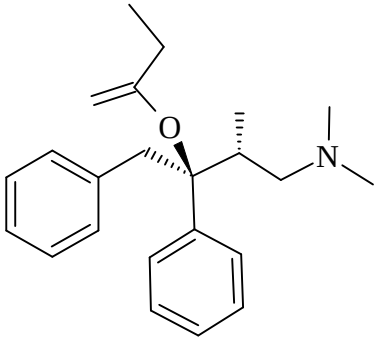
***R*-enantiomer**



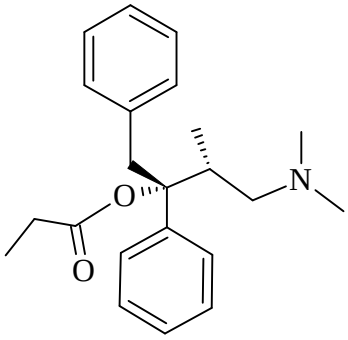
***S*-enantiomer**

A short-acting β2-adrenergic receptor agonist used for the relief of bronchospasm in asthmatics. The *R*-enantiomer produces fewer side effects.

Dextropropoxyphene and Levopropoxyphene ([4-dimethylamino-3-methyl-1,2-diphenyl-butan-2-yl] propanoate)



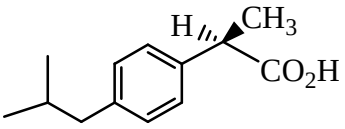
Dextropropoxyphene



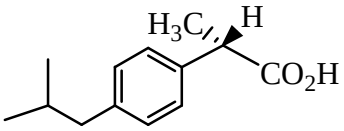
Levopropoxyphene

Dextropropoxyphene ([(2*R*,3*R*)-4-dimethylamino-3-methyl-1,2-diphenyl-butan-2-yl] propanoate) is an opioid analgesic for pain treatment. Levopropoxyphene ([(2*R*,3S)-4-dimethylamino-3-methyl-1,2-diphenyl-butan-2-yl]propanoate) is an anti-cough agent.

Ibuprofen



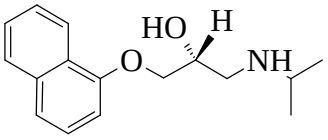
(*R*)-Ibuprofen (inactive)



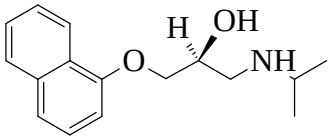
(*S*)-Ibuprofen (active)

Ibuprofen (2-[4-(2-methylpropyl)phenyl]propanoic acid) is a non-steroidal anti-inflammatory drug. Activity is confined to the *R*-enantiomer, the *S*-enantiomer being active.

(*R* and *S*)-Propranolol (1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-ol)



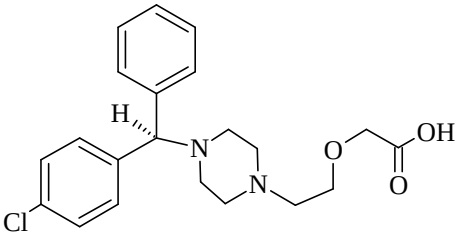
(*R*)-propranolol



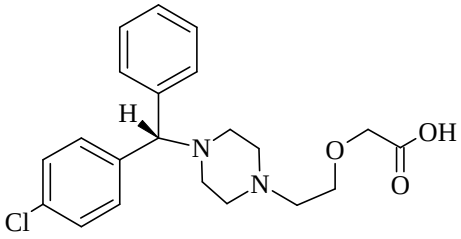
(*S*)-propranolol

Propranolol β-Blocker: (*S*)-propranolol is the effective drug, the (*R*)-propranolol is not active.

