Development of Molecularly Imprinted Polymers for Amphetamine Type Substance Recognition in Aqueous Environments.

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Declaration

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RYAN SHAW

Date: 2/5/2012
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<td>--------------</td>
<td>-----------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>$\Delta E_{\text{int}}$</td>
<td>estimated energy of interaction</td>
<td>COC</td>
</tr>
<tr>
<td>2HEMA</td>
<td>2-hydroxy ethylmethacrylate</td>
<td>COD</td>
</tr>
<tr>
<td>2VP</td>
<td>2-vinyl pyridine</td>
<td>DEAMA</td>
</tr>
<tr>
<td>4VP</td>
<td>4-vinyl pyridine</td>
<td>DFT</td>
</tr>
<tr>
<td>AA</td>
<td>Acrylic Acid</td>
<td>DVB</td>
</tr>
<tr>
<td>AAM</td>
<td>Ally amine</td>
<td>Dye A</td>
</tr>
<tr>
<td>AIBN</td>
<td>azobisobutyronitrile</td>
<td>Dye B</td>
</tr>
<tr>
<td>AM</td>
<td>Acrylamide</td>
<td>Dye C</td>
</tr>
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<td>AMPH</td>
<td>Amphetamine</td>
<td>Dye D</td>
</tr>
<tr>
<td>AMPSA</td>
<td>Acrylamido-2-methylpropanesulfonic acid</td>
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</tr>
<tr>
<td>AN</td>
<td>Acrylonitrile</td>
<td></td>
</tr>
<tr>
<td>APTES</td>
<td>aminopropyltriethoxysilane</td>
<td>EGDMA</td>
</tr>
<tr>
<td>ATS</td>
<td>Amphetamine type substance</td>
<td>$E_{\text{HOMO}}$</td>
</tr>
<tr>
<td>B</td>
<td>analyte bound</td>
<td>$E_{\text{LUMO}}$</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic Adenosine monom phosphate</td>
<td>F</td>
</tr>
<tr>
<td>CC</td>
<td>Coupled cluster</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>Configuration interaction</td>
<td>FT-IR</td>
</tr>
<tr>
<td>CL</td>
<td>clenbuterol</td>
<td>EGDMA</td>
</tr>
<tr>
<td>$E_{\text{HOMO}}$</td>
<td>Highest Occupied Molecular Orbital</td>
<td>MOR</td>
</tr>
<tr>
<td>$E_{\text{LUMO}}$</td>
<td>Lowest Unoccupied Molecular Orbital</td>
<td>MAM</td>
</tr>
<tr>
<td>F</td>
<td>analyte free</td>
<td>MAMPH</td>
</tr>
<tr>
<td>FM</td>
<td>functional monomer</td>
<td>MCSCF</td>
</tr>
<tr>
<td>FT-IR</td>
<td>fourier transform Infrared spectrophotometry</td>
<td>MDMA</td>
</tr>
<tr>
<td>FM</td>
<td>functional monomer</td>
<td>MeCN</td>
</tr>
<tr>
<td>FT-IR</td>
<td>fourier transform Infrared spectrophotometry</td>
<td>MeOH</td>
</tr>
<tr>
<td>GBMV</td>
<td>Generalised Born using Molecular Volume</td>
<td>MIP</td>
</tr>
<tr>
<td>GBSW</td>
<td>Generalised Born with Simple Switching</td>
<td>MM</td>
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<td>GCMS</td>
<td>gas chromatography mass</td>
<td>MP$_x$</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>GDP</td>
<td>Guanine di Phosphate</td>
<td>NIP, non-imprinted polymer</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-Protein Coupled Receptor</td>
<td>NMBA, N,N-methylene-bis-acrylamide</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanine Tri Phosphate</td>
<td>N-MPEA, N-methyl phenylethylamine</td>
</tr>
<tr>
<td>HBA</td>
<td>hydrogen bond acceptor</td>
<td>NMR, nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>HBD</td>
<td>hydrogen bond donor</td>
<td>PB, Poisson-Boltzmann</td>
</tr>
<tr>
<td>HER</td>
<td>3,6-diacetoxy morphine</td>
<td>PES, potential energy surface</td>
</tr>
<tr>
<td>HF</td>
<td>Hartree Fock</td>
<td>PYR, Pyrrole</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
<td>QCM, Quartz Crystal Microbalance</td>
</tr>
<tr>
<td>IAM</td>
<td>N-isopropyl acrylamide</td>
<td>QM, Quantum mechanics</td>
</tr>
<tr>
<td>IMS</td>
<td>Ion Mobility Spectrometry</td>
<td>QSAR, Quantitative structure activity relationship</td>
</tr>
<tr>
<td>ITA</td>
<td>Itaconic acid</td>
<td>RIFS, Reflectomeric Interference Spectroscopy</td>
</tr>
<tr>
<td>ITO</td>
<td>indium tin oxide</td>
<td>SAW, Surface Acoustic Wave</td>
</tr>
<tr>
<td>LDA</td>
<td>Linear Discriminant Analysis</td>
<td>SCF, Self consistent Field</td>
</tr>
<tr>
<td>MAA</td>
<td>Methacrylic Acid</td>
<td>SE-MO, semi empirical molecular orbital</td>
</tr>
<tr>
<td>SLBO</td>
<td>strictly localised bond orbital</td>
<td></td>
</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
<td></td>
</tr>
<tr>
<td>STY</td>
<td>Styrene</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Template</td>
<td></td>
</tr>
<tr>
<td>TFMAA</td>
<td>2-(trifluoromethyl) acrylic acid</td>
<td></td>
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<tr>
<td>TOL</td>
<td>Toluene</td>
<td></td>
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<tr>
<td>TRIM</td>
<td>2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate</td>
<td></td>
</tr>
<tr>
<td>UV-VIS</td>
<td>Ultra violet visible spectrophotometry</td>
<td></td>
</tr>
<tr>
<td>VAC</td>
<td>Vinyl acetate</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Vinyl imidazole</td>
<td></td>
</tr>
<tr>
<td>VOH</td>
<td>Vinyl alcohol</td>
<td></td>
</tr>
<tr>
<td>XLM</td>
<td>crosslinking monomer</td>
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Abstract:

Amphetamine type substances are a legal concern for many jurisdictions across the world, and as such enforcement agencies require tools to easily identify these compounds in field settings.

Synthetic receptors are an interesting tool for this purpose, and they come in many types. Of these varieties, Molecularly Imprinted Polymers are a cheap, fast, stable, selective and viable tool to perform this recognition.

As compounds analogous to the amphetamine type substances are involved in the neuroregulation of homeostasis and stimulus response in natural systems, the techniques and interactions used by natural systems may be mimicked in a synthetic environment to create bio-mimetic, synthetic receptors for amphetamine types substances.

This work comprises of an initial phase of in silico screening where a library of commercially available monomers was screened against several amphetamine analogues to determine the identity and the number of functional monomers associated with the template molecules. Subsequently, in silico screening was carried out on these equilibrium geometry structures after freezing the monomer units in place to mimic the polymerisation step. Screening of a library of drugs and indicator substances was carried out to determine the direct interaction between functional monomer imprint site and the ligands.

NMR investigations were carried out to confirm the interactions predicted in the initial screening and polymers synthesised in a variety of porogens to probe the effect of solvent on the polymerisation process.

Subsequently, the synthesised polymers were introduced to the template in a variety of solvents to probe the adsorption behaviour of the polymers in a variety of solvents. Those which demonstrated the most selective behaviour in aqueous solution were subjected to varied techniques to optimise the selective adsorption of amphetamine type substances using both a pH buffer and ionic solution for the ionisable polymers and a dye displacement scheme to reduce the observable non-specific adsorption component of the interaction between analyte and adsorbent.

A dye displacement motif was incorporated into the adsorption to reduce the non-specific binding component by reporting only specific binding events. Using this
scheme, an imprinting factor “I” of 10.71 was obtained for ephedrine in aqueous solution by a methacrylamide co-EGDMA polymer at a T-FM ratio of 1-5 synthesised as a bulk monolith by thermally activated, free radical initiation in toluene.
Chapter 1: **Introduction.**

Identification of amphetamine type substances through the design and implementation of synthetic receptor protocol.

Amphetamine Type Substances (ATS) are a major focus for law enforcement and border control organisations regardless of country of origin. Current methods for presumptive identification of ATS presence in unknown street samples of drugs suffer from several major drawbacks. These include the destructive nature of reaction-based tests, the subjective nature of the colourimetric discrimination between different drug molecules and classes, and cross reactivity between molecules that contain similar functional groups. A field test should not only provide a fast and easily discernible positive result for the desired compound, but should immobilise in preference to destroying the compound to enable further conclusive analytical testing regimen in an accredited laboratory setting.

Requirements for successful field-testing protocols are high levels of safety for both operator and environment, cheap, reusable, fast, sensitive and reliable. The Molecularly Imprinted Polymer (MIP) approach has led to advances that offer a viable option fulfilling most, if not all of the desired characteristics of a presumptive field testing system. However, they are only one technique through which synthetic receptors can be constructed for specific, targeted molecules.

This review will provide a general introduction to in-field testing of illicit substances in a bulk solid/liquid testing environment, an overview of how synthetic receptors could assist law enforcement and border control; the variety of synthetic receptor designs presented in the literature and the use of molecular modelling to theoretically design the synthetic receptors. It will conclude with a focus on molecular imprinting and the opportunities it offers to the scientific community as a whole, but specifically to scientific forensic organisations.

In order to adequately determine the most appropriate synthetic receptor for the task of presumptive identification of ATS, the range of possible synthetic receptors recognition environments needs to be assessed. In addition, the environments in which
these sorts of synthetic receptors are utilised, and the conditions to which the synthetic receptor may be exposed are also of importance. The stability of the synthetic receptor in these environments will affect its utility. The types of interactions exhibited in natural, receptor-ligand systems; specifically those exhibited by the neurotransmitter receptors (GPCR) are of critical importance, due to the fact that ATS bind to the active sites of these receptors and subsequently modulate their physiological response.

The ultimate goal of this project is to design and describe a synthetic receptor which is capable of selectively recognising amphetamine type substances from a variety of other illicit drug molecules in an aqueous environment.

1.1 - Presumptive methods of unknown substance identification.

Current methods of presumptively identifying illicit substances outside of a laboratory comprise of a series colourimetric, reagent based tests, which involve the chemical reaction of the unknown compound with an array of reagents. These reactions produce a colourimetric response in the presence of specific functional groups. \(^{[1]}\) Subsequently, the produced colour must be subjectively assessed by the operator. Comparative results are provided via a reference colour card. Produced colour and intensity can depend on many factors; from analyte concentration, the protonation state of the functional groups, type of salt, the identity of any contaminants, the conditions in which the test is performed, and the skill of the operator. \(^{[2,3]}\)

1.2 - The desire for synthetic receptors.

The ability to selectively recognise one substance from another has been greatly desired as demonstrated by the use of a variety of chemical reactions as indicators in early scientific research. \(^{[4]}\) It was first attributed to Robert Boyle’s use of plant juices which were observed to change colour under different conditions. \(^{[5]}\) The drive for selective recognition and identification has not waned, and the increase in analytical and instrumental power has forced the invention and validation of many new techniques.
For every leap in identification, a subsequent increase in complexity of the device needed to make the analytical assessments is required. In 1961, Moncrieff et al. developed the first instrument capable of detecting the presence of certain vapour phase compounds by coating thermistors with a film forming compound to act as the adsorbent material.\[6\]

“Polyvinyl chloride, cellulose acetate, milk casein, peanut protein, gelatine, glue, cellulose acetate-butyrate, viscose cellulose, paraffin wax, vegetable fat, sodium alginate, calcium alginate, and rubber. So far as odour detection is concerned, the nature of the film hardly matters; the odour detection process is physical rather than chemical and almost any film-forming material will exhibit behaviour of the kind required”.

This investigation into synthetic noses measured the enthalpy of adsorption as the odourant molecules adsorbed onto the surface of the film coated thermistors. As the thermistors heated, their resistance fell and the effect of the odourant adsorbing onto the surface could be measured.

Several other groups conducted research into chemical noses over the next two decades, but it was not until the publication of a chemical array sensor system by the groups of Persaud and Dodd at Warwick University, [7] and Ikegami et al. at Hitachi P/L, [8] that such techniques became widely available.

By design, these types of sensors do not exhibit any specificity. Modern electronic noses utilise an array of recognition elements, which lack individual specificity, but do not share the same properties. Therefore they will interact with the vapour phase molecules that come into contact with each group of recognition elements in a different manner.

To make sense of such varying reactivities and cross-reactivities, complex computational methods are required to differentiate between the responses created across the chemical array by different chemical entities. In olfactory systems, this role is played by the brain, which receives the stimulus response from the receptor. It relates the information to previously encountered compounds to enable general identification of the vapour phase molecule. Thus, the architecture of the natural olfactory sense is a non-specific array, comprised of different groups of recognition elements whose response patterns are analysed and fitted to a pre-existing database or training set of
previously encountered response patterns. Artificial pattern recognition could be easily achieved with an artificial neural network depending upon an accurate training set being applied to characterise the neural network prior to unknown sample introduction.

1.3 - Non-specific synthetic receptors; Ion Mobility Spectrometry (IMS) and molecular noses/tongues.

Many compounds such as ions or those having a low vapour pressure can only be measured in aqueous phase. For many online or inline applications it is only possible to use systems that measure the solution directly. The development of electronic tongues offers the possibility to study the combination of olfactory recognition with other types of artificial senses.

1.4. - Electro-analytical techniques.

Within the scope of electronic noses and electronic tongues there are several analytical techniques which provide robust, reliable and easily manipulated sensory transduction elements. Voltammetry, potentiometry and conductometry are the most readily applicable and easily decipherable. The most common potentiometric devices, the pH or calomel electrode has found use in almost every field imaginable. The technology, which provided the ability to selectively assess the concentration of a single ion in solution, has progressed to be incorporated in many other ion selective electrodes.

A zero-current technique, the potentiometer consists of two different electrodes. One, known as the reference electrode does not change in potential and provides a baseline response for the working electrode to be able to detect the amount of potential difference as the analyte ion interacts with the working electrode. To reach the working electrode, an analyte ion must be able to pass through the membrane which separates the working electrode from the bulk of the solution. The construction of a variety of membranes that are selective for a variety of differently charged species has allowed this technique to become widely used and applicable to any ionic analyte.
Voltammetry is not a zero-charge technique. It utilises electrode potential to elicit an electron transfer reaction and subsequently measures the current produced by this reaction. Voltammetry is capable of assessing the concentration of any molecular species that has the ability to be an electron acceptor in the charge transfer reaction. Thus it is capable of assessing the concentration of polar substances in solution.

Quartz Crystal Microbalances (QCM) undergo a quantifiable change in their resonance frequency upon the adsorption of analyte species onto the surface of the crystal or the increase in pressure on the crystal due to increased mass on the surface. Surface Acoustic Wave (SAW) analysis uses a frequency that is perpetuated along a polymer chain, which again changes upon the adsorption of the analyte species onto the detector. Optics are also capable of being incorporated into such sensor designs, so long as the neural net responsible for the characterisation of a sensor response to the analyte in question is capable of recognising the response to a certain stimuli – i.e. has been conditioned with an appropriate training set before analysis.

1.4.2 - **Optical analytical techniques**

These sorts of receptor designs are subject to two significant shortcomings independent of the signal creation element. First, they must be coupled to a device that has the ability to instantaneously determine the pattern of response to the sensor. Secondly, they must have experienced the pattern produced by the analyte in an accurate training set. This results in a direct increase in the cost per unit produced because of the increase in computation required. Without the ability to discriminate between similar and complex pulse patterns generated by electrochemical analysis tools, such sensors have little ability to provide real information about the systems being analysed.

1.4.3 – **Electronic noses and tongues**

Electronic noses and tongues have been used to characterise the effluent flow from waste water treatment plants, the assessment of red and white wine, beer, honey, olive oil, the presence of mycological and the associated contamination of foodstuffs,
monitoring of air quality and composition on the international space station, and a large
variety of other tasks.\cite{11-15}

1.5 - **Ion mobility spectrometry. (IMS)**

The underlying principles of ion mobility spectrometry were first postulated in
the late 19\textsuperscript{th} century, and the general theory of the movement of gaseous ions through
electric fields solidified by 1910.\cite{16-18} However, it was not until the publication of a
paper on plasma chromatography in 1970 by Cohen et al.,\cite{19} (as the technique was
known at the time, along with ion chromatography and gaseous electrophoresis), that
the first commercial device was realised.

Traditionally, IMS utilises time of flight analysis of ions along an electronic
field that runs anti-parallel to the carrier gas flow. This prevents the progress of non-
ionic contaminants and increases the separation of analytical components as they
progress to the detector plate and requires several features for the technique to provide
useful information about the sample. The sample must be introduced to the ion source
(typically a chemical ionisation using $^{63}$Ni) in the gas phase, at atmospheric pressure.
The shutter grid prevents the entry of any un-ionised sample into the drift tube by
allowing only electro-statically charged entities to pass unhindered. The gas flow of the
drift tube exists so that only molecules that are transported by the electronic field reach
the detector, remaining independent of gas flow.

Volatilisation steps are required for solid or liquid samples to be analysed. The
addition of a liquid sample to a heated glass permeation tube provides a simple method
of liquid volatilisation, which was used for the introduction of sample into mass
spectrometers in the past.\cite{20, 21} The use of a gas chromatograph front end is a viable
technique for liquid introduction, and the separation provided by this front end provides
distinct improvement for the analysis of complex samples. Such techniques, in addition
to headspace extraction, solid phase micro-extraction, and membrane extraction
techniques have all been used to introduce organic components of aqueous samples for
IMS analysis.\cite{22-29}.

The advent of electrospray ionisation has permitted the direct introduction of
liquid samples into IMS and at the same time removed the need for subsequent sources
of ionisation. Due to the fact that illicit drugs generally contain an amine group, (offering [M+H]^+ product ions) they are excellent targets for IMS screening using even handheld devices.

Explosives form [M-H]^- ions due to the presence of multiple nitro groups, which can be assessed in the negative ion mode. It is with the analysis of explosives that the technique incorporates a pseudo-synthetic receptor.

The screening technique used in bench-top IMS devices does not use a direct liquid injection. A PDMS (polydimethylsiloxane) fibre or pad is swabbed over a surface to adsorb trace particles and residues, before being inserted into the thermal desorption chamber where the sample is volatilised and passed into the ionisation chamber. Is it possible to remove the need for instrumental analysis by using an easily prepared and observed selective adsorbent? Would such a technique alleviate the requirement to run the instrument in positive ion mode or negative ion mode separately?

One of the most utilised applications of IMS is found in customs and transit security settings. Screening for the presence of trace amounts of explosives, and other restricted or dangerous compounds has become a necessary preventative technique to decrease the likelihood of attack. IMS is useful not only for the identification of commonly encountered explosives and drugs, but also in the monitoring of chemical weapons and related compounds, and has found application in areas as diverse as petrochemical analysis, environmental monitoring, and pharmaceutical process monitoring.[31-47]

1.6 - Biological Receptors.

Homeostasis is a critical task for all living systems as the need to be able to respond to external stimuli of a chemical nature is essential. To perform these tasks, the ability of a specific feedback sensor (receptor) to recognise a specific molecule and provide the trigger for the response mechanisms of the body in relation to the stimuli is essential. Not only is the recognition critical, but accurate reporting of the recognition event is also critical.

This recognition is based upon a collection of individually weak intermolecular interactions such as shape, van der Waals, hydrophobic/hydrophilic, hydrogen bonding,
acid base, cation - π and π-π interactions. Natural receptors are generally flexible, to allow variation in conformation caused by the interaction of the binding pocket and a variety of analogous molecular guests. The subsequent change upon the host-guest interaction provides the trigger for specific signal transduction, based upon the molecule that is bound to the receptor. If a collection of individually weak, non-bonding interactions such as these can provide the significant selectivity of interaction and subsequent response, it follows rationally that specificity may also be produced biomimetically by the utilisation of similar interactions.

Receptors are not the only sorts of natural systems that utilise such recognition elements. The function of catalytic enzymes within natural systems also points to the applicability of synthetic receptor/enzyme moieties to hold reagents in a defined steric position, allowing transformation from the component parts, through the stabilised reaction intermediate and finally to the product of such a reaction.

Applications of chemosensory receptors range from industrial indicators to purification systems, transit and customs sensor screening, to use in field by intelligence operatives assessing the performance of illegal synthesis at clandestine sites.

The mimicry of neurotransmitter receptors (generally G-protein coupled receptors (GPCRs) and ligand gated ion channels) has been hindered by the difficulty in obtaining enough of the purified receptor to conduct x-ray crystallographic investigations into the structures of the bound and unbound active sites of these receptors.

As the ATS modulate endogenous neurotransmitter receptors, an understanding of how such receptors behave is essential to creating a successful bio-mimetic receptor for these neurotransmitter agonists.

1.6.1 – G-Protein Coupled Receptors (GPCR).

Many neurotransmitters, endocrine agents, local modulators and external stimuli all bind to GPCRs and cause GTP-dependant (Guanine Tri-Phosphate) signalling-response cascades. Sensitivity to specific agents that activate these receptors is due to the G-proteins, which are coupled to the receptors, and produce signals specific to the receptor and the ligand that induces the signalling cascade.\[^{48}\]
The crystal structure of bovine rhodopsin shows a grouping of seven helices surrounding a central core cavity bounded by Helices I-III and V-VII. Helix IV does not exist as a boundary to the cavity but makes structural contact only with helix III. Though the cavity is accessible through the cytoplasm, the loop from helix IV to helix V makes any access to this cavity through the membrane proximate periplasm impossible. This loop creates interactions with most of the side chains attached to the backbone of the other helices, most prominently a disulfide bridge with helix III.