Dextrocetirizine and Levocetirizine ((±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy]acetic acid)



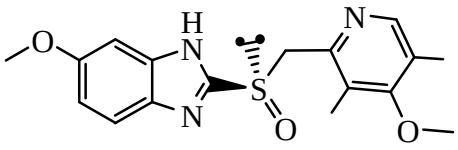
Levocetirizine



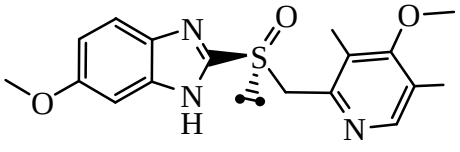
Dextrocetirizine

Cetirizine, (±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy]acetic acid, is a racemic selective H_1 receptor antagonist used as an antihistamine for the treatment of allergies. Levocetirizine is the active enantiomer of cetirizine.

Omeprazole (5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridin-2-yl)methylsulfinyl]-3H-benzimidazole)



(*R*)-omeprazole



(*S*)-omeprazole

Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcers and gastroesophageal conditions. Efficacy of (*S*)-omeprazole over the racemic mixture is claimed.

As a result of altered properties of enantiomeric forms exemplified in Table 1, possibly separation science commands the leading share of the research and commercial interest in the emerging MIP technology, and with it, a huge multi-billion dollar global market potential (Piletsky *et al.* 2001). Using self-assembly MIP protocols, highly efficient chirally discriminating sites have been prepared, possessing large separation factors between enantiomers when the polymers are used as chiral

stationary phases in chromatography (Fig. 2) (Wuff 1995, Mosbach and Ramström 1996).

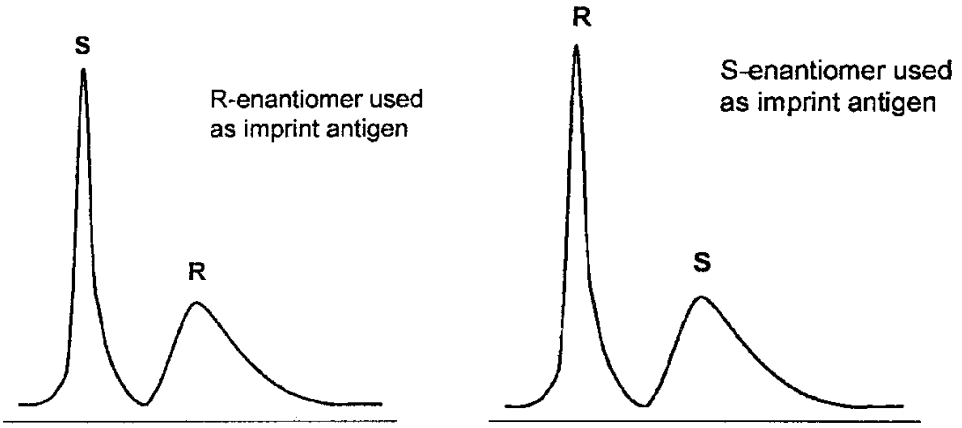


Figure 2: The predetermined chromatographic elution order for enantiomers, using MIP as stationary phases

As typified in Fig. 2, the characteristic predetermined elution order of the enantiomers depends only on which enantiomeric form was used as imprint antigen. For instance, when the *R*-enantiomer is used as imprint antigen, the *S*-form will be eluted first, and vice versa. The benefit of this fabrication is to resolve a racemic compound into its enantiomers using MIP synthesized in the presence of either one of the enantiomers. MIP technology is thus a promising important tool in the production of optically active drugs.

Molecular imprinting technology in sensor development

Sensor performance is characterized by selectivity, sensitivity, stability, and reusability. Selectivity measures the sensor's discrimination capability for the analyte among similar and different species. Selectivity is therefore governed by the recognition component. MIPs are especially suited for sensor technology. A chemical sensor selectively binds a target molecule in a complex matrix and generates an output signal using a transducer that correlates to the concentration of the analyte (Fig. 3). The recognition element is responsible for the selective binding (and in some cases, conversion) of the analyte in a matrix containing both related and unrelated compounds. The transducer translates the chemical event into a quantifiable output signal. MIPs exhibit good specificity for various compounds of medical, environmental, and industrial interest; and they have excellent operational stability. The recognition properties of well fabricated MIPs are unaffected by acid, base, heat or organic treatments, making them superior recognition elements (Vasapollo *et al.* 2011).