Receptor response is generated via the change in conformation associated with ligand binding. Upon the binding event, a signalling cascade is created and the subsequent signal transmission occurs along secondary messenger cascades. It has been proposed that the specific response to a receptor may be related to the exact composition of the G-protein’s three subunits; however this is not yet fully resolved. Upon receptor activation, the exchange of GDP for GTP further triggers an increase in the concentration of cAMP (cyclic Adenosine Mono-Phosphate), and the phosphorylation of this second messenger system to promote the physiological effects due to receptor activation.

Figure 1.1: The ligand binding pocket of the human β2AR-T4L receptor with carazolol bound. Residues within 4 Å of the ligand are shown as sticks. Residues which form polar contacts with the ligand are shown in green, all other residues are grey.

The structure of the proposed active site in the β2-adrenergic receptor, obtained through homology modelling of the bovine rhodopsin GPCR, identified the active
site to be composed mainly of hydrophobic residues. Although the crystal structure of the same receptor was released in 2007, the nature of the binding site is not predicted to vary in character significantly. (52) Hydrophobic effects appear to be a major component in the interaction between receptor and ligand, in addition to specific interactions between hydrogen bond donor/acceptor capable functional groups.

1.7 - Computer Based Molecular Modelling.

To optimise the environment of potentially useful monomers for the creation of synthetic polymer receptors, or further probe the behaviour of natural receptors after x-ray diffraction crystal structures have been developed, *in silico* (in the computer) investigations have become of fundamental importance in the study of receptor-ligand interaction.

The application of computer-based investigation consists of two major approaches. The first approach uses information about the biological activity of a compound and its structure without knowledge of the three dimensional conformation of the receptor, while the second concentrates on the interactions that occur within the endogenous ligand-receptor complex to design new ligands that attempt to enhance those interactions present in the natural complex. The latter approach, Structure or Direct Computer Aided Drug Design, requires a high level of knowledge of the tertiary and possibly quaternary structures of the receptor’s active site, not only in the inactive conformation, but also in any of the various states of activation or inhibition. This structure is known as a pharmacophore, and allows a novel ligand to be tailored to interact with an active or inhibiting site of the receptor being investigated.

The majority of the GPCR family have still not had their structures experimentally determined. In order to develop a potentially effective medicinal agent against these receptors, the use of computer-developed models of these receptors has proven to be an effective method. (53, 54) Due to the size of the systems involved and current processor speed, protein modelling cannot be carried out efficiently using the detailed and accurate Quantum Mechanical (QM) approach as the number of variables is simply too great for an efficient and therefore useful method of structure determination.
The QM method considers all atoms involved as well as all electrons (depending on the level of theory, only valence electrons may be considered), and uses discrete functions to predict their behaviour. Unfortunately, due to the complexity of these calculations, the current upper limit for simulations of this type is \( \approx 100 \) atoms. Consequently ab initio (from the beginning) quantum mechanical methods are unable to accurately model the behaviour of a large protein in vivo (within the living), including solvation effects and indeed such methodologies are incapable of modelling even small molecular units in large amounts of solvent molecules. However, with the progression of processing power, the humble desktop computer is more than capable of performing complex modelling using molecular mechanics (MM), and depending on the system being investigated is capable of performing some semi empirical calculations.

The application of these computer aided modelling approaches to the design of synthetic receptors, and more specifically MIPs, is currently restricted to the former approach, due to the template governed geometry of the functional monomers in the pre-synthetic solution.

### 1.7.1 - Ab initio Quantum Chemical Methods (QM).

The level of complexity that is able to be introduced into efficient in silico screening has risen in conjunction with the increase in processor power. While the computing power has increased, the equations that describe quantum theory to allow submission into software packages have also undergone improvement; which are commercially available for QM calculation e.g. Gaussian\(^{[55]}\) Schrödinger\(^{[56]}\) Gamess.\(^{[57]}\)

Quantum chemical modelling is based upon solving the molecular Schrödinger equation and refers to the Hamiltonian associated with this molecule. The Hamiltonian operator in QM is associated with the observable total energy of the electrons and nuclei within the modelled system.

### 1.7.2 - Types of QM method.

The least complex application of the QM method is the Hartree-Fock (HF) method. The HF method is used in the determination of the ground state wave-function...
and the energy state of a given system. Its application produces non-linear results and must be solved numerically in an iterative process. Hartree-Fock theory is also known as Self Consistent Field (SCF) theory. Importantly, the electrostatic repulsions related to the electrons involved are not calculated discretely, but are instead averaged over the entire system.

Such assumptions have led to the development of methods, which have been termed post HF methods. These theoretical models utilise a correlation between the electrons involved in the system, and thus are a more accurate method of calculation than the HF method. As a direct result of the increased level of specificity the computational power required is increased, with reference to the ‘pure’ HF method. These methods are essentially HF calculations, which are subsequently corrected for the higher level of electron related theory.

Examples of post HF methods include:

Möller–Plesset perturbation theory of order \(x\) (MP\(_x\));

This theory deals with the electronic correlation via the Raleigh-Schrödinger perturbation theory that was originally postulated in 1934\(^{58}\)

Coupled–Cluster method (CC);

This theory was developed by Coester and Kümmel in the 1950s for the theoretical explanation of observations made in nuclear physics. It specifically deals with electron correlation and its use has increased after its form was slightly altered in a 1966 publication by Paldus \textit{et al.}\(^{59}\)

Configuration Interaction (CI);

Discretely identifies every electron and introduces them into the electronic correlation for the HF theory. It is highly computationally intensive that results in its application being restricted to small molecules.

Multi–Configuration Self–Consistent Field (MCSCF);

A combination of the varied configurations developed in CI methods and HF methods to couple the best aspects of both techniques.

Density functional theory (DFT) develops functions to describe the three dimensional electron density of a molecule/system. The theory uses functionals
(functions of functions) to describe this electron density. While originally believed to be too inaccurate for specific quantum calculations, improvements over time have advanced DFT to a method that experiences high levels of use in electronic structure calculation. It suffers from an inability to adequately describe intermolecular interactions such as van der Waals forces, global energy conformation, reaction intermediates and charge transfer interactions. Such inadequacies mean that its application to the modelling of biological or pseudo-biological systems is almost non-existent.

1.7.3 - Molecular Mechanics and force fields.

In comparison to QM, molecular mechanics is easier to use, requires lower computational power and still provides high similarity of results compared to the quantum methods, particularly when applied to molecular geometries.

Molecular mechanics uses Newtonian physics to represent an atom as a discrete ball of specific mass and radius. Bonds are represented as springs that contain a specific energy depending on the atoms to which they join and the type of bond. This formula attempts to calculate the geometries and surface electrostatic potential by modifying the bond lengths, angles and torsions to values that exist at equilibrium. These equilibrium values are determined by the identity of the atom in question, and its orbital hybridisation e.g. for carbon atoms, \( sp^3 \), \( sp^2 \), or \( sp \). Within molecular mechanics, electrons are ignored, while the electro-negativities and dipoles are represented by a static charge. Strain energy related to bond stretching, angle bending, rotations, torsion and interactions between non-bonded pairs of atoms all contribute energy to the molecule’s global energy, and it is this value that molecular mechanics is most useful in determining.

A force field is a mathematical representation of the forces that contribute to the energy contained within a molecule or a molecular system.

The Køllmann force field was developed specifically for use with nucleic acids and proteins, and has three variations on the original force field. The Køllmann United atom force field treats groups of specifically defined atoms as a whole unit, e.g. CH$_3$ as a single entity, Køllmann All Atom force field treats all atoms specifically, and the
Køllmann Hybrid force field allows the use of the United and All Atom variants simultaneously. The energy expression used in the Køllmann force field is shown in equation 1.1: where the terms are respectively: total energy term; energy of bond stretching term; energy of angle bending term; energy of rotation about bonds term; van der Waals/electrostatic energy term; and energy of hydrogen bonding term.

\[ E_{\text{total}} = E_{\text{stretch}} + E_{\text{bend}} + E_{\text{tor}} + E_{\text{vdw/ele}} + E_{\text{H-bonds}} \]  

**Equation 1.1**

The use of such force fields is not applicable for the calculation of small molecules due to the abbreviations of group definitions required for efficient calculation of large macromolecules. Other force fields exist such as MMFF 94, which more adequately handle small molecules:

\[ \text{MMFF 94} = \sum E_{B_{ij}} + \sum E_{A_{ijk}} + \sum E_{BA_{ijk}} + \sum E_{OOOP_{ijkl}} + \sum E_{T_{ijkl}} + \sum E_{\text{dist}_c} + \sum E_{\text{ang}_c} + \sum E_{\text{tor}_c} + \sum E_{\text{range}_c} + \sum E_{\text{multi}} \]  

**Equation 1.2**

Where in a four atom system:

- \( i, j, k, l \) are all terms relating to specific atoms involved within the force field.
- \( E_{B_{ij}} \) energy of bond stretching, related to the compression or extension from the natural (parameterised) bond length.
- \( E_{A_{ijk}} \) energy of angle bending away from the natural (parameterised) value.
- \( E_{BA_{ijk}} \) combination of both angle bending and bond stretching energies.
- \( E_{OOOP_{ijkl}} \) energy of bending of atom ‘l’ out of the plane defined by the boundaries \( i, j, \) and \( k. \)
- \( E_{T_{ijkl}} \) torsional energy relating to the rotation about a bond. This term is specifically related to double bonds.
- \( E_{O_{ij}} \) energy related to electrostatic interactions within the system of interest.
- \( E_{\text{dist}_c} \) distance constraint energy term
- \( E_{\text{ang}_c} \) angle constraint energy term
- \( E_{\text{tor}_c} \) torsion constraint energy term
- \( E_{\text{range}_c} \) distance range constraint energy term
- \( E_{\text{multi}} \) Multifit energy term, associated with multifit atom constraints.
Another force field that is commonly utilised to deal with the interactions between small organic molecules and their bio-macromolecular receptor targets is CHARMm\(^{[61]}\) (Chemistry at HARvard Macromolecular Mechanics), where:

\[
V = \sum_{bonds} k_b (b - b_0)^2 + \sum_{angles} k_\theta (\theta - \theta_0)^2 + \sum_{dihedrals} k_\phi [1 + \cos(n\phi - \delta)] + \\
\sum_{impropers} k_\omega (\omega - \omega_0)^2 + \sum_{Urey-Bradley} k_u (u - u_0)^2 + \sum_{non-bonded} \varepsilon \left[ \left( \frac{R_{\text{min}_{ij}}}{r_{ij}} \right)^{12} - \left( \frac{R_{\text{min}_{ij}}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{\varepsilon r_{ij}} \tag{Equation 1.3}
\]

- \(k_b\) is the bond force constant and \(b - b_0\) is the distance from equilibrium that the atom has moved.
- \(k_\theta\) is the angle force constant and \(\theta - \theta_0\) is the angle from equilibrium between 3 bonded atoms.
- \(k_\phi\) is the dihedral force constant, \(n\) is the multiplicity of the function, \(\phi\) is the dihedral angle and \(\delta\) is the phase shift.
- \(k_\omega\) is the force constant and \(\omega - \omega_0\) is the out of plane angle.
- \(k_U\) is the respective force constant and \(U\) is the distance between the 1,3 atoms in the harmonic potential.

Nonbonded interactions between pairs of atoms \((i, j)\) are represented by the last two terms. By definition, the nonbonded forces are only applied to atom pairs separated by at least three bonds.

The van Der Waals (VDW) energy is calculated with a standard 12-6 Lennard-Jones potential and the electrostatic energy with a Coulombic potential. In the Lennard-Jones potential above, the \(R_{\text{min}_{ij}}\) term is not the minimum of the potential, but rather where the Lennard-Jones potential crosses the x-axis \(i.e.\) where the Lennard-Jones potential is zero.)
1.7.4 - Semi Empirical Molecular Orbital Theory. (SE-MO)

One of the most robust and longest surviving (SE-MO) models available for simulative chemistry is AM1. This theoretical model based on experimentally determined parameters (enthalpies of formation and dissociation constants) has experienced a widespread popularity throughout the chemical profession, and is found as a simulative method for SE-MO in a variety of commercially available and independently developed software packages including MOPAC, Gaussian, Hyperchem, SPARTAN, AMBER, Dynamo, VAMP, GHEMICAL, and BOSS amongst others.

Despite such improvements and optimisation, familiarity and availability coupled with reliability and confidence in the model have kept the AM1 model at the forefront of the research area. Although further parameterisation has been developed that has the potential to be more accurate, a lack of full parameter optimisation or distribution has not allowed such methods to pass into common usage. The techniques used to parameterise and improve the PMx SE-MO model however have assisted the optimisation of other semi empirical methods, specifically the AM1 method, which was published in an almost unchanged format with the exception of parameter optimisation under the moniker RM1.

Table 1.1: Description of the parameters for the AM1 semi-empirical molecular orbital method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(U_{ss})</td>
<td>s atomic orbital one-electron one-centre integral</td>
</tr>
<tr>
<td>(U_{sp})</td>
<td>p atomic orbital one-electron one-centre integral</td>
</tr>
<tr>
<td>(\beta_s)</td>
<td>s atomic orbital one-electron two-centre resonance integral term</td>
</tr>
<tr>
<td>(\beta_p)</td>
<td>p atomic orbital one-electron two-centre resonance integral term</td>
</tr>
<tr>
<td>(a_A)</td>
<td>atom A core-core repulsion term</td>
</tr>
<tr>
<td>(G_{ss})</td>
<td>s–s atomic orbitals one-centre two-electron repulsion integral</td>
</tr>
<tr>
<td>(G_{sp})</td>
<td>s–p atomic orbitals one-centre two-electron repulsion integral</td>
</tr>
<tr>
<td>(G_{pp})</td>
<td>p–p atomic orbitals one-centre two-electron repulsion integral</td>
</tr>
<tr>
<td>(G_{pp'})</td>
<td>p–p’ atomic orbitals one-centre two-electron repulsion integral</td>
</tr>
<tr>
<td>(H_{sp})</td>
<td>s–p atomic orbital one-centre two-electron exchange integral</td>
</tr>
<tr>
<td>(a_i)</td>
<td>Gaussian multiplier for the (i^{th}) Gaussian of atom A</td>
</tr>
<tr>
<td>(b_i)</td>
<td>Gaussian exponent multiplier for the (i^{th}) Gaussian of atom A</td>
</tr>
<tr>
<td>(c_i)</td>
<td>radial centre of the (i^{th}) Gaussian of atom A</td>
</tr>
<tr>
<td>(\zeta_s)</td>
<td>s-type Slater atomic orbital exponent</td>
</tr>
<tr>
<td>(\zeta_p)</td>
<td>p-type Slater atomic orbital exponent</td>
</tr>
</tbody>
</table>
The utility of semi empirical methods is apparent in some significant applications; molecular dynamics simulations in which a large system undergoes iterative calculations to model the dynamic behaviour of its components; the calculations of large systems which contain segments that are inefficiently described by the approximations used in MM calculations, and the submission of large quantities of smaller molecules. Accuracy in such systems is dependent on the effective combination of its formalism and approximations, the training set of molecules, and the quality of the numerical techniques used for parameterisation.\cite{71}

Although the optimisation of such parameters (Table 1.1) provided a significant decrease in the errors obtained by the AM1 method, it is interesting to note that when concerning the bond angles, AM1 is still the most accurate of the methods studied by Rocha et al. \cite{71}

1.7.5 - **Hybridisation of modelling methods.**

Hybrid methods (termed Quantum Mechanics/Molecular Mechanics [QM/MM] methods) describe an approach where the majority of the system is defined by a MM approach with the exception of the active site, or the area of interest, which is defined and modelled via QM methods. For these systems, any QM approach desired can be applied to the smaller critical area, while the majority of the system is defined by the molecular mechanical description. The embedding of QM fragments in MM models was first proposed by Warshel and Levitt to investigate the mechanism of action exhibited by lysosyme. \cite{73}

Accurate definition of the boundary condition between the QM and MM regions is of the utmost importance in these hybrid methods. Without adequate and accurate definition of this area, artefactual error is introduced during computation. A variety of differing methods for the definition and treatment of these boundary areas have been proposed and include:

The link atom approach; uses hydrogen atoms to cap any “broken” bonds that cross the QM/MM boundary.\cite{74} Such link atoms do not participate in the MM segment of the model, though whether or not this should be undertaken has been questioned. Such combination requires the QM/MM boundary to be sufficiently removed from the
active area to prevent any artefactual errors encroaching upon the QM calculation at this point.

The local-Self Consistent Field approach: (Figure 1.2) where a frontier bond (i.e., one that is crossing the QM/MM boundary) is described by a strictly localised bond orbital (SLBO). The SLBOs are obtained from model compounds and are assumed to be transferable as long as the QM/MM boundary is far enough from the reaction site. The rest of the MOs, which are orthogonal to the SLBO are obtained by a local self–consistent calculation.\[^{[75, 76]}\]

The connection atom approach: where Antes and Thiel (Figure 1.2) assigned a \(sp^3\) orbital that contains one electron to every MM atom that binds to a QM atom. Parameterisation of these atoms is based on the QM method used.\[^{[77]}\]

The pseudo-bond approach expanded upon the Thiels and Antes approach and rather than defining the QM/MM boundary bonds as \(sp^3\) orbitals containing one electron they are substituted by pseudo-atoms, which contain effective core potentials and one free valence electron.\[^{[78]}\]

![Figure 1.2: Schematic representation of (A) Connection atom approach, (B) Semi Consistent Field theory approach.\[^{[79]}\)](image)

Hybrid methods, such as the ones listed above have been used to study a large number of biological systems. Such models have been the major target of the coupled approaches to molecular modelling, especially in relation to the action and structure of enzymes. For such systems, the classical MM approach for larger molecules will not suffice. Almost all enzyme reactions can be adequately summarised using the Born-Oppenheimer approximation, in which the sum of the electronic energy and the nuclear repulsion provides a potential energy function, or potential energy surface (PES), governing the inter-atomic motions.\[^{[79]}\]
The potential energy surface for the atoms involved in the enzyme bond making and breaking processes is usually treated via the QM fragment of the calculation. Traditional force fields that describe the energy surface of substrates have been very successful in optimizing equilibrium structure and give reasonably accurate results for the energy contours for small-amplitude vibrations around such structures. However, the necessary increase in complexity of the enzyme active site requires a better description in the calculations.\textsuperscript{[80]} The majority of accurate quantum mechanical studies refer to equilibrium structures in the gas phase, whereas experiments are usually performed in condensed phases and include dynamic averaging effects by both intramolecular (e.g. solute vibrations) and intermolecular (e.g., solvent vibrations) degrees of freedom.\textsuperscript{[81]}

1.8.1 - Synthetic receptor design schemes.

A crucial factor in creating a synthetic receptor, especially in biomimetic designs, is ensuring that the areas of interaction between the host and guest are maintained in a structurally preorganised environment. Failure to maintain such rigidity results in the collapse of the binding ‘pocket’ upon desorption of the ligand from the receptor surface preventing later re-adsorption into the cavity.

A simple synthetic receptor design, which is a good host, if slightly unselective for small organic molecules in aqueous solution, is cyclodextrin. (\textit{Figure 1.3}). These receptor mechanisms are cyclic carbohydrates, which exhibit a hydrophobic core region and three hydroxyl groups, one on the smaller rim of the ring, and two on the larger rim of each sugar unit.

\textbf{Figure 1.3: Schematic of the Cyclodextrin design of synthetic receptor.}\textsuperscript{[82]}
The hydroxyl groups are viable sites for simple synthetic modification to create a selective binding environment for the targeted guest. Cyclodextrins have been modified by the addition of a lariat arm, where a long hydrophobic arm is covalently linked to the upper rim of the cyclodextrin. Once in an aqueous environment, the lariat arm is encapsulated by the cyclodextrin and is displaced by the guest molecule when it enters the hydrophobic pocket. Though cyclodextrins are a very simple receptor design, their inherent asymmetry creates the ability for specific chiral recognition. Dimerisation of cyclodextrins has been shown to enable selective binding of aromatic guests.

The crown ether family (Figure 1.4) are macrocyclic, polyethers and have been used as receptors for cationic and anionic species of guests as well as neutral species, especially in biological environments. Following their development by Pederson in 1967, the crown ether design has been utilized to selectively recognize many types of ionic guests amongst many other small molecular guests. In their simplest examples, they are cyclic molecules whose structure is derived from the repetition of ethylenedioxy \((\text{CH}_2\text{CH}_2\text{O})_n\), where any unit whose \(n \geq 4\) is named by the \(3n\)-crown-\(n\) form.

![Figure 1.4: Crown Ether synthetic receptor design.](image-url)
The cryptand family of macrocycles (Figure 1.5) is composed of two tertiary amine anchors joined by aliphatic chains allowing the orientation of the amine’s lone pair of electrons to point either inwards or outwards. Thus, the macromolecule either encapsulates the lone pairs of the amine groups with the aliphatic chains, or points them along the axis of the macrocycle. Subsequently, Lehn used the amine anchor units, but used the (CH$_2$CH$_2$O)$_n$ linker units.

A combination of the two designs, comprising the more constrained crown ether backbone coupled with a flexible “lariat” or “pendant” arm (largely the same technique used with cyclodextrins), for the encapsulation of the guest molecules upon its interaction with the crown backbone were also developed. Such arrangements generally selected the attachment of the pendant arm to a nitrogen atom located within the main ring system. Attachment here is preferred due to the potential for interaction with the guest regardless of the side of the ether backbone from which the guest approaches.

Calixcrowns (Figure 1.6) are a combination of the cyclical oligomerisation of phenol and formaldehyde (calixarene; Figure 1.6) and the crown ether design. These macrocyclic, synthetic receptors have been instrumental in the study of electrostatic, hydrogen bonding and cation-π interactions. Although such macromolecules are capable of significant anionic and neutral molecule binding, it was their ability to bind cations that was the ultimate goal of the original researchers. Upon this realisation, the pioneers Pedersen, Lehn and Cram were jointly awarded the 1987 Nobel Prize in chemistry. Their application has also been found in the selective binding of Caesium, specifically due to its presence in radioactive waste. It has also been found in ion selective electrodes due to the ability of the macromolecule to selectively detect certain types of cations including alkali metals, lanthanides, both transition and heavy metals,
and also alkyl ammonium ions. Any polyoxa- and polyaza- macrocycles containing a trigonal symmetrical arrangement of binding sites have been shown to be capable of complexing catecholamines by interaction with their ammonium ions. \cite{90-113}

![Figure 1.6: Calixarene-tube design (left), Calix-crown design (right).\cite{96}]

A cyclophane (Figure 1.7) is any aromatic structure bridged by an aliphatic chain between non-adjacent positions on the aromatic structure. Such bridging is generally associated with significant bond angle and bond length strains. As a result, they generally exhibit a cavity, which is generated underneath the bridging chain. Cyclophanes have been shown to be capable of chiral discrimination, selectively binding L-amino acids eight times more favourably than D-amino acids.\cite{114} This property has major implications within natural systems due to their inherent chirality, but also to separate racemic mixtures of chiral, aromatic guests thus providing tools for improved workup and faster synthetic process for the synthetic chemist.

Proteins that interact with steroidal hormones and small molecule neurotransmitters contain very large hydrophobic cavities. They are characterised by the aromatic amino acid side chains, which are directed into this cavity and promote the binding of steroids, and encapsulate these molecules inside the cavity. The effect of this encapsulation on the selectivity of a biomimetic receptor has been demonstrated by Deidrich et al. via the use of a tri-cyclic cyclophane (Figure 1.7).\cite{115} In this study, an increase of 2Å in cavity depth was shown to exhibit an increase of 0.9 kcal mol\(^{-1}\) in the free binding energy when the experiments were conducted in \(d\)MeOH, (deuterated methanol).
A full understanding of neurological function remains unknown; however the implications for probing the endogenous action of neurotransmitters are far reaching if a biomimetic receptor can be constructed for these structurally conserved aromatic amines. Once a highly selective and efficient mimic has been created, the possibility of using a similar process and design for the biomimetic binding of other important hormones and neurotransmitters within the body becomes more significant. Schrader et al. [116] have investigated the use of molecular clip, xylylene bisphosphonates, as a class of synthetic receptor that mimics the host-guest interaction environment of the $\beta_2$-adrenergic receptor (Figure 1.8). Both electrostatic and hydrogen-bond interactions are evident in the complexation of the ammonium functional group by the aspartate residue. This interaction is reinforced by stabilisation of the ammonium cation by the three surrounding aromatic rings. Both the aromatic hydroxyl groups and the aliphatic hydroxyl group are H-bonded to a serine hydroxyl group and the aromatic ring is bound by entry into a hydrophobic cleft, flanked by two phenylalanine residues that not only create hydrophobic van der-Waals interactions, but also $\pi-\pi$ stacking and electron interactions.

Although the $\pi-\pi$ interactions may be present in this proposed representation, it is most likely that they are the weakest of the intermolecular forces present due to low dipole-dipole strengths produced by having an electron poor, catechol ring flanked by two electron rich phenylalanine rings.

Another form of synthetic receptor that has been extensively studied is the molecular clip/tweezer molecule. In an aqueous environment, hydrophobic attractions are often the major force in the interaction of host-guest complexes.
Reek et al. demonstrated that the complexation strength of 1, 3-dihydroxybenzenes is a combination of many smaller, weaker intermolecular forces such as π-π ring current interactions, cavity hydrophobic effect, and hydrogen bonding (Figure 1.9). This study was completed using molecular clip style molecules with one side wall, no side walls or two side walls. The effect of increasing the size of the aromatic moiety on the walls of the clip was found to be a two edged blade in that although the van-der Waals interactions were significantly increased, the electrostatic repulsion was also increased.
Through synthetic variation on the xylylene bisphosphonate backbone, it was found that the \textit{m-} and \textit{p-} variants were both effective in binding noradrenaline and adrenaline. The \textit{m-} variant showed a higher binding affinity. Molecular modelling studies indicated that this was due to the reduction of the PO$\cdots$HN$^+$ distance that strengthened both the hydrogen bonds, and the electrostatic interactions. The proposed chelate binding method was confirmed in this study by a $\sigma=0.2$ ppm downfield shift in the $^1$H NMR of the lone aromatic proton, which shows that like many natural receptors, when the guest molecule becomes proximate to the receptor an induced fit is created. This work represented the first time that a synthetic receptor had been created that was selective for the 1, 2- amino alcohol functional groups.\cite{118}

Further modification of this structure to achieve a biomimetic state was later performed by the incorporation of this xylylene bisphosphonate design into a macrocyclic system. It included more preorganisation and consequently, maximisation of the intermolecular interactions in the host-guest complex. The cyclisation of this receptor removed the opportunity for competitive formation of hydrogen bonds between the bisphosphonate moiety and the catecholic OH groups. By cyclisation, the receptor was able to specifically bind to the amino alcohol tail of adrenaline effectively shielding the catecholic OH groups inside the hydrophobic cavity.

Another similarity to the biological receptor was shown to exist upon shaking. The formation of micelles was observed, indicating the hydrophobic nature of the synthetic receptor, another biomimetic similarity to the natural receptor state. Unfortunately, this molecule exhibited a very high self-association in water, and was unable to distinguish between amino acids and adrenaline-like molecules.\cite{118}

The addition of further hydrogen bonding sites to selectively bind the catechol group of adrenaline resulted in the generation of the first shape selective, synthetic adrenergic receptor, which is biomimetic and functions in water.

Further modification and experimentation within this framework led to the discovery of the receptor molecule shown in Figure 1.9. This receptor exhibits a very high selectivity for adrenaline and noradrenaline, and the structural rigidity of the hinge segment was confirmed by molecular dynamics calculations. The lower segments of the receptor still exhibit small amounts of mobility, however this enables an induced-fit style binding mode. The lipophilic nature of this molecule prompted its inclusion into
membrane layers, where it was discovered to aggregate in a similar fashion to the natural adrenergic receptors.\textsuperscript{119, 120}

1.8.2 - Molecular Imprinting design for synthetic receptor construction.

The first use of a molecular imprinting technique was performed by Dickey in the 1940-50s, where his use of Pauling’s theory of antibody formation led to the use of a silica gel matrix to imprint the shape of dye molecules.\textsuperscript{121}

Mosbach \textit{et al.} described the possibilities for techniques related to the creation of template specific synthetic antibodies and receptors.\textsuperscript{122} It was from this paper that MIPs were shown to exhibit similar behaviour to antibody based immunoassays, and the potential for such a screening technique became evident. This involved the imprinting of a MethAcrylic Acid (MAA)-co-EthyleneGlycolDiMethAcrylate (EGDMA) co-polymer with mimics for 2 different classes of drugs (both in their method of action and their structure), theophilline (a bronchodilator) and diazepam (valium, a benzodiazepine tranquiliser). Post polymerisation, the monolith was ground with a mortar and pestle before the imprinted particles were washed with methanol/acetic acid to remove the template molecules, before the polymer particles were dried and stored. The milling process is necessary to allow faster kinetics for the extraction and eventual re-introduction of the template molecule into the imprinted cavities.

In the most common form of molecular imprinting, a target molecule (or template) is added to a solution of functional monomer in a specific stoichiometry. Such stoichiometries are generally implemented at arbitrary ratios or determined through the application of \textit{in silico} screening using molecular dynamic modelling of the template/functional monomer transient associations. Such modelling is unable to give a 100\% accurate representation of the energy of interaction between the template and functional monomer units. However, the values obtained in such modelling experiments are capable of a comparative selection of the T: FM ratios that exist in the lowest global energy state of the selections modelled.

Subsequently, the stable association is further probed by performing NMR titrations, whereby the chemical shift of the protons involved in the template functional
monomer interactions can be tracked to confirm the predicted interaction ratio from the MM simulations. This titration involves the addition of molar equivalent aliquots of functional monomer to a solution of template molecule. NMR investigations also provide the opportunity for the calculation of the association constant ($K_a$) of the T:FM cluster. This value is obtained through the Benesi-Hildebrand treatment where the reciprocal change in chemical shift ($1/\Delta \delta$) is plotted against $1/[FM]$, which results in a linear function of gradient $1/K_a \Delta \delta_{\text{max}}$ and ordinate intercept of $1/\Delta \delta_{\text{max}}$. Such techniques are the link between the *in silico* screening and the subsequent synthesis of the imprinted polymer.

**Figure 1.10:** Thermal initiation of the radical state in AIBN.

Polymerisation is generally started by the cleavage of an initiator molecule to form a highly reactive free radical defined as the presence of un-paired electrons, which attack areas of electron density, thus transferring the radical state to the site from which the electron was scavenged. The most commonly used radical initiator in MIP synthesis is AIBN (azobisisobutyronitrile, *Figure 1.10*). The exposure of such initiator molecules to energy is usually achieved through the provision of heat or UV light.

By performing this polymerisation in the presence of the target molecule, a matrix is created through which, once the target molecule is removed, selective binding of the analyte can occur (*Figure 1.11*). A factor in the use and development of molecularly imprinted polymers is the formation of the same non-covalent interactions that are found in native receptors in the same spatial orientation.
There have been two distinct types of molecular imprinting published, though variants of the type of polymerisation and form of the end product polymers also exist. Essentially these variants refer to the type of template/functional monomer interactions that are used to induce the imprinting effect and the resultant specificity.

Covalent imprinting, first realised by Wulff *et al.*, relies on the covalent synthesis of a linkage between functional monomer and the template.\cite{124} This creates a homogeneous dispersion of binding sites. However there is a need for the covalent bond to be cleaved through harsh chemical reaction such as acid/base hydrolysis, for the template to be extracted from within the highly cross-linked matrix. Thus the template extraction is generally kinetically unfavourable. Due to covalent linkage to the template, fewer non-specific cavities are produced in the polymer, which theoretically makes the polymer more specific, and capable of outperforming the interaction in a non-covalently imprinted polymer. As the desired outcome is a specific cavity related to a specific template, it is often necessary to either purchase the template already bonded to the functional monomers or to synthesise both the monomers themselves and the monomer/template entity for polymerisation. Both of these routes can be both expensive and time consuming.

The second method was described by Mosbach *et al.* and involves the utilisation of van der Waals interactions, hydrophobic interactions, electrostatic interactions, hydrogen bonding interactions, cation-π and π-π interactions.\cite{122, 125} The non-covalent methodology is the more biomimetic of the two techniques, and as a result of the weaker interactions a combination of several interactions must be used to induce selectivity. This is one of the strengths, but also one of the drawbacks of the technique. Due to the necessity of adding stoichiometric excesses of functional monomer, and the transient nature of the pre-synthetic complex, binding site homogeneity is generally not exhibited.

The plethora of choices that present themselves to an imprinting technician with respect to the functional monomer and the ratio of T:FM, have placed this technique at the forefront of the research in this field. Reductions in binding site heterogeneity and disorder have occurred due to the use of powerful *in silico* screening methods and the power of multiple parallel syntheses offered via combinatorial synthetic methods.
The two methods (covalent and non-covalent) have been coupled via use of the binding site pre-organisation, imparted to the polymer by the covalent method of imprinting, in tandem with elegantly designed non-covalent interactions for analyte recognition. Whitcombe et al. reported the creation of a molecularly imprinted polymer for a tripeptide (Lys-Trp-Asp) combined with a sacrificial spacer (o-hydroxybenzamide; Figure 1.12), which was subsequently removed by hydrolysis of the covalent bond between the polymer and the spacer, leaving a carboxyl group in the exact position needed to create a non-covalent interaction with the tripeptide. In this case the sacrificial spacer can be easily cleaved producing CO₂ through the hydrolysis of cholesterol-4-vinylphenyl carbonate ester. This combination method provides utility, whereby molecules with few useable points for interaction can be imprinted and later non-covalently adsorbed into the specific cavities.

1.8.3 - Physical forms of Imprinted polymers.

Historically, MIPs have been synthesised as bulk polymer monoliths that are subsequently milled to form micrometre diameter particles, however specific applications require the adhesion of the MIP to a solid support in the same fashion as ion exchange resins and immunoassay columns for binding assays. Such polymers have been used as chromatographic stationary phases for chiral separation of small molecules and capillary electrophoresis of specific molecular entities. The monolith that is produced in such reactions is milled to enhance the kinetics of the mass transport of the template/analyte molecules from the solution phase onto the solid phase adsorbent.
Although the polymer monolith must be subsequently ground to the desired particle size, it is perhaps the simplest and most widely applicable physical structure for imprinted polymers, many other physical forms have been and are currently being investigated. Tailoring of the physical properties of the synthetic receptor to the environmental conditions in which such selective and specific recognition must occur provides another avenue for optimisation of the host guest interaction. Such synthetic tailoring to control the properties of the product polymer has resulted in a variety of product forms.

Suspension and emulsion polymerisation creating micro-bead and nano-bead spheres have been developed.\textsuperscript{[126-132]} Such techniques produce a uniform distribution of particle size, and a high surface area to volume ratio in comparison to the monoliths produced during bulk polymerisation. Precipitation polymers are created through significantly decreasing the concentration of the polymer reagents in the synthesis solution. This results in higher rates of polymer chain termination than in the bulk polymers as the concentration is significantly lower than the bulk polymerisation process, and is also subject to slower polymerisation due to increased dispersion of monomers.

Surface grafted MIP films and core-shell particulate MIPs have been developed as attempts to overcome the kinetic issues associated with the template traversing the cross-linked polymer network to reach the active site (experienced by larger and sterically bulky template/analyte molecules).\textsuperscript{[133-136]} Fabricated macro and microstructures and thin polymer films (MIP\textsubscript{f}) have been methods through which the mass transfer from the solution phase onto the solid phase is enhanced.\textsuperscript{[137, 138]}

Shea \textit{et al.}, have successfully reported the coupling of the bulk polymerisation design with the specific imprint layer existing upon the surface of the polymeric structure. Their work showed that it is possible to imprint the epitope of a target protein on the surface of a MIP\textsubscript{x}, allowing specific interaction to occur while removing the need for the macromolecule to negotiate its way through the polymer network.\textsuperscript{[139]}

1.8.4 - \textbf{Performance of MIP – Analysis: Specificity and selectivity}
The analysis of MIP is generally carried out using analytical instruments such as GCMS, FT-IR, UV-VIS or HPLC. Polymer samples of known quantity are added to a container, where a quantified aliquot of analyte is added to the polymer for a controlled period of time. The particulates are filtered out and the analyte sample quantified by the analytical method of choice.

Solid Phase Extraction (SPE) has also increased in use, as not only does flow past a surface increase the rate of adsorption onto the surface, but the sequestration of the polymer particles makes for easier handling.

In addition to SPE, MIP particles have found application as the stationary phase for chiral separation, or separation of similar analogues using HPLC. MIP selectivity is classified by comparison with a non-imprinted polymer partner – the NIP. The NIP is produced in the same fashion as the MIP except for the absence of the template molecule. A simple comparison using the equation below gives a selectivity factor (I) that can be used to compare the performance of a group of MIPs quickly and efficiently.

\[ I = \frac{B_{\text{MIP}}}{B_{\text{NIP}}} \]  \hspace{1cm} \text{Equation 1.4}

1.8.5 - Quantification of interaction between analyte and MIP Isotherms

The rate at which the analyte is able to interact with the imprinted polymer can be characterised mathematically by the application of an applicable model for the binding event. Such methods utilise the instrumental quantification of the binding by plotting the fraction of the analyte concentration that remains in solution (\(F\)) and by subtraction from the initial concentration (\(B\)), the quantity that has adsorbed onto the polymer.

As the interaction with the surface of the polymers is governed by the structure of the imprinted cavities vs. non-specific surface, the accurate modelling of the interaction relies on the choice of the most applicable view of the structure of these sites. While the Langmuir isotherm is a good model for homogeneous interaction
(through covalent imprinting), the non-covalent pre-polymerisation solution’s dynamic nature generally results in the formation of heterogeneous sites. Although linear regression analysis is possible if the recognition sites are homogeneous and results in a line of single slope that is equal to the $K_a$ and an abscissa intercept equal to the total number of imprint cavities, heterogeneous sites of interaction present a more complex model to fit.

Most MIP exhibit a curved Scatchard plot (Figure 1.13), which indicates that heterogeneous binding takes place.

A homogeneous interaction is one in which the surface and all adsorbed molecules interact in the same way due to the equivalent nature of each interaction site. Conversely, heterogeneous interaction describes the interaction between a surface and adsorbed molecules in which there are multiple types of interaction occurring, caused by fundamental differences in the sites available for interaction.

The Langmuir isotherm is a semi-empirical model for gaseous adsorption, and requires adherence to conditions for accuracy. It assumes that the surface of the adsorbent is uniformly structured, and that all the adsorption sites are equivalent. Adsorbed molecules are assumed not to interact with each other. It assumes that all adsorption occurs through the same mechanism and that the maximum adsorption possible creates only a monolayer.

One of the first techniques to address this issue was the bi-Langmuir plot, or limiting slopes method (Figure 1.14). This technique only allows for the existence of two binding sites that differ in their interaction strength, the high and low affinity sites.

![Figure 1.13: a) Langmuir analysis and b) the Scatchard plot of the saturation binding curve.](image)

One of the first techniques to address this issue was the bi-Langmuir plot, or limiting slopes method (Figure 1.14). This technique only allows for the existence of two binding sites that differ in their interaction strength, the high and low affinity sites.
This method was shown to provide unreliable data when applied to the same system in repeated experiments.

![Bi-Langmuir technique demonstrating high and low affinity sites.](image)

The Freundlich isotherm is more suited to modelling these types of systems and involves the logarithmic plot of $\log_{10} B$ v $\log_{10} F$, and the isotherm parameters are obtained through the Scatchard equation (Equation 1.5), given by the linear function that takes the form of $y=mx+b$.

$$\frac{B}{F} = \left( \frac{1}{K_d B} \right) + \left( \frac{N}{K_d} \right)$$  \hspace{1cm} \text{Equation 1.5}

1.9 - Sensors.

The general sensor design idea is one that involves a recognition element and a signal transduction element coupled to a reporting element. In the case of synthetic receptors, specifically MIPs, while the recognition element is selective and specific, reporting such events in real time has proven difficult and is thus the target of much research effort across the world.

1.9.1 - Signal transduction from MIP recognition elements
While molecularly imprinted polymers are an efficient and simple method of recognition for molecular and macromolecular guests, they have generally lacked the ability to easily communicate such recognition events. Historically, complex and costly laboratory analysis equipment such as Gas Chromatography (GC) and High Pressure Liquid Chromatography (HPLC) coupled with various detector designs has been the manner in which the observation of adsorption events has been quantified. Such instrumental techniques require high financial cost, and suffer lack of portability.

For fast and effective notification of recognition events, a variety of approaches are available. The majority of such signal transduction elements have used either optical or electrochemical methods to propagate the signal transfer between the recognition and reporter elements of the sensor systems.

The ability to provide quantitative information about the target molecule is probably not of the utmost importance for a presumptive test. If the cut-off limit is variable, the sensor would have a wider application and its potential application to varied tasks would be similarly enhanced. The ability to detect and signal the presence of a given target above that threshold concentration is of the utmost importance for sensor development. In most realistic settings, the ability to detect presence would likely result in subsequent analytical investigation of the concentration of the analyte species. Such analysis is essential not only for the identification of the presence of analyte but as a step to confirm the sensor signal.

Optical methods of signal transduction have included the use of fluorescently labelled guest molecules. D-glucose was fluorescently derivatised and the kinetics of the adsorption of the labelled analogue to a D-glucose imprinted polymer was monitored via FTIR and fluorescent microscopy; The derivitisation of polymer particles grafted to a silica base-structure using 3-aminoo quinoline showed that higher levels of this graft caused significant reductions in analyte binding presumably due to the cavities being sterically ‘blocked’ by the fluorescent graft. The incorporation of iodinated monomers into the MIP, which enhanced chemical sensing, resulted in a new MIP design approach, allowing for room temperature phosphorescence. Fluoranthene was shown to create phosphorescence upon interaction with the iodine in the polymer structure in water. In addition to the long (100µs) delay time, this significantly reduces the response produced by non-specifically bound analyte or interfering agents in the
sample matrix. Similarly, Jenkins et al. showed that incorporation of Europium into a MIP framework for the hydrolysis product of the nerve agent soman, produced excitation and subsequent emission at the wavelength specific to Europium(III) upon the binding of the analyte at the polymer’s active site.\[146\]

Fluorescent components can also be incorporated into the polymer makeup by using fluorescent monomers. The use of 2, 6-bis (acrylamido) pyridine was shown to have increased fluorescent intensity at the time of the template adsorption event at the templated sites.\[147\] This work showed that a zinc (II) porphyrin functionalised monomer produced dramatic reduction in fluorescence upon the template recognition event when incorporated into a system templated by cinchonidine. A phenylboronic acid anthracene monomer has been utilised by Gao et al. to produce selectively increased fluorescence upon the reintroduction of D-fructose to the related MIP.\[148\]

Generic light based analysis tools have also been used to quantify the recognition and selectivity of MIPs. UV-VIS spectrophotometry and FTIR being two of the most commonly used instrumental techniques. However, these systems are subject to operational interference based on impurities etc. in the sample matrices; FTIR being especially sensitive to the presence of any aqueous material within the sample. UV-VIS spectrophotometry is subject to the nature of the target molecule. For such a species to absorb light in the UV-visible spectrum, the target must possess functional groups capable of absorbing light within the UV-Vis spectrum. Without this property, monitoring the change of concentration over time is not possible.