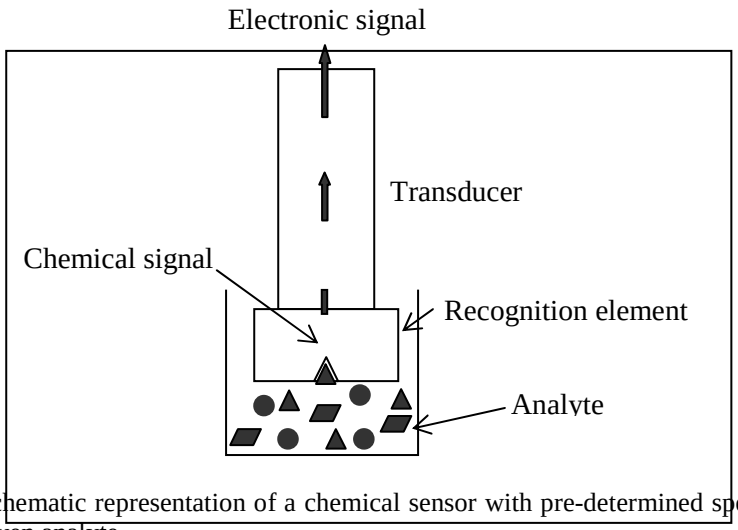


Figure 3: Schematic representation of a chemical sensor with pre-determined specificity for a given analyte

A chemiluminescent MIP-based flow-through sensor for 1,10-phenanthroline is among recent advances in sensor development and aptly illustrates the essentials in the recognition mechanism (Lin and Yamada 2001). The design (Fig. 4) is based on a divinylbenzene based molecularly imprinted polymer with both the molecular recognition and chemiluminescence (CL) reaction catalytic properties. A ternary complex, 4-vinylpyridine-Cu(II)-l,10-phenanthroline, was used as the functional monomer. Upon completion and oxidation by peroxide, which is the CL source, a recognitive cavity for rebinding 1,10-phenanthroline remains. The system serves as a flow-through sensor for the determination of 1,10-phenanthroline.