Techniques used to study the recognition of biologically active molecules by biological receptors are intrinsically useful in the study of biomimetic receptors such as MIPs. Although used previously to study biological systems, Surface Plasmon Resonance (SPR) has experienced few, but growing applications in MIP research. A surface plasmon is an electromagnetic wave on the surface of a material that propagates parallel to the metal/solvent surface. Such waves are highly sensitive to the nature of the surface through which the wave is propagated and changes the refractive angle of the light beam used to create the plasmon. The application of this technique to biological systems involves the coating of the surface with receptor proteins, whose refractive index changes upon the addition of analyte. The first application of this technique to imprinted polymers was in 2002 where Ping et al. imprinted a 1,4-
butanediol diacrylate-methacrylic acid polymer with L-phenylalanine ethyl ester. \textsuperscript{[149]}

The elution and adsorption procedures were investigated \textit{in situ} (in the place) by surface plasmon resonance. The changes of refractive angle during elution procedure suggest that the MIP is prepared through non-covalent interactions.

Reflectometric Interference Spectroscopy (RIfS) utilises a beam of light directed onto a recognition element coated, glass transducer. Characteristic interference patterns are formed and the uptake of an analyte by the layer will result in a shift of these interference patterns. This shift corresponds to the change of the optical thickness layer (the product of the physical thickness and the refractive index). \textsuperscript{[150]}

A fluorescent indicator (\textit{N,N}-dimethyl ethylenediamine 7-nitrobenz-2-oxa-1,3-diazole) was used as an indirect method of signalling analyte adsorption by Shimizu \textit{et al.} to identify varied molecularly small aromatic amines from each other using an array of MIPs and Linear Discriminant Analysis (LDA). \textsuperscript{[151]} The ability to couple a MIP array and a simple competitive displacement led to a 94\% success rate at analogue identification. This technique relied on the UV-Vis absorbance of the supernatant solution and a comparison of the absorbance before and after analyte addition.

An example of a sensor based on a flow chemiluminescence application was related to the detection of pesticide 2, 4-dichlorophenoxyacetic acid residues. \textsuperscript{[152]} The luminescence was enhanced via the addition of luminol. The continuation of this research was also published in Feng \textit{et al.} \textsuperscript{[153]}

Soon after, a clenbuterol (CL) sensor based on MIP was published. \textsuperscript{[154]} First a MIP was created for CL, followed by sample extraction and readsorption. Following this, a reaction that involved the addition of potassium permanganate and formaldehyde in a solution of phosphate ions was conducted in which photons are emitted. This emission was assessed and found to be linearly related to the concentration of CL. This design made the detection of ng.L\textsuperscript{-1} concentrations of CL in animal urine possible.

Electronic signal transduction may take on several forms. From an organic conductive element that is attached to a metallic, usually Au\textsubscript{(s)}, structure to allow easy current conduction, to the use of polymeric conducting materials such as non-cross linked polyvinyl chlorides, polyporphyrins, polyphenols, and polyphenylenediamines, and polypyrroles, all of which have found application as recognition elements for anionic species. \textsuperscript{[155]} From this group, polypyrroles have significant utilisable benefits.
They may be used in neutral solutions, and polymerisation may be controlled easily onto a variety of surfaces to allow further sensorisation. In a non-imprinted methodology, halo acetic acids can be detected at conducting polymer electrodes. The electrochemistry of the polymer in the presence of analyte shows slow diffusion of the anions, whose magnitude changed with the identity of the analyte. This study also found that the currents arising at the electrodes are created by the ion-exchange between the analyte ions and the polymer units. Interestingly, the performance of such electrodes can be manipulated by varying the polymer composition, the potential applied and also the solution identity used to conduct the electrochemistry. Two lipophilic derivatives of a macrocyclic hexamine were tested in this work. The potentiometric response properties of the macrocyclic hexamine based electrodes were compared to those of an electrode containing a non-selective quaternary ammonium salt as anion exchanger.

Zielinska et al. reported the combination of a non-selective polyvinyl chloride with two macrocyclic hexamines. In a similar fashion to many biological and biomimetic recognition elements, these selective receptor elements are lipophilic, incorporating not only shape or functional group reciprocity, but also the combination of hydrophobic interactions. These polymer derivatised electrodes were used in aqueous solution to detect small organic acid molecules.

Liang et al. showed that it was possible to incorporate molecular imprinting into these sorts of designs, and successfully imprinted a polypyrrole solution with D-tyrosine using spin coating to place the solutions on a Ni electrode followed by subsequent thermal polymerisation. Rebinding in this study was shown to be generated by applying a potential, the subsequent analysis showed significant selectivity for the imprinted enantiomer of tyrosine (Figure 1.15).

Dickert et al. used a similar approach to create a polypyrrole coated QCM, imprinted with trichloroacetic acid and other halogenated acetic acid compounds. QCM wafers were shown to provide a sensor transduction element for coating with nanoparticulate MIPs synthesised with MAA and TRIM for the sensing of either of the components of a racemic mixture of R and S-propranolol.
Figure 1.15: Electroconductive polymer exhibiting a polypyrrole conductive scaffold on Ni electrode and a recognition element selective for D-tyrosine.  

Indium Tin Oxide Electrodes (ITO) have been used by Dong et al.\textsuperscript{159} Aminopropyl- derivatised organosilane aminopropyltriethoxysilane (APTES), was used as the functional monomer for a dopamine imprinted polymer attached to the ITO surface (Figure 1.16). Although the authors state that the technique is still in its infancy, the ability to sense the binding event of a neurologically active species such as dopamine suggests that such a technique has a large potential with further fine tuning.

Figure 1.16: Schematic of hypothetical dopamine imprinted polymer on ITO electrode TMOS-tetramethoxysilane; PTMOS-phenyltrimethoxysilane; MTMOS- methyltrimethoxysilane.\textsuperscript{159}
Molecularly Imprinted Polymers present themselves as a very useful tool for future biological receptor studies and synthetic antibody assays, but also as cheap, sensitive and reliable sensors for use in any molecular recognition environment. The ability to tailor their chemical characteristics and also their physical properties implies that not only is it currently possible to tailor make polymers for a specific target or species of interest, but that application based optimisation will be possible in the future. Wulff et al. demonstrated that it was possible to enantioselectively retain phenylmannose in a H₂O/MeCN/MeOH mobile phase. This work used L-o-phenylmannopyranoside and vinyl phenylboronic acid as the MIP.¹⁶⁰

The work of Sellegren et al. highlighted some major issues in the behaviour of MIPs.¹⁶¹ This work used phenylalanine-L-anilide to imprint the enantiomer phenylalanine-D-anilide. These polymers used MAA and EGDMA, and were synthesised in CHCl₃. They provided enantioseparation in MeOH and MeCN, demonstrating that the synthesis solvent (porogen) is of significant importance to the final performance of that imprinted polymer. From this work, the following determinations were made: The porogen should provide porosity such that the imprint sites are accessible; The porogen should be as non-polar as possible whilst retaining the ability to dissolve the components of the pre-synthesis solution; If the eventual polymer is to rely on H-Bonding to interact with the reintroduced template, the porogen should not disrupt these interactions in the synthesis phase. Sellegren postulates that the “best” rebinding performance for a MIP is encountered in the porogen. Maintenance of the steric environment around the imprint sites is the reason given for this conclusion.

Mosbach et al. have conducted many studies on phenylalanine-anilide and have shown that amino acids or esters can be imprinted with MAA MIPs, and that the use of polar mobile phases suggests that in similar conditions, the interactions are likely to be ion-pairing.¹⁶² They have also shown that N-protected amino acids can be imprinted and retained on MAA MIPs but the interaction is reduced due to H-bonds being the only available interaction.

Mosbach and Yu showed that some acidic templates gave better response in a polymer that utilised amide functional groups rather than acidic ones.¹⁶³ Although these polymers showed overall a lower retention of the template, the selectivity and separation of racemates was increased. In comparison with MAA, acrylamide is a
hydrogen bond acceptor (HBA) rather than a hydrogen bond donor (HBD). If an excess of FM is used (as shown by Yu et al. above) the levels of non-specific interaction with the analyte molecules are significantly reduced.

Ephedrine has been the model compound of choice for models of adrenaline, serotonin and dopamine receptors. There have been many studies conducted using ephedrine specific polymers. The majority of these studies view ephedrine as not only a model compound for neurotransmitters, but for amphetamine type substances and other basic amine containing molecules. The work of Suedee et al. demonstrated the usefulness of using pseudoephedrine and norephedrine as Thin Layer Chromatographic stationary phases to separate a variety of adrenergic drugs and enantiomers of the mentioned drugs.\[164\] This study utilised methacrylic acid and itaconic acid as functional monomers with an EGDMA copolymer to provide the cross linked matrix in a stoichiometric ratio of 5:18:540 (T: FM: XLM). The synthesis was carried out in chloroform or THF and underwent UV initiated radical polymerisation via the addition of a small amount of AIBN. The mobile phase for the subsequent chromatography was comprised of methanol or acetonitrile with 0, 1, 5 or 10% v/v acetic acid.

From these experiments, it appeared that the addition of acetic acid provided the required conditions for enantiomeric resolution to occur. This effect is demonstrated by the increase in the $R_f$ (Retardation factor) values upon increasing concentrations of acetic acid, and is exhibited more strongly by the itaconic acid polymers. The simple manipulation of the pH generates not only increased selectivity between molecules but also of the enantiomers. The increase in the polarity of the mobile phase was demonstrated to decrease the amount of analyte bound to the polymer, highlighting that the interaction between the acidic monomers and the basic adrenergic drugs has a large role played by ionic interactions. That this effect was more noticeable in the itaconic acid polymers confirms this type of interaction.

Due to the structural similarity of amphetamines and neurotransmitters, both in regards to their steric layout and their functional groups, the binding of such molecules must be carried out in very similar fashions. The work of Shea et al. investigated the use of a MIP for the 5-HT receptor antagonist WAY-199635,\[165\] which showed general agreement between the behaviour of the natural serotonergic receptor and that of the synthetic mimic. However, the rebinding experiments were conducted in chloroform,
which may be the factor that induces the variation between the natural and synthetic receptor behaviour.

The work of Okutucu and Telefoncu demonstrated that MIPs made with amide functional groups in DMSO showed much better recognition properties than MAA MIPs in the same environments. This work also suggests that the more non-polar the porogen is, the stronger the recognition will be. The hydrogen bonding ability of the solvent was assessed to have the largest effect of the solvent properties on the final exhibited behaviour of the MIP.
Chapter 2 - Investigation of template and functional monomer association using molecular modelling.

The use of in silico screening allows high-throughput, preliminary analysis of the interaction between template and a library of functional monomers. It is a fundamental thermodynamic principle that energy cannot be created or destroyed. Thus, differences in the calculated energies of each component system in these equations can be regarded as reporting the interaction between the component parts. This allows selection of the stoichiometry that provides the most favourable and stable association. It is hypothesised that the best potential cluster identity and stoichiometry will be observed where the greatest template/functional monomer (T:FM) association is observed. Thus, the highest level of interaction and selectivity exhibited by the imprinted polymer for the template can be inferred to occur at this ratio.

In this study, individual clusters of functional monomer units in a range of stoichiometries were modelled both in the presence and absence of the template molecule. Assessment of the interactions between molecular components through the estimated interaction energies ($\Delta E_{\text{int}}$) of clusters in the presence and absence of the template is possible. It is also important to note that while the cluster stabilities derived from these calculations are expressed in kJ/mol, the output cannot be directly correlated with experimentally obtained enthalpies. Although the values reported are presented in terms of enthalpy of formation ($\Delta H_f$) by the SPARTAN package, their direct relationship to experimentally derived enthalpies of formation is indicative only. Although such methods may report the enthalpy of formation, the real value may be different due to the incorporation of estimated parameters into the Semi-empirical method.

Such calculations commonly apply the following formulae where, in each equation every component is minimised individually, followed the calculation of the $\Delta H_F$ via the equilibrium geometry values. While the calculation of both the $\Delta H_{F, \text{monomer unit}}$ and $\Delta H_{F, \text{template}}$ involve the calculation of $\Delta H_F$ of single molecules, the terms $\Delta H_{F, \text{monomer cluster}}$ and $\Delta H_{F, \text{system}}$ involve the initial minimisation of the respective molecular clusters under MMFF94, followed by the equilibrium geometry calculation of the $\Delta H_F$ of these poly-molecular systems:
Estimated $\Delta E_{\text{interaction}} = \Delta HF_{\text{system}} - (\Sigma(\Delta HF_{\text{monomer cluster}} + \Delta HF_{\text{template}}))$\textsuperscript{[167]} \textbf{Equation 2.1}

While a description of the interaction within the whole system including any functional monomer self-association/repulsion and template - functional monomer (T:FM) interaction is described by:

Estimated $\Delta E_{\text{interaction}} = \Delta HF_{\text{system}} - (n.(\Delta HF_{\text{monomer unit}} + \Delta HF_{\text{template}}))$ \textbf{Equation 2.2}

where ‘n’ is the number of individual functional monomer units present in the cluster.

As the lowest global energy conformation is the most stable, a net attractive interaction is one that exhibits a negative magnitude and a net repulsive interaction exhibits a positive value. The contribution of the FM:FM self-association can be delineated from the cluster calculations via the formula that is the result of subtracting the specific interaction energy (T:FM) from the total system interaction energy \textit{(Equation 2.2 - Equation 2.1)}. A comparison of the estimated energy of interaction using these approaches should provide insight into the identity of the interacting molecular species in the simulated pre-polymerisation clusters.

Thus, it is possible to alleviate the influence of the interaction of monomer units between themselves, and also to compare the interaction between molecules. Initially the functional monomer clusters were modelled in isolation to assess the level of self-association that the monomers display. This initial step allows an insight into the likelihood of the monomer units dispersing as single units throughout the system upon the application of synthesis conditions, or the possibility of the monomer units associating in clusters before such dispersal in the synthesis solvent.

It must be emphasised that the models were not placed in such an orientation as to artificially select possible interactions. To do so would be to circumvent the entire purpose of the in silico screening process which in this case, is to investigate the likelihood of the aggregation of monomer and template molecules at given ratios. The positions of all molecules in the calculation of the heats of formation were generated through the iterative calculation process. All models were also conducted \textit{in vacuo} (in a
vacuum) to avoid the complication of interactions between all components and the solvent molecules at this early stage. Each system was constructed in triplicate and the results averaged.

Commercial monomers possessing a range of functional groups capable of interacting with the template were selected for screening. For a potential interaction to be selected from the in silico screening, two criteria were considered essential for the successful selection: (i) Cluster formation as a whole being energetically favourable, and (ii) The cluster possessing a significant net negative energy of interaction. The ability to undergo polymerisation is also a necessary feature of the functional monomer, without such functionality, incorporation of the T:FM association cluster into the imprinted polymer network is impossible.

Thus the monomers displayed in Table 2.1, Table 2.3, Table 2.7, and Table 2.9 were built in SPARTAN, and the global energy minima calculated, before interactions were compared after calculation with Equations 2.1 and 2.2.

2.1 Ephedrine – Functional monomer association.

The precursor for much illicit amphetamine synthesis, ephedrine (EPH; Figure 2.1) contains two vicinal, appropriate functional groups (the α-hydroxyl group and the 2° amine) to create reciprocal, point interactions with the functional monomers during the synthesis of the MIP. Both of these groups are capable of hydrogen bonding and electrostatic interaction, given the approach of a complementary functional group. Although the amine is more prone to protonation and thus electrostatic interaction, the lone pairs and resulting dipole of the OH group make interaction with a positively charged moiety possible. The amine is easily protonated in acidic environments, and the lone pair of electrons in the hydroxyl group provides a localised area of negative charge. As EPH is an identified precursor for the illicit synthesis of methamphetamine, a neuromodulator, and a restricted compound (Australia, Drug Misuse and Trafficking Act, 1985, Schedule 1) in its own right, it is a perfect model compound for the imprinting of a polymer specific for amphetamine type substances.
A number of functional monomer classes (acidic, basic, neutral and aromatic) were chosen for the semi-empirical modelling scheme, the best performing of which were selected for synthesis of an amphetamine type substance (ATS) MIP.

![Figure 2.1: (-)-ephedrine (EPH)](image)

### 2.1.1 Ephedrine - Basic Monomers

Molecules that possess proton accepting, hetero atoms (hydrogen bond acceptor sites) have great potential for the creation of reciprocal hydrogen bonding interactions with certain template molecules. Basic functional monomers possess an ability to interact with the two donation sites on the EPH molecule, the OH and NH. Depending on the protonation state of the template and monomers, ionic interaction may also be possible between these sites of varying electrostatic potential. The monomers shown in Table 2.1 were screened for the level of interaction with the template – ephedrine, and the energy of interaction between the template and functional monomer cluster calculated using *Equations 2.1 and 2.2*. The ratio of FM-T represents the number of monomer molecules modelled around 1 template molecule.
Table 2.1: Basic functional monomers chosen for screening. Density potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green).

<table>
<thead>
<tr>
<th>Monomer Name</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-vinyl pyridine (2VP)</td>
<td>OH</td>
</tr>
<tr>
<td>4-vinyl pyridine (4VP)</td>
<td>H2N</td>
</tr>
<tr>
<td>Vinyl imidazole (VI)</td>
<td>O</td>
</tr>
<tr>
<td>2-hydroxy ethylmethacrylate (2HEMA)</td>
<td>COOCH2OH</td>
</tr>
<tr>
<td>Allyl amine (AAM)</td>
<td>H2N</td>
</tr>
<tr>
<td>Vinyl acetate (VAC)</td>
<td>O</td>
</tr>
<tr>
<td>3-isopropyl-N,N-diethylamino methacrylate (DEAMA)</td>
<td>N</td>
</tr>
</tbody>
</table>
Three of the basic molecules modelled were aromatic bases, of which VI displays the largest self-association (Figure 2.3). It is likely that this association is due...
to the presence of HBD and HBA groups on this FM. VI also appears to produce the largest interaction with ephedrine of these aromatic bases (Figure 2.2) except at a stoichiometry of 1-3, where the 4-vinyl pyridine cluster is calculated to exhibit ca. -10 kJ.mol\(^{-1}\) larger net interactions with the template. The variation of the position of the vinyl group with respect to the hydrogen bond acceptor site on the vinyl pyridine molecules shows that at 1-3 stoichiometry, the less sterically crowded pyridine produces a larger (ca.-17 kJ.mol\(^{-1}\)) interaction. At higher stoichiometries, the association of the VP functional monomers show little observable difference between either the T:FM or the FM:FM associations.

A comparison of the estimated energy of interaction within the T:FM clusters (Figure 2.2) and the estimated functional monomer: functional monomers (FM:FM) interaction energy (Figure 2.3) shows that the addition of monomer units to the system does not necessarily equate to a significant increase in either the T:FM or FM:FM levels of interaction. AAM and the pyridine monomers modelled demonstrate a much lower predilection for FM:FM interaction, while VAC, VI, DEAMA and 2HEMA display much greater interaction between functional monomer units than with EPH.

Although 2HEMA contains hydrogen bond donor and acceptor sites, the 1-2 cluster finds both alkanol groups interacting with the amine group of EPH’s tail, with the suggestion of a slight stabilising interaction from EPH’s hydroxyl group. (Table 2.2)

### Table 2.2: Separation between hydrogen bond donors [D-H···A] and hydrogen bond acceptors from AM1 level simulations.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>separation (Å)</th>
<th>Hydrogen bond donor</th>
<th>Hydrogen bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2.162</td>
<td>2HEMA OH</td>
<td>EPH N</td>
</tr>
<tr>
<td>1:2</td>
<td>2.150</td>
<td>EPH OH</td>
<td>2HEMA O</td>
</tr>
<tr>
<td></td>
<td>2.181</td>
<td>EPH NH</td>
<td>2HEMA O</td>
</tr>
<tr>
<td>1:4</td>
<td>2.223</td>
<td>EPH OH</td>
<td>2HEMA O</td>
</tr>
<tr>
<td>1:7</td>
<td>2.153</td>
<td>2HEMA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td></td>
<td>2.199</td>
<td>EPH OH</td>
<td>2HEMA O</td>
</tr>
<tr>
<td>1:8</td>
<td>2.183</td>
<td>EPH OH</td>
<td>2HEMA O</td>
</tr>
<tr>
<td></td>
<td>2.143</td>
<td>EPH OH</td>
<td>2HEMA O</td>
</tr>
</tbody>
</table>

DEAMA is not observed to participate in any significant, favourable interactions with EPH. A small favourable interaction is observed at 1-3 (Figure 2.3), presumably a hydrophobic interaction, as the geometry of the cluster clearly indicates that the
functional groups of the ephedrine tail are not located in sufficient proximity for a specific, strong interaction to exist.

AAM is capable of both behaving as a hydrogen bond donor and acceptor; additionally its size means that any possible steric hindrance of the interaction between template and monomer will be minimal. Despite this, no hydrogen bonds were predicted between allyl amine and ephedrine at any stoichiometry modelled. Of the basic monomers modelled, allyl amine displays the largest amount of interaction at each stoichiometry except for 1:1. This suggests that solely relying on the number of predicted hydrogen bonds between template and functional monomer is neither the most appropriate, nor the most accurate method of functional monomer selection.

2.1.2 Ephedrine - Acidic monomers

The density potential surfaces (Table 2.3) of the acidic functional monomers show the similarities in charge distribution between the acidic functional monomers screened using this protocol. Large amounts of electron density and thus negative charge centre on the lone pairs of the carboxylate group, and a positive point charge at the location of the mobile proton. The remainder of the molecular surface appears to exist in a partially positive environment suggesting the remainder of the molecules are quite hydrophobic. The induction of electron density towards the electronegative carboxylate groups are the cause of this dipole moment. Acrylamido-2-methylpropane sulfonic acid displays a slightly different potential distribution, due to the AM group causing a more neutral potential surface of the remainder of the functional monomer unit.

The carboxyl groups of the acidic species studied provide the capacity for hydrogen bond donation and acceptance. In addition, strong dipole moments, caused by the presence of the electronegative oxygen atoms in close proximity to the $\pi$ bonds, allow for significant electrostatic interaction to take place if an interaction does exist as a hydrogen bonding interaction. It is likely that due to the close location of multiple lone pairs of electrons, such groups would form cooperative networks of hydrogen bonds, should they participate at all.
Table 2.3: Acidic functional monomers chosen for screening. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green).

<table>
<thead>
<tr>
<th>Acidic Functional Monomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic Acid (AA)</td>
</tr>
<tr>
<td>Methacrylic Acid (MAA)</td>
</tr>
<tr>
<td>Acrylamido-2-methylpropanesulfonic acid (AMPSA)</td>
</tr>
<tr>
<td>Itaconic acid (ITA)</td>
</tr>
</tbody>
</table>

Figure 2.4: Average estimated energy of interaction for acidic functional monomer/EPH clusters. (number of repetitions = 3)
### Table 2.4: Separation between hydrogen bond donors \([D-H \cdot \cdot A]\) and hydrogen bond acceptors from AM1 level simulations. Hydrogen bonds predicted by SPARTAN are highlighted in yellow.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Separation (Å)</th>
<th>H bond donor</th>
<th>H bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:4</td>
<td>2.031</td>
<td>ITA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td>1:5</td>
<td>2.03</td>
<td>ITA OH</td>
<td>EPH O</td>
</tr>
</tbody>
</table>

At lower stoichiometries, ITA demonstrates the largest interaction energy between functional monomer and template from *ca.*-20 kJ.mol\(^{-1}\) at 1:1 to *ca.*-50 kJ.mol\(^{-1}\) at 1:2 (*Figure 2.4*). It is necessary to note that the predicted level of FM:FM interaction is also of a significant magnitude upon the addition of multiple monomer units, suggesting a heightened probability of FM:FM interactions predominating over T:FM. ITA is the only acidic functional monomer studied that possesses the ability to participate in intramolecular hydrogen bonds, a feature which must be overcome should it be chosen as the functional monomer unit for synthesis. It is not observed to participate in identified hydrogen bonds with the template other than in the clusters at higher T:FM ratios (*Table 2.4*). The predicted hydrogen bonds occur at a stoichiometry that is estimated to be a net repulsive interaction. At this stoichiometry, it is clear that although the hydrogen bonds are predicted, the magnitude of the repulsive forces outweigh the favourable hydrogen bonding, and attractive interactions.

AMPSA demonstrates *ca.*-20 kJ.mol\(^{-1}\) interaction with ephedrine at 1:1 however, this interaction does not appear to remain following the interaction of additional monomer units. The FM:FM interactions are seen to increase in magnitude following the sequential additions, implying that the FM:-FM interaction is stronger than the T:FM in this case. Heavier monomers in this class showed significantly increased levels of FM:FM interaction in comparison to acrylic acid and methacrylic acid (*Figure 2.5*). The amount of surface available for van der Waals’ and hydrophobic interaction is likely to be the cause of this result.

### Table 2.5: Separation between hydrogen bond donors \([D-H \cdot \cdot A]\) and hydrogen bond acceptors from AM1 level simulations. Hydrogen bonds predicted by SPARTAN are highlighted in yellow.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>separation (Å)</th>
<th>H bond donor</th>
<th>H bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2.105</td>
<td>AA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td>1:2</td>
<td>2.084</td>
<td>AA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td>1:3</td>
<td>2.095</td>
<td>AA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td>1:4</td>
<td>2.081</td>
<td>AA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td>1:5</td>
<td>2.066</td>
<td>AA OH</td>
<td>EPH O</td>
</tr>
</tbody>
</table>
Figure 2.5: Average estimated energy of FM:FM interaction (kJ/mol) for acidic functional monomer/EPH clusters. (Equation 2-Equation 1) (number of repetitions = 3)

Table 2.6: Separation between hydrogen bond donors \([D-H \cdots A]\) and hydrogen bond acceptors from AM1 level simulations.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Separation (Å)</th>
<th>H bond donor</th>
<th>H bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2.254</td>
<td>EPH OH</td>
<td>MAA O</td>
</tr>
<tr>
<td>1:2</td>
<td>2.221</td>
<td>EPH OH</td>
<td>MAA O</td>
</tr>
<tr>
<td>1:3</td>
<td>2.233</td>
<td>MAA OH</td>
<td>EPH O</td>
</tr>
<tr>
<td></td>
<td>2.499</td>
<td>EPH OH</td>
<td>MAA O</td>
</tr>
<tr>
<td></td>
<td>2.335</td>
<td>EPH NH</td>
<td>MAA O</td>
</tr>
<tr>
<td></td>
<td>2.344</td>
<td>EPH NH</td>
<td>MAA O</td>
</tr>
</tbody>
</table>

MAA produces stronger interactions with EPH than does AA at all stoichiometries, except 1:5 (Figure 2.4). This was unexpected due to the higher number of predicted hydrogen bonds for the AA cluster than the MAA cluster (Table 2.4, Table 2.5) and the steric constraints exhibited by the bulky -CH\(_3\) addition. Although the methylation of the functional monomer appears to increase the separation of the hydrogen bonding donor and acceptor groups, the additional surface interactions must account for the higher predictions of interaction. It is possible that the methyl substituted functional monomer is able to form more stable association clusters than the un-substituted monomer due to a reduction in the degrees of freedom for monomer movement, while maintaining the donor/acceptor interactions with the template.
Additionally, the presence of additional surface area for interaction must play a role in the T:FM cluster observed here. Both 1:2 and 1:3 are the most viable stoichiometries for the use of MAA to imprint a polymer for EPH due to the interaction predicted between functional monomer and template, and also the reduced levels of FM:FM interaction observed in these models.

2.1.3 Ephedrine - Neutral monomers

The neutral monomers studied possessed hydrogen bond donor groups, some acceptor groups and some both, while STY possesses neither (Table 2.7). Yu et al. used AM as a functional monomer for adsorbing amino acids in water.\cite{Yu2008} Four different AM functional group containing monomers were modelled to determine the effectiveness of these monomers for their potential interaction with ephedrine.

AM was observed to generate an estimated interaction energy of \textit{ca.-}20 to -30 kJ.mol\(^{-1}\) at the stoichiometries 1:1 to 1:3 (Figure 2.6). At higher equivalencies, the interaction appeared to become weaker, presumably due to increased levels of preferential FM- FM interaction (Figure 2.7).

Methylation of the monomer reduces the rotational freedom of the cluster’s components without modulating the ability of the hydrogen bond donor/acceptor groups to participate in their respective non-bonding interactions, clearly an area of similarity to the behaviour of the (meth)acrylic acid systems studied above. The single monomer unit interaction shows an electrostatic attraction to be the cause of the interaction at 1:1, as no hydrogen bonds are identified by SPARTAN\textsuperscript{04}. At higher stoichiometries, significantly higher levels of T:FM net interaction occur at 1:4 (\textit{ca.-}40 kJ.mol\(^{-1}\)), 1:5 (\textit{ca.-}60 kJ.mol\(^{-1}\); Figure 2.6). It is only at the 1:5 ratio (Table 2.8) that a hydrogen bond is observed to occur between the template and functional monomer cluster.
Table 2.7: Neutral functional monomers chosen for screening. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green).

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acrylamide (AM)</strong></td>
<td><strong>Methacrylamide (MAM)</strong></td>
<td><strong>N-isopropyl acrylamide (IAM)</strong></td>
</tr>
<tr>
<td>Acrylonitrile (AN)</td>
<td>Styrene (STY)</td>
<td>Pyrrole (PYR)</td>
</tr>
<tr>
<td>$N,N$-methylene-bis-acrylamide (NMBA)</td>
<td>Vinyl alcohol (VOH)</td>
<td></td>
</tr>
</tbody>
</table>

The implication of this result is that the ability for singular interaction with the template is an important feature for a functional monomer to possess, but that the interaction between the template and functional monomer cluster may be increased by
the creation of a co-operative network of interactions between the template and functional monomer cluster.

![Figure 2.6: Average estimated energy of interaction for neutral functional monomer/EPH clusters. (number of repetitions = 3)](figure)

Table 2.8: Separation between hydrogen bond donors [D-H··A] and hydrogen bond acceptors from AM1 level simulations. Hydrogen bonds predicted by SPARTAN are highlighted in yellow.

<table>
<thead>
<tr>
<th>ratio</th>
<th>Separation (Å)</th>
<th>Hydrogen bond donor</th>
<th>Hydrogen bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:4</td>
<td>2.114</td>
<td>EPH OH</td>
<td>MAM O</td>
</tr>
<tr>
<td></td>
<td>2.129</td>
<td>MAM NH</td>
<td>EPH O</td>
</tr>
<tr>
<td></td>
<td>2.379</td>
<td>MAM NH</td>
<td>MAM O</td>
</tr>
<tr>
<td>1:5</td>
<td>2.085</td>
<td>EPH OH</td>
<td>MAM O</td>
</tr>
<tr>
<td></td>
<td>2.118</td>
<td>MAM NH</td>
<td>EPH O</td>
</tr>
<tr>
<td></td>
<td>2.168</td>
<td>MAM NH</td>
<td>EPH O</td>
</tr>
<tr>
<td></td>
<td>2.276</td>
<td>MAM NH</td>
<td>MAM O</td>
</tr>
<tr>
<td>1:6</td>
<td>2.222</td>
<td>MAM NH</td>
<td>MAM O</td>
</tr>
<tr>
<td></td>
<td>2.124</td>
<td>EPH OH</td>
<td>MAM O</td>
</tr>
</tbody>
</table>

IAM is a commonly used monomer that has found uses in aero-gels, and stimulus response polymers. Its steric bulk is greater than MAM, however in this case, higher magnitudes of T:FM interactions are seen at the lower stoichiometries. The large amount of steric hindrance due to the isopropyl group may be the cause of the failure of the favourable interactions to exist at higher stoichiometries. The steric bulk possessed by a functional monomer clearly plays a role in the strength of the association between template and functional monomer, and the organisation of the geometry of the...
association cluster. There is obviously a point at which the favourable shift in interaction due to this increase in steric bulk reverses and becomes detrimental to the strength of the T:FM association cluster.

**Figure 2.7:** Average estimated energy of FM:FM interaction for neutral functional monomer/EPH clusters. (Equation 2.2-Equation 2.1) (number of repetitions = 3)

STY’s interaction with the template is restricted to \(\pi-\pi\) interactions between the monomer units and the aromatic head of ephedrine, and van der Waals interactions. It does not appear (Figure 2.6) to generate sufficiently strong interactions with ephedrine to be used as the FM, although its interaction appears to increase steadily as the stoichiometry increases. Little FM:FM interaction is noted (Figure 2.7).

NN-MBA (Table 2.7) is a symmetrical monomer with 2 AM groups enabling hydrogen bond donation and accepting in addition to multiple sites for polymerisation. It would therefore fulfil the task of acting as a functional monomer, but also the task of cross-linking the polymer scaffold to provide rigidity to the system. It does not appear to generate significant amounts of interaction with ephedrine at the higher stoichiometric ratios studied, with the greatest interaction noted to be ca. -25 kJ.mol\(^{-1}\) at a ratio of 1:2. It appears to undergo little self association (Figure 2.7).
2.1.4 Ephedrine - Cross linking monomers

Table 2.9: Cross linking monomers chosen for screening. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green).

<table>
<thead>
<tr>
<th>2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate (TRIM)</th>
<th>Ethylene glycol dimethacrylate (EGDMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>meta-divinyl benzene (m-DVB)</td>
<td>para-divinyl benzene (p-DVB)</td>
</tr>
<tr>
<td>N,O-bismethacryloyl ethanolamine (NOBE)</td>
<td></td>
</tr>
</tbody>
</table>
The potential cross linking monomers chosen for the screening process (*Table 2.9*) are commonly used crosslinking monomers. As they are the major component (*ca.* 80%) of the imprinted polymer, their interaction, or lack thereof is an essential component in the end product of the *in silico* screening.

There is little observed interaction between the DVB monomers (*Figure 2.8*) and EPH. N,O-bismethacryloyl ethanolamine (NOBE) was developed as a one size fits all monomer, to fulfil both the task of interacting with the template and the task of creating the cross-linking. It is observed to participate in favourable interaction with ephedrine (*Figure 2.8*), although this favourable interaction is not observed in the 1:3 cluster and demonstrates the largest amount of FM:FM interaction of the cross linkers modelled (*Figure 2.9*)

![Figure 2.8: Average estimated energy of interaction for cross linking monomer/EPH clusters. (number of repetitions = 3)](image-url)
Ethylene glycol dimethacrylate (EGDMA) is a monomer that has found uses in material science for many different applications. It is observed to participate in the strongest interaction of the cross linking monomers studied with ephedrine (Figure 2.8), however it also is observed to participate in the largest amount of FM:FM interaction, similarly to NOBE at the highest ratio investigated. The monomer template interaction is favourable at low stoichiometry, while the largest ratio studied displays a net repulsive interaction.

2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate (TRIM) is another popular cross linking monomer. The interaction between TRIM and ephedrine is not observed to be of a significant magnitude and the similarity in the interaction between EPH and TRIM/ EGDMA occurs at the ratio of 1:5. Interestingly, the FM:FM interaction exhibited by TRIM (Figure 2.9) is predicted to be very repulsive. The only apparent explanation of this anomalous result is the size, and structure of TRIM itself. The three- armed, asymmetrical structure has large, flexible chains that may account for the energetic instability indicated by the repulsive interaction predicted between FM:FM. It is also possible that the gas phase nature of these investigations is responsible for this apparently anomalous result, preventing the stabilisation of the side chains by the solvent molecules, subsequently causing the calculation of the increased
estimated enthalpy of formation, also influencing further calculation using this initially anomalous value.

2.2 MDMA – Functional monomer association.

N-methyl-3,4-methylenedioxymethamphetamine (MDMA) (Figure 2.10) is a commonly encountered ATS. The differences between MDMA and ephedrine (removal of the hydroxyl group and addition of methylene dioxy group) are expected to create some differences in the manner and the geometry of the associations between the monomer units and the template molecule. However, the conserved phenylethylamine backbone of the adrenergic receptor modulators suggests that although minor differences in the structure and magnitude of the associated clusters may exist, the overall pattern, location and types of interaction will remain largely the same.

![Figure 2.10: N-methyl-3,4-methylenedioxyamphetamine (MDMA)](image)

2.2.1 MDMA - Basic Monomers

It was expected that MDMA may demonstrate lower levels of interaction than ephedrine with the basic monomers due to the removal of the hydroxyl group – a hydrogen bond donor and acceptor. VAC demonstrates the largest interaction with MDMA of the basic monomers, and also displays the largest levels of FM:FM interaction (Figures 2.11 and 2.12). AAM demonstrates a larger interaction than the remaining monomers at higher stoichiometries. This monomer is not observed to participate in significant levels of FM:FM interaction.

2HEMA appears to participate in an electrostatic interaction with the amine group of MDMA. This interaction does not appear to be dramatically affected by the
addition of monomer units, nor does the magnitude of the 1:1 cluster (Figure 2.11) imply that the interaction is strong. It was expected that at low ratios, the additional template functional groups would provide further electrostatic centres for monomer template to occur. However, the reduction in the number of donor sites in the cluster is likely to be the cause of the lack of interaction. The aromatic bases, 2-VP and 4-VP, do not demonstrate a large propensity for interaction with MDMA. They are not observed to participate in large levels of FM:FM interaction.

Figure 2.11: Average estimated energy of interaction for basic functional monomer/MDMA clusters. (number of repetitions = 3)
2.2.2 -MDMA - Acidic Monomers

MDMA was expected to participate in greater levels of T:FM interaction with the acidic monomers than ephedrine due to the additional HBA atoms possessed by the methylenedioxy ring group.

AA was observed to generate a favourable interaction with MDMA at a ratio of 1:5 (Figure 2.14). Although the absence of the OH functional group (in comparison to EPH) reduces the amount of possible steric hinderance for the groups approaching the secondary amine group of MDMA, the 1:5 cluster (Figure 2.14) was calculated to have the greatest interaction, however this interaction was not observed to take place in a similar position to ephedrine. The interaction (Figure 2.14) is observed to involve interaction with both the amine and methylenedioxy groups, though it was surprising that both available oxygen atoms were not participating in this interaction. Although acrylic acid is capable of participating in hydrogen bonding interactions, none were predicted to exist in these model systems.
Figure 2.13: MDMA / acrylic acid cluster 1:5 showing the interaction between carboxylic acid groups and the amine and methylene dioxy group of the template.

ITA is predicted to participate in the most favourable interactions of the acidic class at low (1:1, 1:2) equivalence (Figure 2.14). This interaction does not increase with the addition of further monomer units, however at 1:5 a large interaction is predicted. This case is similar to acrylic acid. The interaction between MDMA and itaconic acid produces a similar pattern to that generated during the ephedrine modelling experiments. It was again noted that itaconic acid participates in the largest FM:FM interaction of the acidic monomers studied (Figure 2.16). It is likely that the number of carboxylic acid groups available for interaction is a direct cause of the FM:FM association energy. As such, ITA is not predicted to be a suitable candidate for MDMA imprinting.
MAA did not demonstrate an identical pattern of interaction to that observed in the modelling of the ephedrine clusters. The addition of monomer units does not increase the level of interaction between monomer cluster and template, and the location of this interaction is initially unusual. Although the addition of a second monomer unit appears to produce a reduction in the level of interaction, the location of this interaction at the symmetrical methylene dioxy group cannot justify the reduction in interaction as there does not appear to be any hindrance or repulsive interaction generated in this case (Figure 2.15). As the stoichiometry increases, the interaction observed at the lower stoichiometries (Figure 2.15) are no longer observed to involve the methylene dioxy ring, but to centre on the amine group. The cooperative interactions possessed by the template and functional monomer cluster generate the favourable interaction displayed at 1-5, and although the interaction is weaker than observed between ephedrine and the same cluster composition, the lack of the α-OH group explains the reduction in the interaction between the analogous templates.
Figure 2.15: MDMA / MAA clusters 1:2 (left) and 1:5 (right) showing the interaction between carboxylic acid groups and methylene dioxy group of the template, which is not observed as the stoichiometry increases.

Figure 2.16: Average estimated energy of FM:FM interaction for acidic functional monomer/MDMA clusters. (Equation 2.2-Equation 2.1) (number of repetitions = 3)
2.2.4 -MDMA - Neutral Monomers

AM possesses functional groups capable of participating in potentially favourable interaction with MDMA. However, it does not exhibit a strong estimated interaction at any ratio of composition (Figure 2.18).

Figure 2.17: Interaction between AM and MDMA.

Figure 2.18: Estimated energy of interaction for neutral monomer/MDMA clusters. (number of repetitions = 3)

The interaction between AM and MDMA appears to be largely located around the methylenedioxy group (Figure 2.17). Although the amine group is available for interaction, unlike the observed geometry of the cluster when ephedrine is used as the
template the interaction does not appear to occur here. At a ratio of 1:1 and 1:2, AM is observed to participate in a \( \text{ca.} -15 \text{ kJ.mol}^{-1} \) interaction with MDMA, centred at the methylene dioxy functional group. This interaction is not observed to exist at higher stoichiometries.

STY is observed to participate in interaction with MDMA, which is strongest at 1:1 and further at 1:4 (Figure 2.18). This interaction is most likely to be hydrophobic or \( \pi-\pi \) interaction, as the FM lacks any functional groups capable of directly interacting with MDMA in a hydrogen bond donor-acceptor fashion.

MAM demonstrates a similar level of interaction to AM at 1:1. It is however, again at the 1:5 ratio where the favourable interaction is predicted to occur. Although the approach of hydrogen bond acceptor and hydrogen bond donator sites is seen to occur, they do not qualify according to the description possessed by the SPARTAN package and are therefore not identified automatically.

![Figure 2.19: Estimated energy of FM:FM interaction for neutral functional monomer/MDMA clusters. (Equation 2.2-Equation 2.1) (number of repetitions = 3)](image)

AN is observed to participate in favourable interaction with the template that appears to plateau (\( \text{ca.} -15 \text{ kJ.mol}^{-1} \)) at a stoichiometry of 1:2 before becoming stronger again at 1:6 (Figure 2.18). The only possible method of favourable interaction is electrostatic attraction, and the presence of more negatively charged groups on the MDMA in comparison to ephedrine and N-methylphenylethylamine (N-MPEA) explains this larger interaction.
AM and MAM are observed to participate in the largest levels of FM:FM interaction (Figure 2.19). With respect to MAM, it is likely that the increase in T:FM interaction is correlated to the smaller rate of change of the FM:FM interaction between the stoichiometries. Such repetition of the observed interaction at this ratio with molecules of analogous backbones implies that such a structure may be providing stabilisation of the transient association clusters in the presynthesis mixture. It appears unlikely that MDMA would be a suitable candidate for the creation of an amphetamine type substance imprinted polymer due to its significantly larger aromatic moiety. The failure of the strongest interactions to occur at the structurally conserved functional group suggests that this amphetamine type substance is unsuitable for this role.

2.2.5 - MDMA - Cross linking monomers

Neither meta- nor para-divinyl benzene indicate a proclivity for interaction with MDMA. It is also unlikely that a template/functional monomer cluster would be incorporated into the cross link scaffold without either aromatic functionalities present in the functional monomer cluster, or hydrogen bonding/electrostatic functionalities on both the cross linker and functional monomer cluster. p-DVB is observed to cause a significantly repulsive interaction (ca. 90 kJ.mol⁻¹) with MDMA at 1:2 (Figure 2.20).