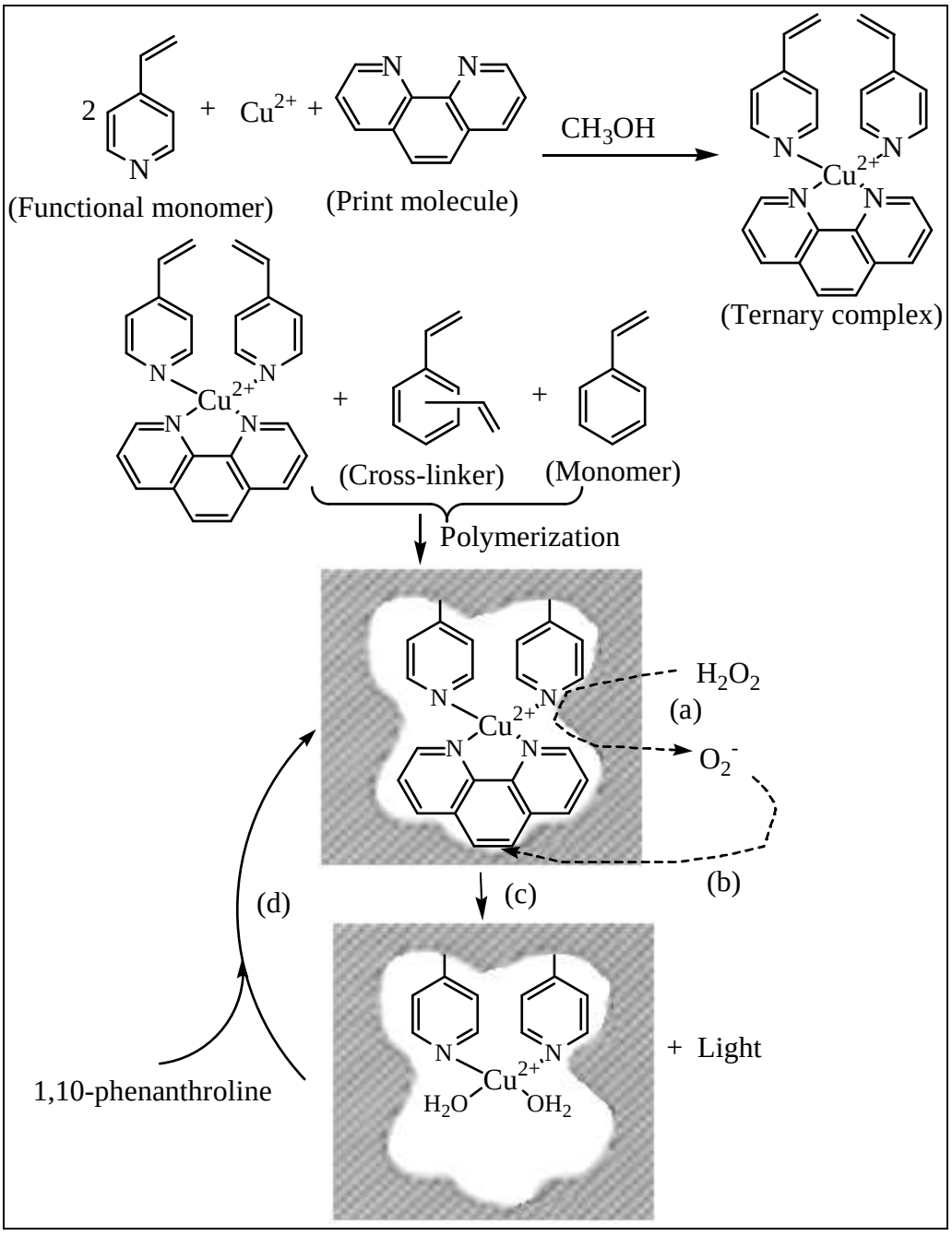


Figure 4: Scheme for a chemiluminescent flow-through sensor for 1,10-phenanthroline (Lin and Yamada 2001)

Molecularly imprinted polymers in catalysis

Investment in the development of artificial enzymes or enzyme mimics based on MIPs is one of the most intriguing challenges of the MIP technology. Despite catalysis enjoying a well established traditional base and competition in zeolites, important advances yielding novel catalysts have emerged in the domain (Vasapollo *et al.* 2011). Synthetic polymeric catalysts have the appeal and potential to become a real complement to natural catalysts. The cost efficiency with which such materials may be produced is a great advantage for catalytic applications.

The imprinted polymer for the Diels-Alder reaction between tetrachlorothiophene-S,S-dioxide and maleic anhydride is among promising advances where MIP serves to guide the product formation via transition state analogues (TSA's) in the imprinting protocol; the reaction transition state is stabilized, saving energy (Liu and Mosbach 1997). The Diels-Alder reaction is a concerted co-addition reaction and has a large entropic barrier. To catalyze the reaction, creation of a substrate selective cavity which functions as an "entropy trap" seems critical, and this is accomplished by molecular imprinting (Fig. 5). The strategy involves the use of a transition state anologue, similar to the one used to generate catalytic antibodies (abzymes) (Liu and Mosbach 1997). The structure of the final product conveniently changes substantially from that of the transition state via simultaneous exclusion of sulphur dioxide. Therefore product inhibition which could be a serious problem for rigid MIPs is minimized. These studies have shown that MIPs can be used to create "entropy traps" for the catalysis of molecular reactions.