NOBE is observed (Figure 2.20) to exhibit no favourable interaction with MDMA. It is again observed to exhibit the largest levels of FM:FM interaction (Figure 2.21).
Figure 2.20: Estimated energy of interaction for cross linking monomer/MDMA clusters. (number of repetitions = 3)

EGDMA does not display a large amount of interaction with MDMA (Figure 2.20) although it appears to generate the largest interaction with MDMA (ca. -30 kJ.mol$^{-1}$) at 1:2 stoichiometry.
TRIM exhibits a similar magnitude of interaction as EGDMA after the initial favourable prediction of interaction (*Figure 2.20*). It is likely that this increase in interaction is generated by the stabilisation of the monomer-template cluster that is not possible with the presence of only one molecule in the simulation. The observed repulsive interaction shown by TRIM is of unclear cause, though the explanation offered for the similar artefact observed in the ephedrine system (three armed, asymmetrical structure has large, flexible components) appears to apply here as well.

### 2.3 - N-methyl phenylethylamine (N-MPEA)

![N-methyl phenylethylamine](image)

*Figure 2.22: N-methyl phenylethylamine – (N-MPEA)*

Because of the conserved backbone of the amphetamine type substances, N-methyl phenylethylamine, is predicted to be a suitable template for the imprinting of an ATS specific MIP. Although the lack of the α-OH group, and the steric bulk of the aliphatic methyl groups are not present (suggesting that the template will not be as useful for the entire family of substances) the observations of the type of monomer and stoichiometries at which the greatest interactions take place should reflect the similarities between the template molecules.

It was predicted that N-methyl phenylethylamine (*Figure 2.22*) would interact in a similar fashion both to EPH and MDMA. It was also predicted that the level of interaction would be of a smaller magnitude to both other amphetamine type substances due to the presence of only one hydrogen bond donor/acceptor site in this template.
2.3.1 N-MPEA - Basic Monomers

VAC is predicted to exhibit the greatest interaction of the basic monomers with N-MPEA, however in this case the interaction is only observed to occur at a stoichiometry of 1:3 (Figure 2.23). There is observed to be a significantly lower FM:FM interaction when this template is considered than in earlier T-VAC models. Comparison of this cluster and the previously modelled template association with the same functional monomer shows the role in the organisation of the T:FM cluster played by the template.

The aromatic, amine monomers exhibit little interaction with N-MPEA. It appears unlikely that either VP molecule will be capable of generating a significant imprinting effect. VI appears to interact favourably at higher stoichiometric ratios (1:5) (Figure 2.23) but not at the lower equivalencies. Although the electrostatic interaction between the nitrogen of the imidazole ring and the amine of the template may produce a favourable interaction, it appears to exist only when a contribution from multiple monomer units is present. This feature is observed for the remainder of the basic functional monomers.

Figure 2.23: Estimated energy of interaction for basic functional monomer/N-MPEA clusters. (number of repetitions = 3)
VAC demonstrates an ability to interact to a small extent with N-MPEA, \( \text{ca.} -50 \text{kJ.mol}^{-1} \) at a stoichiometry of 1:3 (Figure 2.23). This maximum interaction is associated with the smallest separation between hydrogen bond donor and acceptor. The significant increase in FM:FM interaction from 1:4 (Figure 2.24) appears to be associated with the decrease in interaction exhibited between the template and functional monomer at the same stoichiometry (Figure 2.23).

Figure 2.24: Estimated energy of FM:FM interaction for basic functional monomer/N-MPEA clusters. (Equation 2.2-Equation 2.1) (number of repetitions = 3)

AAM shows a slightly repulsive net interaction with other functional monomer units, however a favourable, attractive interaction with the template is predicted at a ratio of 1:5, again suggesting cooperative action between multiple monomer units and the template.

2.3.2 - N-MPEA - Acidic Monomers

AA demonstrates a favourable interaction with N-MPEA at a stoichiometry of 1:2 (Figure 2.25), where the observed geometry between N-MPEA and AA (Figure 2.26) appears to exhibit a co-operative geometry around the amine group of the template molecule. While no hydrogen bonds were predicted by the SPARTAN package, it
appears that a potential network of cooperative, bifurcated hydrogen bonds could potentially exist. Even if this interaction is only an electrostatic dipole-dipole interaction, the number of points of charge located in this one area of interaction should cooperate in a similar fashion to a network of hydrogen bonds. Although only one point for direct interaction exists in the N-MPEA template, the cooperative network of interactions can clearly be seen to benefit the interaction between template and functional monomers.

Further to this, at a stoichiometry of 1:5, an even more favourable interaction is observed that is not present either side of this ratio. The absence of predicted attractive interactions bordering the favourable interaction is yet another suggestion of the cooperative interaction of template, and functional monomers in creating a cluster that exhibits a geometry, which allows greater levels of interaction with the template, than could be achieved with a single monomer alone.

![Figure 2.25: Estimated energy of interaction for acidic functional monomer/N-MPEA clusters. (number of repetitions = 3)](image)

MAA does not exhibit a similar pattern to its interaction with N-MPEA as it does with MDMA and EPH. There appears to be little interaction at both 1:1 and 1:2 stoichiometries (Figure 2.25). The 1:3 model shows the co-operative effect increasing the predicted interaction energy where two monomers directly interact with the amine functional group of the template; the third monomer unit appears not to participate in a
direct interaction with the template but interacts with one of the monomer units (Figure 2.26). This interaction appears to prompt the distance between the cluster and the amine group to narrow, thus increasing the magnitude of the interaction between template and functional monomer cluster. The increased surface area contact due to the additional monomer unit in which van der Waals interactions occur may also go some way to explaining this increase in interaction.

![N-MPEA](image)

**Figure 2.26:** N-MPEA:AA 1:2 (left) and N-MPEA :MAA 1:3 (right)

![Graph](image)

**Figure 2.27:** Estimated energy of $FM:FM$ interaction for acidic monomer/N-MPEA clusters. (Equation 2 - Equation 1) (number of repetitions = 3)

IA displays another example of the cooperative behaviour of the monomer units in relation to the template. The increase in interaction energy estimated for the 1:3 and 1:6 clusters show that although not all of the monomer units interact directly with the template molecule, the favourable orientation of the monomer units allows a greater
level of interaction between two monomer units and the template (Figure 2.25) than occurs when two monomer units only are added. Due to the number of potential interactions between six monomer units the increase at this ratio may be conceivably contributed to by FM:FM interactions. (Figure 2.27). Despite the favourable predictions of T:FM interaction in the IA system, the sheer magnitude of the FM:FM interaction in the same systems suggests that interaction between template and functional monomer are unlikely to exist in large numbers within the pre-synthesis solution. It also makes it unlikely that during the synthesis, such interactions would survive the large amount of energy added during polymerisation due to the initiation through thermal means, or the exothermic nature of the polymerisation itself.

2.3.3 - N-MPEA - Neutral Monomers

Where AM is modelled interacting with N-MPEA, the addition of multiple monomer units results in the interruption of the template monomer interactions in favour of the FM:FM interaction (Figures 2.28 and 2.29). While AM exhibits the largest interaction at a 1:1 stoichiometry, the methylation of this monomer allows the cooperative, repeated interaction between molecules based upon this phenylethylamine backbone and 5 MAM molecules (Figure 2.28). The lack of observed hydrogen bonding with the template suggests that the combination of many weaker interactions can combine to surpass the performance of stronger yet less frequent interactions. This is also suggested by the fewer number of sites for interaction to occur reducing the overall magnitude of the interaction, but not removing its existence.
AN shows little direct interaction with N-MPEA (Figure 2.28). Although the nitrile group does approach the hydrogen bond donor of N-MPEA, the separation between these groups does not indicate the possibility of hydrogen bonding to occur.

STY is observed to participate in a small increase in the interaction with N-MPEA as the number of monomer molecules increases. This interaction appears to plateau at 1:3 (Figure 2.28), with addition of monomer units observed to generate little increase in interaction with the template, and a slight increase in FM:FM self association.

PYR demonstrates a small favourable interaction between the 1:2 cluster and N-MPEA (Figure 2.28) that is diminished as further monomer units are added.
Figure 2.29: Estimated energy of FM:FM interaction (kJ/mol) for neutral functional monomer/N-MPEA clusters. (Equation 2.2-Equation 2.1) (Number of repetitions = 3)

2.3.4 -N-MPEA - Cross-linking Monomers

None of the modelled cross linking monomers is predicted to participate in significantly favourable levels of interaction with N-MPEA. Although a slight favourable interaction is predicted at lower equivalence, the trend appears to suggest a decreasing magnitude of interaction the more molecules of crosslinker are present (Figure 2.30).
Both DVB analogues demonstrated their potential utility as a cross linker for a MIP specific for this template by demonstrating that neither monomer participates in significant levels of interaction with the template (Figure 2.30). At a ratio of 1:5, m-DVB shows a significant decrease in the interaction between monomer units and the template (Figure 2.30).
EGDMA demonstrates a variable level of linked, FM:FM and T:FM interaction. Larger levels of T:FM interaction are observed at lower stoichiometries (1:2) ([Figure 2.30]) that appears to be linked to an unfavourable interaction between the cross linking units ([Figure 2.31]). This oscillation appears to increase towards greater interaction between the cross linking monomers’ self association as the magnitude of the T:FM stoichiometric excess increases.

**2.4 - 3, 6-Diacetyl morphine (HER)**

A commonly encountered illicit substance, heroin was included in the modelling scheme to investigate if there were any differences in the interaction of the library of functional monomers with a drug of a different class to the ATS. 3, 6-Diacetoxymorphine (HER) ([Figure 2.32]) contains six hetero atoms that are capable of participating in hydrogen bonding interactions, only as acceptors. The interactions observed between the functional monomer clusters and the heroin template exhibited similar overall calculated heats of formation to the ATS. Although the starting points were slightly lower than those observed during the ATS modelling, such initial uncertainty does not hinder the comparative value of subsequent models.

![Figure 2.32: Heroin (3, 6-diaceetyl morphine)](image-url)
2.4.1 - Heroin (HER) - Basic Monomers

2VP demonstrates an increase in interaction between template and monomer cluster at a ratio of 1:4 (*Figure 2.33*). This interaction also appears to be located at the acetoxy groups of the template, with the amine group of the template isolated from any monomer units. The increased abundance of $\pi$ electrons possessed by the template molecule and the increased surface area (in comparison to the ATS), suggested that the pyridine molecules would be more likely to associate with this molecule than the ATS molecules. However, it was not expected that 2VP would outperform its analogue, 4VP despite the effect of steric hindrance in ordering the geometry of the association noted during the amphetamine type substance modelling. The pyridine molecules are observed to participate in interactions with the acetoxy groups of the template, presumably due to the mass of localised lone pair electrons in this area (*Figure 2.33*). This interaction is dissimilar in the case of 4VP, with far less direct interaction between acetoxy group and monomer groups.

*Figure 2.33*: HER : 2 VP 1:4 clusters showing the association with the acetoxy groups of the template.

All basic monomers studied interact in a favourable, attractive fashion at one stoichiometry, the exception being VI, whose interaction profile appears to change very little based on the number of monomer molecules present in the model. It is likely that
the availability of groups for electrostatic interaction, and the sheer increase in surface size of the HER molecules in comparison to the ATS is responsible for this, despite the lack of ability for hydrogen bonding.

2HEMA indicates the likelihood of a favourable interaction occurring at a ratio of 1:3 (Figure 2.34) that was not observed at the remaining stoichiometries studied. This cluster demonstrates interaction with the three functional groups expected to be capable of donor/acceptor interaction, each with a single monomer unit. The same interactions appear in the ratio of 1:6, although the net magnitude is less than at 1:3.

**Figure 2.34:** Estimated energy of interaction for basic functional monomer/HER clusters. (Number of repetitions = 3)

**Figure 2.35:** HER: AAM 1:4.
When allyl amine was modelled, little favourable interaction was noted at the lower stoichiometries. There is an increase in the net attractive interaction at 1:4, and then again at 1:6. The failure of the modelling to show an association between the acetoxy groups and the hydrogen bond donating amine monomer cannot explain the significant increase in interaction predicted at a cluster stoichiometry of 1:6. Although the acetoxy groups are free for interaction, the allyl amine monomers appear to be located in a cluster around the bridged nitrogen ring (Figure 2.35).

Vinyl acetate is observed to generate the highest level of estimated interaction (ca. -30 kJ.mol$^{-1}$) with HER at a ratio of 1:2 (Figure 2.34). This net favourable interaction is not exhibited at any stoichiometry above 1:2.

![Figure 2.36: Estimated energy of FM:FM interaction for basic functional monomer/HER clusters. (Equation 2.2-Equation 2.1) (Number of repetitions = 3)](image)

2.4.2 - HER - Acidic Monomers

AA participates in self association in preference to interaction with the template (Figures 2.37 and 2.38). Although hypothesised to be a major site of interaction, the acetoxy groups of the template do not appear to participate.

MAA surprisingly, does not appear to participate in interactions of large magnitude with HER, and at 1:3, appears to suffer a significant repulsive interaction (ca. 20 kJ.mol$^{-1}$; Figure 2.37). It is suggested from the large difference in the observed
pattern of interaction between the opioid molecule and the phenylethylamine backbone that the template plays a large role in the stabilisation of the T:FM cluster. However, at higher stoichiometries, the interaction becomes progressively more favourable.

Figure 2.37: Estimated energy of interaction for acidic functional monomer/HER clusters. (Number of repetitions = 3)

Figure 2.38: Estimated energy of FM:FM interaction for acidic functional monomer/HER clusters. (Equation 2.2-Equation 2.1) (Number of repetitions = 3)
Table 2.10: Separation between hydrogen bond donors [D-H··A] and hydrogen bond acceptors from AM1 level simulations.

<table>
<thead>
<tr>
<th>ratio</th>
<th>Separation (Å)</th>
<th>H bond donor</th>
<th>H bond acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>2.151</td>
<td>ITA OH</td>
<td>HER O</td>
</tr>
<tr>
<td>1:3</td>
<td>2.191</td>
<td>ITA OH</td>
<td>HER O</td>
</tr>
<tr>
<td>1:6</td>
<td>2.233</td>
<td>ITA OH</td>
<td>HER O</td>
</tr>
</tbody>
</table>

IA displays its most favourable interaction at a ratio of 1:2 of ca. -60 kJ.mol\(^{-1}\) (Figure 2.37). This interaction, although not being identified as a hydrogen bond by the SPARTAN\(^{04}\) package (Table 2.10) is the closest identified in this set of experiments. Although the 1:6 cluster exhibits the same interaction between the T:FM, the repulsive contribution must be significantly greater in this case (Table 2.10). The FM:FM interaction of IA (Figure 2.38) suggests that although interaction may occur with the template, the self association is preferred. The greatest prediction of interaction occurs with IA, specifically at 1:2 stoichiometry describing the interaction with the acetoxy groups of the template.

2.4.3 HER - Neutral Monomers

PYR appears to participate in favourable interactions with HER (Figure 2.39). The location of the interaction around the furan ring, and the acetoxy groups suggests that such interaction may be comprised of both electrostatic and π-π interactions. The number of hydrogen bond acceptors on the template molecules makes a small hydrogen bond donor monomer a perfect candidate for the generation of a strong, multi-point association. This level of favourable interaction appears to be greatest at lower stoichiometries, suggesting that the addition of more monomer equivalencies may disrupt these interactions, without necessarily causing a significant rise in the FM:FM interactions (Figure 2.40).
Figure 2.39: Estimated energy of interaction for neutral functional monomer/HER clusters. (Number of repetitions = 3)

Figure 2.40: Estimated energy of FM:FM interaction for neutral functional monomer/HER clusters. (Equation 2.2-Equation 2.1) (Number of repetitions = 3)
STY interacts in a surprising fashion with HER. Rather than interacting with the ring system of the template as was predicted, the interaction is centred on the acetate groups, and is estimated to be a favourable interaction at higher equivalencies (Figure 2.39). The number of lone pairs associated with the four oxygen atoms and the delocalised $\pi$-electrons in the STY molecules must be the responsible properties causing this predicted electrostatic interaction, in conjunction with any surface or hydrophobic interactions.

AM appears to participate in favourable interaction with HER only at stoichiometries lower than 1:3 (Figure 2.40). The methylation of this monomer, to MAM appeared in previous studies (EPH, MDMA, and N-MPEA) to generate a larger interaction; presumably due to the steric hinderance produced by the methyl group resulting in orientations that allowed stronger interactions to occur. The effect is again observed at a stoichiometric ratio of 1:5.

2.4.4 - HER - Cross-linking Monomers

The only direct interaction predicted between HER and NOBE was at 1:1 (Figure 2.42), between the amine of NOBE and one oxygen atom of an acetoxy group on the template. It would appear in this case, like the ATS that NOBE does not
participate in strong enough interactions to allow it to effectively act as both functional monomer and cross linking monomer.

**Figure 2.42:** Estimated energy of interaction for cross linking monomer/HER clusters. (Number of repetitions = 3)

**Figure 2.43:** Estimated energy of FM:FM interaction for cross linking monomer/HER clusters. (Equation 2.2-Equation 2.1) (Number of repetitions = 3)

The DVB analogues chosen as potential cross linking monomers do not indicate that either of these monomers exhibits a significant interaction with the template (*Figure 2.42*). EGDMA indicates that at lower ratios of T: XLM, the interaction
between these components is largely favourable, and it displays the earlier identified oscillating pattern of interaction magnitude. TRIM exhibits similar levels of interaction at all stoichiometries other than 1:1. The lack of a favourable interaction at this ratio is not able to be currently explained, though the size of the TRIM molecule may result in an energetically unfavourable conformation in the gas phase due simply to its size.

2.5 Discussion:

The modelling of the preassociation interactions of template and functional monomers has been carried out to isolate those monomers that demonstrate the greatest potential for interaction with the template molecules. Further to this, investigations have been carried out to determine the stoichiometry of the T:FM cluster that generates the greatest interaction with the template. The utility of these calculations allows interrogation of the difference between the association between the T:FM and quantification of the FM:FM interactions.

Previous computational studies investigating the interaction between the template and functional monomer have utilised many levels of molecular definition from high level QM calculations, SE-MO to Newtonian Molecular Mechanics, to the hybrid QM/MM approach where areas of critical importance are defined and modelled using the more detailed QM methods and others using MM for efficiency of calculation speed.

In a series of papers investigating the pre polymerisation associations between ephedrine and functional monomers MAA, IA, TFMAA, 2HEMA and with cross linking monomers EGDMA and DVB, Ansell et al. identified a range of T:FM clusters were present and characterised via NMR studies.\textsuperscript{[171-173]} Of significant interest in this series of papers was the finding that the speciation was dependant on the solvent in which they were measured (chloroform and toluene producing different cluster stoichiometries). Further, TFMAA was observed to participate with ephedrine in the strongest clusters identified, however it was not found to be the best performing of the synthesised polymers. Such a result makes the ultimate goal of modelling slightly unclear. Is the goal to find the highest level of interaction with the template with one
functional monomer, or to discover the most appropriate cluster stoichiometry? The authors made another interesting finding, for although itaconic acid was determined to exhibit a higher level of interaction with the template, it was not possible to separate the performance of the MIP and NIP when used as a stationary phase in HPLC chiral separations.

Further to these results, the authors found that polymers using EGDMA performed significantly better than those using DVB as cross linking monomer. The ultimate cause of this difference was not explained fully however, it was suggested that the result was most likely due to the physical structure of the polymers created with the two cross linking monomers.

Nicholls identified three areas in which quantum methods have been used to model the design of MIP systems: (i), The characterisation of template/functional monomer association clusters; (ii), screening of functional monomers in a template based suitability calculation and assessment of the cluster stoichiometry; (iii), The use of target analogues to imprint the polymer matrix. Further to this, the use of quantum level calculations can be used to evaluate the post synthesis structures of pseudo-receptor cavities.\cite{174}

While the power and accuracy of quantum mechanical methods of calculation is not questioned, the necessary complexity of the basis sets and semi empirical methods results in neglect of significant factors in the real world synthesis of MIPs. The limitation in the number of electrons in a system before computation becomes too complex to be conducted efficiently means that realistic modelling of the total presynthesis mixture, including the total crosslinking component and the porogen is currently impossible. Additional concerns regarding itaconic acid were identified in this paper. Solubility forced the authors to synthesise their polymers in a polar porogen to avoid the precipitation of itaconic acid dimers and complexes with the template.

One manner in which such computational limits has been surmounted has been to utilise Molecular Mechanics approaches to the systems that exhibit significantly higher populations of atoms. This method and the combined QM-MM methods allow for significantly larger amounts of molecular species to be efficiently modelled in the same system. Such simplification is necessary if effective methods of rationally predicting interactions and associations for the creation of protein imprints are to occur.
The group of Piletsky et al. have successfully utilised a molecular mechanics force field to investigate the interaction between a single monomer unit and the ephedrine.\cite{175} Their study indicates that itaconic acid, methacrylic acid, 2-hydroxymethacrylate and AM produce the highest levels of interaction with the ephedrine molecule. The investigation is guided by the belief that the monomers that can participate in the largest number of hydrogen bonds with the template will be the monomers most suitable for specific MIP creation. This method isolates individual monomers that exhibit high levels of interaction with a template and continues to construct MIPs that utilise a 1:4 T: FM stoichiometry.

Piletsky et al. appear not to have modelled ephedrine, (1\text{R},2\text{S})-2-(methyl amino)-1-phenylpropan-1-ol, but to have modelled a demethylated analogue of ephedrine. They note that ephedrine (1\text{R},2\text{S})-(2)-a-(1-methylaminoethyl)benzyl alcohol was used for the synthesis, however a figure in their modelling section reproduced here (Figure 2.44) suggests that the modelling results may not be what was intended. The effect of this demethylation effect may be considerable in the hindrance of binding especially to the amine group of the template.