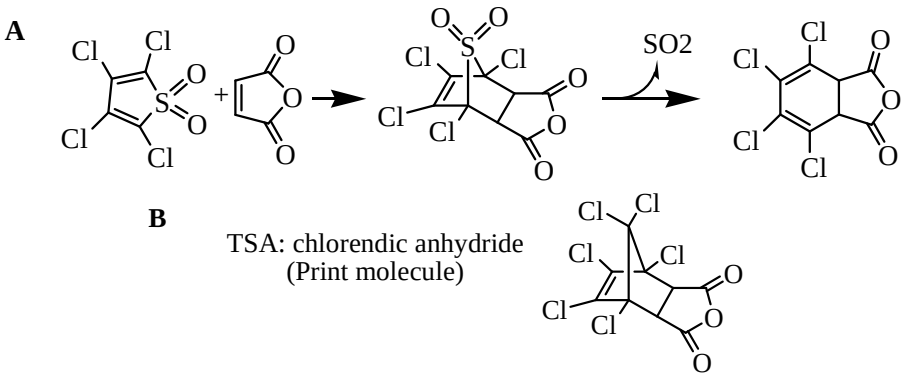


Figure 5: (A) The Diels-Alder reaction between tetrachlorothiophene and maleic anhydride, (B) the print molecule (TSA analogue) chlorendic anhydride (Liu and Mosbach 1997)

The synthesis of aspartame from *Z-L*-aspartic acid and *L*-phenylalanine methyl ester is a typical model for MIP-assisted equilibrium shifting in favour of a product which is continually removed by a pre-imprinted polymer. This enzymatic synthesis is catalyzed by a polymer imprinted against *Z*-aspartame. This results in a considerable increase (~40%) in product yield (Ramström *et al.* 1998). The reaction scheme is shown in Fig. 6.

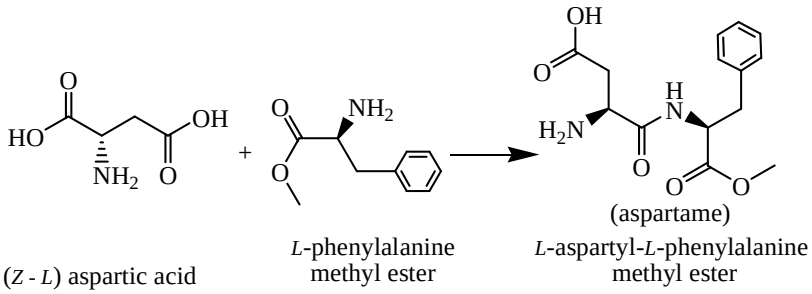


Figure 6: Preparation of aspartame from (Z – L) aspartic acid and (L-) phenylalanine methyl ester. This thermodynamically unfavourable equilibrium is shifted through a polymer pre-imprinted with aspartame (Ramström *et al.* 1998)

MIPs in toxin sequestration

Among important challenges for imprinted polymer technology, non-intrusive therapy for the safe sequestration of specific toxins from systems such as the gastro-intestinal tract (GIT), has been a targeted priority. Target toxins include deoxycholic acid (DCA), as the free acid or as the glycine (GDCA) and taurine (TDCA) conjugates. The insolubility of MIPs and thus their failure to be absorbed as well as their thermal stability, which affords sterilization possibilities, e.g. by autoclaving, have been cited as appealing safety elements (Green *et al*. 2000). DCA is produced by bacterial flora from cholic acid and is implicated as a factor in heart disease, cancer of the colon and esophagus, gallstones and liver cholestasis. To effect the sequestration of DCA form gastro-intestinal tract, use was made of the functional monomer N,N'-diethyl(4-vinylphenyl) amidine (DEVPA), which forms a strong complex with DCA and GDCA for pre-imprinted polymers that are used in successful clinical procedures for their selective elimination from the GIT (Green *et al*. 2000).

Potential for imprinted polymer technology in environmental clean-up applications

The sequestration scheme shown in Fig. 7 is designed to mimic nature's guest-host chemistry whereby certain soil bacteria are known to harvest iron from soil via powerful enzymes *(siderophores)* which bind and deliver the load for recognition and internalization at the cell wall (Zuo *et al*. 2005). The ultra high affinity macrocyclic ligand, *N*,*N*',*N*'',*N*'''-tetra(2-carbamoylethyl) cyclam (see scheme in Fig. 7) is used to mimic nature's *siderophore* bacterial enzyme to bind the metal ion (step-1). With Ni(II) as the prototypic common environmental metal ion, the resulting Ni(II)-complex is bound into recognition sites in an EGDMA based pre-imprinted polymer, accomplishing the step-2 binding, this serving as the cell wall mimic. The combined two-event selectivity caters for enhanced overall system effectiveness. To benefit from superior hydrogen bonding affinity in the design, acrylamide monomer was chosen for the binding while the three isomers of vinyl pyridine (either 4-,3- or 2-vinyl pyridine) (in synergy) yielded information on the efficacy of the added metal coordinate interaction.

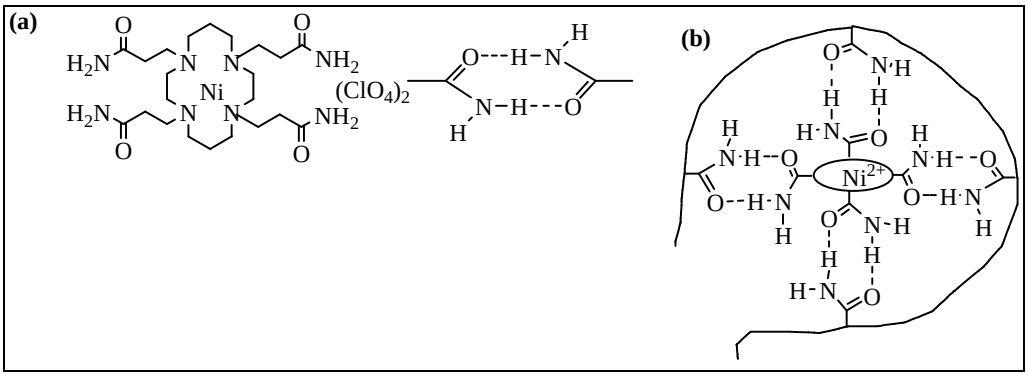


Figure 7: (a) The high affinity ligand *N,N',N'',N'''*-tetra(2-carbamoylethyl) cyclam and the 4-centre-2 hydrogen bonding interaction with acrylamide monomers; (b) schematic representation of the "receptor-within-receptor" for the binding in the MIP precognitive cavity. Ni(II) represents common prototypic environmental metal ions (Zuo *et al*. 2005)

The acrylamide, 4-vinyl pyridine and 3-vinyl pyridine MIPs have yielded binding affinities that compete or excel those documented for most systems relying on non-covalent interactions. Affinities improve with increasing MIP monomer excess and are additionally enhanced by synergism via use of simultaneous monomers (methacrylic acid + vinyl pyridine) in the same MIP. No imprinting effect is elicited by MIPs utilizing the 2-vinyl pyridine monomer on account of prohibitive structural stress. As further reported by Zuo *et al*. 2005, the product MIP test materials exhibit proven durability, robustness and endurance to thermal stress (120 °C) and endure continuous re-use without significant performance loss. Thus, an envisaged soil poultice system based on the above would require treating irrigated metal-contaminated soil with both the MIP material and the high affinity ligand and allowing for the two-event binding to proceed. Thereafter, the loaded polymer is collected for extraction into a concentrated waste form for disposal while the MIP is recycled.

CONCLUSIONS

Since MIPs are generic, facile and cost effective, they pledge to be one of the best synthetic polymers for molecular recognition in assorted scientific applications. MIPs have been widely studied as stationary phases, especially for resolving racemic compounds into their enantiomers. Although in its toddler stage, an interest has been directed to the development of MIPs as selective material for sensor developments. The application of MIPS in catalysis, especially in drug delivery, has been successfully studied, yet not thoroughly explored. In addition, the use of MIPs in toxin sequestration as well as in environmental clean-up has been explored. Despite numerous outstanding challenges, not only the principles of molecular imprinting technology have to be established, but also their relevance to the real world.

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