![Figure 2.44: Template modelled by Piletsky et al. 2001][175]

2.6 - Conclusions:

AM1 level SE-MO theory has allowed an efficient screening process not only of the monomer identity, but also cross linker, and stoichiometry of the T:FM cluster before going into the laboratory. Not only has the \textit{in silico} investigation prevented much laborious processing of non-viable imprinted polymers after synthesis, but the monomers that are predicted to produce the highest levels of interaction with the template, and the ratios at which this interaction is greatest have been identified.

Ephedrine is predicted to participate in favourable interactions, with methacrylic acid at ratios of 1:2 and 1:3, and for MAM at a ratio of 1:5. These favourable
interactions do not appear to be related to higher levels of FM:FM interaction. Although itaconic acid demonstrates favourable interaction, it appears to be more prone to inter- and intra- molecular self association. As a direct result, it is not suggested here as a “good” functional monomer in this case for this reason.

EGDMA and DVB appear to be the most appropriate cross linkers. It was not possible to effectively model the number of crosslinking monomers in a normal synthesis due to hardware limitations. Although all crosslinking monomers display little interaction with the template, the apparent failure of TRIM to self associate suggests that this monomer is not suitable for use in this case as the principal component of the imprinted polymers.

MDMA produces similar results to that of ephedrine in the template association studies that was expected due to the analogous structures of the ATS. The variation of functional groups did not appear to have a significant overall impact on the magnitude of the interactions. The location of these interactions does appear to change, with significantly more interaction being located at the methylene dioxy ring group than the secondary amine of the aliphatic tail.

It was expected that the level of interaction would be reduced between N-MPEA and the functional monomers due to the reduction in number of functional groups in the molecule. The effect of multiple cooperating monomers cooperating to increase the interaction between the functional monomer cluster and template molecule was most evident here, given the reduced number of sites for interaction precludes interaction at a single site. A 1:3 ratio of template: VAC, as well as 1:3 template: ITA was also predicted as potential stoichiometric ratios for further investigation of N-MPEA imprinted polymers.

A T:FM ratio of 1:2 IA molecules was determined to be the most promising stoichiometric ratio for the imprinting of HER. In this case, the interaction between itaconic acid molecules is not as large as that between the itaconic acid molecules and the template, in contrast to the behaviour noted in the EPH investigations.
Chapter 3 - Modelling of the pseudo-MIP cavity and in silico screening of competitor drugs classes and dye indicators for binding activity.

Investigation of the pre-synthetic association energy and geometry of a T:FM cluster provides insight into the nature of associations. Much effort and many different approaches have been directed towards simplifying the process of selecting the best functional monomer(s), the stoichiometry of the pre-synthesis mixture of template, functional monomer and cross-linking agent.\cite{162, 167, 171-173, 176-186} The commercial availability of a selected monomer is important as the ease of acquisition and the costs associated with synthesising or purchasing synthesised to order monomers may increase the cost of utilising such monomers, to the point that such techniques become commercially unviable.

This chapter aims to further investigate the nature of interactions between the template, ephedrine and functional monomer (FM) clusters identified in previous modelling experiments (Chapter 2). The interactions of these optimised, EPH-FM systems (both with the reintroduced template molecule and with species from other drug classes) are assessed computationally for comparison against experimental trials. In addition, the interactions of several indicators with the frozen clusters are studied to rank their estimated binding efficacy relative to EPH, with a view to employing these materials as an infield colour based detection system. Semi-empirical computational investigations have previously been explored by Wu and Li, who found their interaction energies correlated favourably with experimental findings relating to observable differences in analyte retention times.\cite{187}

Extending upon the technique of Wu and Li, the impact of solvent polarity on the nature of template/functional monomer interactions was also investigated, by introducing a solvent dielectric parameter to mimic different solvent environments. While it would be more realistic to explicitly incorporate a finite number of solvent molecules into the computational paradigm, to do so would create such computational demands that the efficient evaluation of the model would be improbable.
A number of methods have been proposed for the implicit incorporation of solvent molecules into the energetic calculation of molecules using the polarisable continuum. Such methods have gained popularity in the investigations of many bioactive substances by utilising the Poisson-Boltzmann (PB) equation, and the generalised Born methods (Generalised Born with Simple Switching - GBSW and Generalised Born using Molecular Volume - GBMV).\cite{188,193}

Unfortunately, direct numerical solutions to the Poisson equation are of little use in situations other than isolated, single point calculations. Thus for larger systems that are undergoing such calculations at many points and many steps (such as Molecular Dynamics simulations) the Generalised Born methods allow calculation of the solvation free energies using the following pair-wise sum:

\[ \Delta G_{\text{sp\rightarrow ov}}^{\text{elec}} = \sum_{i,j} \frac{q_i q_j}{\sqrt{r_{i,j}^2 + \alpha_i \alpha_j \exp(-r_{i,j}^2 / F \alpha_i \alpha_j)}} \] \text{ Equation 3.1} \]

where \( q_i \) is the charge of atom \( i \) of the solute, \( r_{i,j} \) is the distance between two atomic sites \( i \) and \( j \), \( \varepsilon_p, \varepsilon_w \) are the interior and exterior dielectric constant, \( F \) is a constant usually set to 4 or 8 and \( \alpha_i, \alpha_j \) are the effective born radii for atomic sites \( i \) and \( j \) respectively.\cite{195}

In both the PB and GB methods, the definition of the dielectric boundary given by the solute volume is of critical importance. The most simplistic definition is the van der Waals radius, which assumes superposition of atomic van der Waals spheres though it occasionally leads to overestimation of solvation energies due to the formation of micro-cavities exhibiting high dielectric constants.\cite{196} By modifying the van der Waals radius based volume, improvements are made to the accuracy of the calculations, whereby the spheres are extended by a small fixed value of a smooth interface function with the result being that the small interior cavities become filled.\cite{197,198} The molecular volume (MV) is obtained by rolling a sphere with a radius corresponding to the size of a solvent molecule, typically 1.4 Å for water, around the molecular Van der Waals surface.\cite{199} In an attempt to mimic the PB, most Generalised Born methods mimic the molecular volume.
The GBMV method approximates this volume by integrating overlapping van der Waals spheres and adds contributions from interstitial, solvent excluded volumes through a vector scaled algorithm given by Onufriev et al. \cite{199}

The Generalised Born methods were assessed in conjunction with the PB method by Chocholousova and Faig, where it was concluded that dielectric continuum model can be sufficient for generating realistic trajectories of nucleic acid systems. \cite{200} They found close agreement between the highly accurate generalised Born methods with the Poisson-Boltzmann theory existed. This was based on the commonly used sharp molecular surface definition, and compromises energy conservation and stability in molecular dynamics simulations of protein and nucleic acid systems.

Although the current system does not involve the level of complexity present in a protein system, the approach is likely to be still a valid method of quickly and efficiently screening the interactions between pseudo receptor and ligand to gauge the effect that solvent has on template- - functional monomer interactions.

3.1 – **Drug molecules for screening molecules – computed properties from in vacuo, AM1 calculations.**

The drug molecules used in the interaction screening process were selected from different drug classes (opioids, amphetamines etc) as illicit chemical species that might be expected to be encountered by law enforcement officers in their field activities. *Figure 3.1* shows their structures, while associated properties such as molecular surface area, volume and dipole moment are displayed in *Table 3.1*.

The amphetamine type substances (ATS) exhibit molecular surface areas and volumes in agreement with each other, as was expected with the relationship between their structures. EPH has a slightly larger molecular volume and area than the remaining ATS other than MDMA, whose volume is larger due to the presence of a methylenedioxy substitution on the phenyl ring. EPH exhibits the largest dipole moment of the ATS, which may be due to the presence of a second hetero atom on the tail of the molecule when compared to the other ATS. It is likely that MDMA’s lower dipole moment is due to the location of the polarisable groups at opposing ends of the molecule. This feature may also be responsible for the observed $E_{HOMO}$ (Highest
Occupied Molecular Orbital) and $E_{\text{LUMO}}$ (Lowest Unoccupied Molecular Orbital) differences between MDMA and the other amphetamine type substances. The $E_{\text{HOMO}}$ and $E_{\text{LUMO}}$ values give an estimate of the electron donating or accepting character of a given compound respectively.\textsuperscript{[201-203]} A compound is considered more electron donating the higher the value of its $E_{\text{HOMO}}$ and more electron accepting the lower the value of its $E_{\text{LUMO}}$.\textsuperscript{[204]}

Opioid compounds heroin (HER), morphine (MOR) and codeine, (COD) are significantly larger than the ATS and are observed to have higher dipole moments (ATS=0.75-2.27, OPI=2.85-6.16). Substitution of the C3 and C6 positions of the morphine skeleton appears to generate a higher dipole moment, regardless of the nature of the substituent (e.g. methoxy (COD) or acetoxy (HER) substitution). Cocaine (COC) has a larger volume and surface area than EPH, with a dipole moment that is greater than the members of the ATS group with the exception of ephedrine.

Table 3.1: Properties of drug molecules from \textit{in vacuo}, minimised AM1 calculations.

<table>
<thead>
<tr>
<th>Molecule Identity</th>
<th>Area (Å(^2))</th>
<th>Vol (Å(^3))</th>
<th>Dipole. (debye)</th>
<th>Max $\sigma^+$ Charge</th>
<th>Max $\sigma^-$ Charge</th>
<th>$E_{\text{H}}$ (eV)</th>
<th>$E_{\text{L}}$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephedrine (EPH)</td>
<td>214.46</td>
<td>192.45</td>
<td>2.27</td>
<td>22.91</td>
<td>-66.68</td>
<td>-9.27</td>
<td>0.48</td>
</tr>
<tr>
<td>Methamphetamine (MAMPH)</td>
<td>206.58</td>
<td>185.07</td>
<td>1.11</td>
<td>20.54</td>
<td>-75.15</td>
<td>-9.35</td>
<td>0.43</td>
</tr>
<tr>
<td>Amphetamine (AMPH)</td>
<td>185.93</td>
<td>164.82</td>
<td>1.35</td>
<td>23.02</td>
<td>-74.90</td>
<td>-9.4</td>
<td>0.46</td>
</tr>
<tr>
<td>N-methyl phenylethylamine (NMPEA)</td>
<td>191.02</td>
<td>167.11</td>
<td>1.14</td>
<td>19.93</td>
<td>-72.25</td>
<td>-9.39</td>
<td>0.45</td>
</tr>
<tr>
<td>N-Methyl-3,4-methylenedioxy amphetamine (MDMA)</td>
<td>231.9</td>
<td>210.72</td>
<td>0.75</td>
<td>21.84</td>
<td>-71.42</td>
<td>-8.89</td>
<td>0.15</td>
</tr>
<tr>
<td>Morphine (MOR)</td>
<td>279.6</td>
<td>281.92</td>
<td>2.85</td>
<td>27.58</td>
<td>-63.62</td>
<td>-8.78</td>
<td>0.21</td>
</tr>
<tr>
<td>Codeine (COD)</td>
<td>300.71</td>
<td>302.19</td>
<td>3.51</td>
<td>23.98</td>
<td>-64.38</td>
<td>-8.6</td>
<td>0.36</td>
</tr>
<tr>
<td>Heroin (HER)</td>
<td>363.12</td>
<td>363.96</td>
<td>6.16</td>
<td>21.42</td>
<td>-74.56</td>
<td>-9.11</td>
<td>-0.09</td>
</tr>
<tr>
<td>Cocaine (COC)</td>
<td>327.58</td>
<td>313.89</td>
<td>1.72</td>
<td>17.80</td>
<td>-69.72</td>
<td>-9.56</td>
<td>-0.39</td>
</tr>
</tbody>
</table>

The surface potential of the drug molecules investigated is indicated by the differential colouration of the density potential surfaces of the molecules listed in \textit{Figure 3.1} and the values for the maximum (\textit{red (-)}) and minimum (\textit{blue (+)}) surface potentials are listed in \textit{Table 3.1}. Molecules within each drug class were found to exhibit similar surface potentials, with the ATS exhibiting maximum positive charge in the range +19.93 (N-MPEA) to +23.02 (AMPH) and a maximum negative charge of between -66.68 (EPH) to -75.15 (MAPMH). Opioid compounds exhibited a maximum positive surface charge of between +21.42 (HER) to +27.58 (MOR) and a maximum negative surface charge of -63.62 (MOR) to -74.56 (HER).
Figure 3.1: Drug molecules used in screening experiments A) EPH, B) N-MPEA, C) MDMA, D) AMPH, E) MAMPH, F) COC, G) COD, H) HER, I) MOR
The similarity between the maximum and minimum estimated charges for the drug molecules investigated suggests that the interactions between the ligands and the functional monomer molecules will be very similar if there are no constraints on their interaction. The similarity in the functional groups present in the alkaloid molecules (O and N containing groups) means that some other facet is responsible for the differing interactions of these molecules with their native receptors. However, the constraints induced to simulate this receptor ligand system means that single point interactions are unlikely to produce the differentiation required in MIPs. Thus, the spatial separation and orientation of these groups on each molecule plays a large role in governing the interaction between synthetic receptor model and each ligand.

3.1.2 Benzoxadiazole Indicators - Computed Properties from \textit{in vacuo, minimised AM1} calculations.

The work of Shimizu and Greene demonstrated that it is possible to employ a benzoxadiazole based dye to indirectly report specific adsorption phenomena in MIPs.\textsuperscript{[151]} They successfully employed a fluorescent dye (\textit{N,N}-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine) to display the relative binding efficiencies of a group of small aromatic amines towards an amphetamine imprinted MIP. The imprint cavities of the MIP were exposed to the dye prior to exposure to the amine analyte, which resulted in competitive displacement of the probe. Analysis of the findings was not a trivial matter, requiring Linear Discriminant Analysis (LDA) to correlate changes in adsorption to MIPs selectivity for each analyte. The study did however succeed in discriminating between structurally similar analogues to a reported 94\% accuracy.

The indicators, with the exception of Dye G (Figure 3.2, Table 3.2) display similar skeletal structures and possess multiple areas of positive and negative polarity on the head group as well as aromatic areas of neutrality, and an aliphatic tail with amine groups contained on the tail. The modelling studies reported in \textit{Chapter 2} showed significant interaction around the amine group of the amphetamine tail. As a result, modulation of this functional group with differing steric and electronic environments was undertaken in the dye compounds to give insight to this effect.
Fluorescein was included as a potential displacement indicator for opioid imprinted MIPs. Its significant volume and constrained structure were hypothesised to be a good mimic for this class of compounds. Dye G shares a similar, albeit smaller molecular volume to HER (Dye G = 313.18, HER = 363.96), and a slightly lower maximum negative charge (Dye G = -72.22, HER = -74.56). The dipole moment observed for fluorescein is however higher than all opioid compounds, as is the maximum positive charge, with fluorescein displaying almost double the maximum positive charge of the opioid molecules (48.13 vs. 27, 23 and 21 for MOR, COD and HER respectively). Therefore, Dye G may be useful as a discriminator of HER, but it is unlikely to be useful to discriminate either COD or MOR, due to the lower charge magnitudes, and its larger molecular volume in comparison to these opioid molecules.

Table 3.2: Properties of indicator molecules from in vacuo, minimised AM1 calculations.

<table>
<thead>
<tr>
<th>Molecule Identity</th>
<th>Area (Å²)</th>
<th>Volume (Å³)</th>
<th>Dipole (debye)</th>
<th>Max ‘+’ Charge</th>
<th>Max ‘-’ Charge</th>
<th>E_H (eV)</th>
<th>E_L (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediame</td>
<td>266.2</td>
<td>236.96</td>
<td>8.71</td>
<td>50.46</td>
<td>-66.24</td>
<td>-9.18</td>
<td>-1.96</td>
</tr>
<tr>
<td>N,N-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediame</td>
<td>264.38</td>
<td>236.55</td>
<td>8.64</td>
<td>31.14</td>
<td>-66.68</td>
<td>-9.11</td>
<td>-1.93</td>
</tr>
<tr>
<td>4-fluoro-7-sulfamoyl-2,1,3-benzoxadiazol</td>
<td>187.42</td>
<td>161.51</td>
<td>3.54</td>
<td>59.16</td>
<td>-92.95</td>
<td>-10.31</td>
<td>-2.22</td>
</tr>
<tr>
<td>4-(2-aminoethyl amino)-7-N,N-dimethylsulfamoyl-2,1,3-benzoxadiazol</td>
<td>287.47</td>
<td>257.59</td>
<td>6.92</td>
<td>48.62</td>
<td>-101.76</td>
<td>-9.13</td>
<td>-1.73</td>
</tr>
<tr>
<td>4-(3-amino pyrrolidino)-7-N,N-dimethylsulfamoyl-2,1,3-benzoxadiazol</td>
<td>308.41</td>
<td>283.6</td>
<td>8.6</td>
<td>38.83</td>
<td>-105.16</td>
<td>-8.89</td>
<td>-1.59</td>
</tr>
<tr>
<td>4-piperazino-7-N,N-dimethylsulfamoyl-2,1,3-benzoxadiazol</td>
<td>305.08</td>
<td>283.88</td>
<td>7.35</td>
<td>42.77</td>
<td>-102.45</td>
<td>-8.97</td>
<td>-1.68</td>
</tr>
<tr>
<td>Fluorescein (Dye G)</td>
<td>313.18</td>
<td>315.4</td>
<td>6.63</td>
<td>48.13</td>
<td>-72.22</td>
<td>-8.64</td>
<td>-1.89</td>
</tr>
</tbody>
</table>

The dyes exhibit a similar direction of dipole moment (towards the electronegative arene substituent) and away from the amine containing “tail”. Dye C exhibits a slightly different vector of the polarisation (with reference to the conserved aromatic system) due to the substitution of fluorine in place of the aliphatic section of the other dye molecules. The dipole moment of fluorescein is directed towards the carboxyl group from the phenyl substituted carbon and follows the plane of the molecule.
Figure 3.2: Inserted possible indicator molecules a) Dye A, b) Dye B, c) Dye C, d) Dye D, e) Dye E, f) Dye F, g) Dye G. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green)
Analysis of benzoxadiazole Dyes A-F showed that they possess greater surface area and molecular volume than the ATS with the exception of Dye C, which is smaller in molecular volume than all ATS, but has a greater surface area than N-MPEA. The indicators exhibit similar $E_{\text{HOMO}}$ values to the ATS, Dye C showing the lowest value for all molecules at -10.31 eV, likely to result from the electron withdrawing effects of the halogen substituent. All indicator molecules exhibit lower $E_{\text{LUMO}}$ potentials than any of the drug molecules investigated suggesting that the indicator molecules will be capable of stronger charge transfer interactions with the receptor sites than the drug molecules. However, this feature is also suggested by the higher dipole moments for this class of compounds, and also higher maximum positive charge. This electron accepting property is presumably due to greater presence of electronegative atoms (N, O, S and F) on the dye molecules in comparison to the drug molecules.

The surface charge of the indicator molecules used in this screening protocol are listed in Table 3.2, and refer to the scale for the colouration of the red (-) and blue (+) regions of the density potential surface shown in Figure 3.2. The analogues of the Shimizu and Greene dye display much higher positive potentials than the drug molecules investigated (Table 3.1), the lowest of these is observed for Dye B, at +31.1366. Generally the indicator molecules exhibited significantly higher negative charges, the only exceptions being Dye A and Dye B, at -66.2438 and -66.6774 respectively. The remaining dye molecules exhibited negative potentials almost double this amount – from -92.9464 (Dye C) to -105.158 (Dye E) that implies electrostatic interactions between the cluster and the indicator substances could play the largest role in the outcome of the calculations.

### 3.1.3 Imprinted Clusters - Computed Properties from *in vacuo*, minimised AM1 calculations.

The properties of the imprinted clusters after the removal of the template molecules were calculated to show the electronic properties of the sites that are responsible for the direct interaction with introduced ligands in real polymers. The monomers were subjected to translational and rotational constraints so that their structures based on interaction with the template are preserved.
The imprinted cluster geometries (Figure 3.3) show that unsurprisingly the volume and surface area (Table 3.3) of each cavity is dependent upon the number of functional monomers of which it is comprised. The largest dipole moment is observed to exist in the EPH-MAA 1:3 cluster, despite more carboxylic acid groups being present in the HER-ITA 1:2 cluster. The orientation of the EPH-MAA 1:3 cluster, with all carboxyl groups being present on the same side of the cluster is suspected to be responsible for this feature of the imprint cluster.

The lowest dipole moment is observed in the HER-ITA 1:2 cluster, presumably due to the orientation of the carboxylic acid groups being oriented in opposing directions. The EPH-MAM 1:5 cluster is observed to have the largest surface area and molecular volume of the pseudo imprinted sites. Thus, in this imprint cavity (as defined by the functional monomers alone), the effect of dispersion forces will be the greatest in this imprint site.

Table 3.3: Properties of imprint cavities from in vacuo, minimised AM1 calculations.

<table>
<thead>
<tr>
<th>Molecule Identity</th>
<th>Area (Å²)</th>
<th>Volume (Å³)</th>
<th>Dipole Moment (debye)</th>
<th>Maximum + Charge</th>
<th>Maximum - Charge</th>
<th>HOMO (eV)</th>
<th>LUMO (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH-MAA 1:2</td>
<td>238.09</td>
<td>188.52</td>
<td>3.76</td>
<td>33.9288</td>
<td>-68.3986</td>
<td>-10.54</td>
<td>-0.07</td>
</tr>
<tr>
<td>EPH-MAA 1:3</td>
<td>333.89</td>
<td>280.09</td>
<td>4.51</td>
<td>33.2117</td>
<td>-77.7338</td>
<td>-10.29</td>
<td>-0.16</td>
</tr>
<tr>
<td>EPH-MAM 1:5</td>
<td>572.02</td>
<td>481.51</td>
<td>3.95</td>
<td>36.6110</td>
<td>-81.8166</td>
<td>-9.95</td>
<td>-0.05</td>
</tr>
<tr>
<td>HER-ITA 1:2</td>
<td>288.66</td>
<td>243.00</td>
<td>2.62</td>
<td>52.9232</td>
<td>-67.0945</td>
<td>-10.78</td>
<td>-0.78</td>
</tr>
</tbody>
</table>

The spatial orientation of the imprinted cluster monomers is a key factor in the selective ability of the imprint sites to preferentially bind the template and analogues over different molecular species. As the comparison of the electrostatic properties of the ligands and MIP theory suggests, the functional monomer is capable of interacting with a large range of template molecules. It is fixed in position during the polymerisation step, thus inducing the selectivity of the imprinted site. This feature is likely to include the cross linking monomer as a restriction on the imprint site for the molecular volume of the species being recognised. The functional monomers play an important role in this discrimination, through repulsive electrostatic interactions with the proximate molecular species. The more functional monomer units present, the more likely that such a feature would be possible due simply to the additional numbers of functional groups present in the imprint site and the associated increase in preorganisation.
It is unlikely that the EPH-MAA 1:2 cluster will demonstrate size exclusion in its interaction with the compounds to be screened against the pseudo receptor cavities. This is likely to be due to the separation between the functional monomer units. Similarly, the EPH-MAA 1:3, EPH-MAM 1:5 and HER-ITA 1:2 clusters may also be subject to lower levels of size exclusion than real world imprinted polymers, thus the interaction energies calculated represent only the interaction with the functional monomer aspect of the imprint site.

It is therefore not useful to compare the volume of the monomer units comprising the imprint site and the molecular species around which it was templated. Further development of accurate models of the polymerisation process may eventually allow for in silico discrimination of compounds like this, however there is no current method that allows for this. Despite this possible confounding influence, Wu and Li successfully implemented such a design of in silico screening for the imprint cluster and confirmed its utility using HPLC and analogues of their template (nicotinamide). It therefore remains a viable approach to investigating the effect of the positioning of the monomer units on the interaction with a group of potential ligands that a field-based MIP detection/presumptive identification technique would be expected to encounter. More importantly, it is predicted that based on the interactions with the functional monomers alone that the template and its analogues may not exhibit the greatest magnitude of interaction.

The colouration of the density potential surfaces of the imprint sites is annotated in Table 3.3. The MAA imprint sites exhibit similar positive potentials of +33.93 and +33.21 for the 1:2 and 1:3 clusters respectively and their maximum negative potentials are -68.40 and -77.74. The EPH-MAM cluster exhibits a maximum positive potential of +36.61 and a maximum negative potential of -81.82. Unexpectedly, the HER-ITA 1:2 cluster demonstrates the largest positive potential +52.92, and a maximum negative potential of -67.10. The interaction with charged species for the MAA clusters will be largely similar, although it is likely that the 1:3 cluster will exhibit a greater affinity for cationic species. In comparison, the MAM cluster is expected to participate in greater electrostatic interactions with the ligands than the MAA cluster, with greater positive and negative calculated maximum point charges and greater available surface area for weak intermolecular interactions.
3.2 Screening of MAA 1:2 cluster in the gas phase using AM1 and CHARMm, and using the GBSW method of implicit solvation using CHARMm.

The EPH: MAA cluster (Figure 3.4) was identified in earlier experiments (Chapter 2) as a system that demonstrated a significant level of attraction between the monomer units and template. The distance between the monomer units is unlikely to prevent the interaction between this cluster and any molecular species involved in the screening process. Reintroduction of the template species is essential to determine the ability of the constrained site to replicate the interactions that determined the location of the monomer molecules.

As these molecules are free to move in the initial monomer screening simulations as they are in the pre-polymerisation solution, potential steric hindrance of entering/exiting the imprint site is avoided. However, after polymerisation such freedom of movement is no longer possible. Thus, as the recognition is desired in the synthesised polymer the behaviour of the template and the post-polymerisation model of
the imprint site are of critical importance. If a molecule is unable to enter the imprint site as defined by the functional monomers such that the reciprocal interactions created through the polymerisation in presence of the template, the addition of further restrictions on the ability of a molecule to approach the imprint site will only hinder the interaction of ligand and receptor cavity further.

![Image](figure3.png)

**Figure 3.4**: Orientation and separation of the MAA receptor cluster from ephedrine template studies (Chapter 2).

3.2.1 - **Screening of Drug Molecules against EPH- MAA 1:2 imprint cavity using AM1 Semi-Empirical Molecular Orbital Theory, (SPARTAN04) and CHARMM Molecular Mechanics Force Field (AccelrysDS)**

EPH was not seen to participate in identical interactions upon reintroduction as were observed as the template/monomer cluster was screened (Figure 3.5). A rotation of the template of approximately 90° is apparent between the two models. Reintroduction of the EPH molecule into the imprinted cluster geometry results in a conformation preventing interaction between the hydrogen bond donor and acceptor sites of the amine group; however the hydroxyl group appears to interact with the imprint cluster. It is likely to be a result of the reduced degrees of freedom exhibited by the monomer cluster – drug system with no variation of the favourable geometry possible due to the freezing of the monomer units in place.

There appears to be a significant difference between the magnitude of the interaction between screened molecule and the EPH- MAA 1:2 imprint cavity in the gas phase depending on the force field used. **Figure 3.6A and 3.6B** demonstrate the ca. +300 kJ.mol⁻¹ differences in interaction magnitude with the constrained monomer cluster. In both forcefield descriptions, EPH is not the molecule that interacts most strongly with the cluster.
In the AM1 simulations, AMPH demonstrates a favourable interaction with the cluster of 180% that of EPH, while the remaining ATS exhibit interactions within 20% of the reintroduced template (*Figure 3.6C*). N-MPEA binds less strongly to the cluster than does the template, while MDMA binds more strongly (20%). This variation may be due to the interaction with only one of the functional monomers (N-MPEA, *Figure 3.7*) and a greater amount of surface involved in the MDMA interaction than EPH as there appears to be no hydrogen bond donor acceptor involved in the interaction (MDMA, *Figure 3.7*). COC demonstrates the least favourable interaction with the imprinted cluster in comparison to EPH, its interaction magnitude only 50% of the template’s. The values for the simulated, comparative binding were derived using Equation 2.1.
Figure 3.6: Comparison of interaction estimates from gas phase molecular screening experiments
A) drug molecules AM1, B) Drug molecules CHARMM, C) interaction magnitude relative
to template (EPH) AM1, D) interaction magnitude relative to template (EPH) CHARMM.
(A and B) Lower values represent stronger interaction, (C and D) while a stronger
interaction than the template is represented by positive values, normalised to the
interaction of the template.

Although the CHARMM screening appears to indicate that the interaction is
repulsive, reliance on the absolute magnitude of the abbreviated descriptions of
energetic properties and the calculations being a combination of strain energies rather
than enthalpies of formation makes reliance on these magnitudes unwise. However, as
each molecule in this series of experiments is described by the same definitions of physical properties, the relationship between the interactions calculated for different ligands is the important facet of these results. This second software package (AccelrysDS) was used in the gas phase to give a comparison between the two methods used for the calculation, as the SPARTAN package did not allow for the modulation of the dielectric constant to create an implicit solvent environment. The gas phase modelling was undertaken to give some comparison between the values presented from each forcefield, and to demonstrate the perils of considering calculations such as this as presenting actual enthalpies of formation. The calculations, although reported as kcal.mol⁻¹ enthalpies of formation, are actually global bond and parameter strain energies. While comparative value remains between these calculated values, to think of them as representative of empirical calorimetric results is unwise.

The CHARMM results (Figure 3.6B and 3.6D) demonstrate a very similar level of interaction for the ATS family, while the remaining drug molecules exhibit higher levels of interaction with the cluster. This unexpected result is likely to be due to the lack of size exclusion ability for this cluster, for no other property could prevent the interaction of the non-imprinted molecules with the cluster. In all ATS, there appears to be an interaction between the aromatic head of the molecule and the labile H of the functional monomers.

![Figure 3.7: Eph-MAA 1:2 cluster with inserted drug molecules from AM1 calculations A) N-MPEA, B) MAMP, C) MDMA. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge(green).](image)
3.2.2 - **Gas Phase Screening of Indicator Molecules against the EPH-MAA 1:2 imprint cavity model using AM1 Semi-Empirical Molecular Orbital Theory. (SPARTAN\textsuperscript{04}) and CHARMM Molecular Mechanics Force Field (AccelrysDS).**

The interaction of the dye molecules with the EPH-MAA 1:2 cavity (*Figure 3.8*) shows that in the AM1 calculations that all dye molecules are capable of favourable interaction with the monomer geometry associated with EPH. Dye B and Dye F are observed to participate in less favourable interactions with the cluster than the template, whilst the remaining dyes show an increased magnitude of the interaction with the constrained monomers.

There is little supporting literature identifying appropriate adsorption properties for a dye displacement indicator. One hypothesis is that a species of similar size but capable of forming only weak interactions with the binding cavity (thus being easily displaced by the template) might be appropriate. Such an approach may however lead to poor selectivity due to competitive displacement by other analytes. A second plausible option is that the indicator possess slightly greater (but less directed) interactions with the imprint cavity, limiting displacement only to species fitting securely within the binding pocket. A third is to use a molecule with an area of similarity to the template, but with substituents at one point that make it sterically much larger, thus inhibiting its interaction with the entire cavity. Thus, as the interaction is only occurring at one point, and a significant portion of the indicator molecule will be directed into the solvent space outside the cavity, the dye may be leveraged out of the cavity by the combination of interaction exhibited by the template and its analogues.

Joshi *et al.* suggests that the imprint site discriminates ligands through a combination of reciprocal point interactions (between template and monomer) and size/shape exclusion effects.\textsuperscript{178} As the surface area and volume of all dye species examined is significantly larger than the ATS, non-specific hydrophobic and van der Waals forces may play a significant role in determining overall selectivity.
Although the greatest estimated interaction with the MAA 1:2 cluster was shown to be produced by Dye G, no hydrogen bonding interaction is predicted by either the AM1 or the CHARMM forcefield. Interactions observed between Dye A and the monomer cluster was shown to take place at the secondary amine group of this molecule, in preference to any other hydrogen bond donor acceptor present on the molecule. A change in the position of the methyl substitution on this molecule (Dye A → Dye B) resulted not only in a change in the location of the interaction between dye and receptor cavity model, to the N of the dioxazole ring, but also resulted in a reduction in the magnitude of the interaction.

Dye F demonstrates the lowest interaction with the imprint cluster, which is also less than 50% of the interaction of EPH, potentially due to the apparent interaction between the sulfamoyl groups with one MAA unit. Similar to Dye B, (Figure 3.9) this molecule appears to not be enveloped by the monomer units, and visually appears to have a lower amount of surface area in contact with the monomer units than the other dye molecules shown– all of which interact more strongly and exhibit greater visual levels of surface contact. Dye C is the only indicator to exhibit a hydrogen bond with the cluster between the sulfamoyl and carboxyl groups, the separation of which was observed to be 2.072 Å (Figure 3.9E).

From the interactions observed, it is apparent that the sulfamoyl group is capable of increasing the interaction of the molecular species in comparison to the nitro group in the same position, due to the polarity of the molecule, and the increase in size in comparison to the nitro group given the addition of the sulphur atom to the functional group. This effect is tempered by the dimethylation of the sulfamoyl moiety due most likely to the steric crowding of the sites by the methyl groups. Species containing linear alkyl amines appear to participate in higher levels of interaction with the receptor site model than those with bulkier (pyrrolidino and piperazino).
Figure 3.8: Comparison of interaction estimates from gas phase molecular screening experiments
A) indicator molecules AM1, B) indicator molecules CHARMM, C) interaction magnitude relative to template (EPH) AM1, D) interaction magnitude relative to template (EPH) CHARMM. (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented by normalised values based on the interaction of the template.
3.2.3 Screening of MAA 1:2 cluster using GBSW method to implicitly model the solvent environment using CHARMM Molecular Mechanics Force Field (AccelrysDS).

The GBSW method of incorporation of the solvent environment as a polarisable continuum can provide insight into the potential for solvent based modulation of the interaction between the monomer cluster and the screened. Although the solvent
molecules are not explicitly modelled, the use of a polarisable continuum to predict the solution to the Poisson–Boltzmann equation is a useful approximation when more than a single point is being calculated (such as in MD simulations).

3.2.3.1 - Influence of Solvent on Drug-Cluster Binding Interactions

The increase of dielectric constant value ‘\(\varepsilon\)’ is observed to generate a decrease in the magnitude of the interaction between the monomer cluster and the screened molecules. The template was not observed to participate in the largest interaction with the monomer cluster, and the opioid class of compounds significantly outperformed the template and its analogues. The most pronounced outperformance being in the implicit toluene environment (\(\varepsilon = 2.38\)) where MOR exhibits a 4 fold higher interaction than EPH.

The calculated magnitude of the interaction between ATS and synthetic receptor cavity decreases as the polarity of the implicitly modelled solvent is increased. MDMA is observed to exhibit a slightly different pattern of interaction as the polarity gradient is incorporated into the calculations. It follows the other members of the ATS group in the organic solvents, however when water is implicitly incorporated, it is observed to exhibit a significant increase in the prediction of the interaction.

The only other ATS to exhibit an increase in interaction at higher \(\varepsilon\) was N-MPEA; however the magnitude of the change was relatively small. N-MPEA interacts more strongly with the imprint site than the template in every solvent except for MeCN. It was expected that the primary amine of AMPH would exhibit a larger interaction with the imprinted site monomers, however this was not observed. In addition, EPH possesses a hydroxyl group that was shown to be available for a chelating interaction with MAA by Ansell et al.\textsuperscript{171-173} but interactions of this nature were not observed in these modelling experiments.

The interaction between cocaine and the monomer cluster is observed to increase as the solvent continuum value increases from toluene to chloroform (\(\approx 2-4\)) however this association of increasing polarity and increasing interaction is not observed for the remainder of the solvent profile. The opioid molecules also exhibit
deterioration of the interaction as solvent polarity increases. Despite this, the opiates are observed to participate in the most favourable interactions with the EPH-MAA 1:2 cluster.

**Figure 3.10:** Comparison of interaction estimates from molecular screening experiments using CHARMM A) Amphetamine Type Substances, B) Other types of drugs, C) Amphetamine Type Substances shown as relative to the template (EPH), D) Other drug types shown as relative to the template (EPH). (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented normalised values based on the interaction of the template.
3.2.3.2 Influence of Solvent on Dye - Cluster Binding Interactions.

The interaction of the dye indicators A to G with the frozen MAA 1:2 cluster was observed to follow a similar pattern to that observed for the ATS. Interactions increase in all cases with increasing dielectric constant. In toluene, Dyes B, C and E exhibit a weaker interaction with the imprint cluster than EPH, while the remaining indicator molecules Dyes A, D, F and G interact more strongly with the cluster than EPH. In chloroform, all indicator molecules interact more strongly with the cluster than EPH, however there is little observable difference between the magnitude of interaction of Dye C and Dye E. In the model of the MeCN environment, Dye A exhibits no difference in the binding magnitude to the cluster than the template, while Dyes B, D, F and G exhibit stronger interaction with the cluster than the template, while the remaining Dyes C and E bind less strongly than the template to the imprint site.

Figure 3.11: Comparison of interaction estimates from molecular screening experiments using CHARMM A) Indicator substances, B) Indicator substances relative to the template (EPH). (A) Lower values represent stronger interactions, (B) stronger interaction than the template is represented by normalised values based on the interaction of the template.
The model of an aqueous environment shows that Dye A binds marginally less strongly to the cluster than EPH, Dye B binds less strongly than the template, but slightly more strongly than Dye A to the imprint site and Dye C again binds less strongly to the imprint site. Dye E shows little separation from EPH, while the remaining dyes bind more strongly to the imprint site, Dye G binding significantly more strongly to the cavity than EPH.

3.3 **Screening of EPH-MAA 1:3 cluster in the gas phase using AM1 and CHARMm, and using the GBSW method of implicit solvation using CHARMm.**

The MAA monomers that make up the EPH-MAA 1:3 imprint site (*Figure 3.12*) exhibit a more proximate relationship to each other than the EPH-MAA 1:2 component monomers. All 3 monomer molecules are involved in obvious interaction with each other. The charge distribution and location of the carboxylic acid functional groups are located on the same side of the cluster, resulting in greater restrictions on the vector from which a ligand can approach the imprint site and interact favourably than in the EPH-MAA 1:2 imprint site model.

Due to the closer proximity in which the monomer units exist, the likelihood of size exclusion or electrostatic repulsion effects on non-template analogues is higher, with fewer degrees of freedom existing for the ligand molecule to orient itself for favourable interaction with the imprint site model.

*Figure 3.12:* (A) EPH-MAA 1:3 cluster from original AM1 experiments and (B), monomer cluster separation. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge(green) Distances are shown in Å.
3.3.1 - **Screening of Drug Molecules against EPH-MAA 1:3 imprint cavity using AM1 Semi-Empirical Molecular Orbital Theory (SPARTAN\textsuperscript{04}) and CHARMM Molecular Mechanics Force Field (AccelrysDS)**

Reintroduction of the template (EPH) to the EPH-MAA 1-3 imprint site model produces a similar outcome to that obtained from the monomer screening (*Figure 3.13*). The presence of a third MAA monomer unit has a significant influence on ligand interactions by forming a cooperative network with the other functional monomer units.

The interaction of the imprint site with the ATS is conserved (*Figure 3.14*), with the aliphatic segment of the molecules interacting with the carboxylic acid groups of the imprint site monomers. AMPH interacts least strongly of the ATS with the imprint site suggesting that the imprint site demonstrates selectivity for the methylated, secondary amine exhibited by the template in comparison to the primary amine of AMPH. MDMA exhibits a slightly different orientation to the other ATS, which could be due to the differences in charge density brought about by the electron donating character of the methylenedioxy unit. MDMA’s interaction is however still dominated by the secondary amine unit in the tail of the molecule with the MAA units of the imprint site.

![Figure 3.13: EPH-MAA 1:3 cluster from original AM1 models (A) and reintroduced (B). Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge(green)](image)
Figure 3.14: Comparison of interaction estimates from gas phase molecular screening experiments. A) drug molecules AM1, B) Drug molecules CHARMM, C) interaction magnitude relative to template AM1, D) interaction magnitude relative to template CHARMM. (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented by normalised values based on the interaction of the template.
The acetyl substituents of the HER molecule appear to interact with the carboxyl groups resulting in a favourable interaction magnitude between imprint site and ligand. The remaining opioid molecules are not observed to participate in energetically favourable interactions with the imprint site. COD exhibits the least interaction followed by MOR, which suggests that the methoxy group of COD inhibits the interaction with the imprint site more than the hydroxyl groups of MOR in the same position. The difference in orientation of the opioid backbone is shown (Figure 3.15),
and suggests that a size exclusion feature would further increase the ability for selective
discrimination of ATS by the imprint site by utilising the molecular surface area and
volume of the screened ligands (Table 3.1, 3.1, 3.3).

COC is observed to participate in favourable interaction in the AM1 experiments
(Figure 3.14) with the imprint site, though not to the same extent as EPH. The acetate
and tropane groups are involved in the interaction with the imprint site, as these groups
appear to be the most accessible for the imprint site to interact with. The close
proximity of N and O atoms (similar to EPH) may go some way to explaining why this
molecule still interacts favourably with the imprint site although it belongs to a different
class to the template.

The CHARMM screening in the gas phase is consistent with the behaviour of
the ATS class. EPH interacts more strongly than the rest of the ATS class, although
little difference is observed in the interaction magnitudes of EPH and MDMA. N-
MPEA demonstrates the lowest level of interaction. AMPH and MAMPH demonstrate
that the absence of the OH results in only a small decrease in interaction magnitude (in
comparison to EPH). AMPH demonstrates a higher magnitude of interaction than
MAMPH, which is presumably due to the substitution differences of this amine group
between AMPH and the rest of the class.

COC is the only drug molecule screened that interacts more strongly with the
imprint site than the template. The opioids show a significantly different interaction
with the imprint site than both the ATS and COC. None of the opioids indicate a
favourable interaction with the imprint site and a significantly lower level of interaction
than EPH. Again, it is noted that in order of increasing interaction magnitude the opioid
molecules are COD, MOR and HER (Figure 3.14). Presumably this propensity for
interaction relates to the available sites for interaction, which is precluded by the
methoxy group in COD (in comparison to MOR) and increased again for HER with the
additional O atoms and polarisation due to the acetoxy groups.
3.3.2 Screening of Indicator Molecules against EPH-MAA 1:3 imprint cavity using AM1 Semi-Empirical Molecular Orbital Theory (SPARTAN\textsuperscript{04}) and CHARMM Molecular Mechanics Force Field (AccelrysDS)

In the AM1 experiments, the indicator compounds generally show greater levels of interaction with the imprint site than EPH. Dyes E and F are the exceptions to this pattern, with Dye F exhibiting the lowest level of interaction with the imprint site and Dyes A, B and C exhibiting similar interaction magnitudes with the imprint site (Figure 3.16). The interaction of Dye C involves the sulfamoyl amine groups and the oxygen of the benzoxadiazole ring as HBD and HBA species respectively, with the oxygen of the carboxylic acid groups of the functional monomers providing the reciprocal donor acceptor interactions. Dye A exhibits a similar type of interaction to EPH and the ATS (Figure 3.17) with the aromatic head of the molecule facing away from the imprint site, and the tail unit interacting with the carboxylic acid side of the imprint site. Dye B interacts in a similar fashion to Dye A, and the ATS, however in comparison to Dye A, there appears to be a rotation of the molecule through approximately 90°. It is possible that this rotation may be due to the variation of the position of methylation between these two molecules.

The two dye molecules with the cyclo-alkyl amine substituents in place of the linear alkyl amine chains (Dye E and F) exhibit lower levels of interaction with the imprint site that EPH, and also the lowest level of interaction with the imprint site in the AM1 screening. Thus, it would appear that accessibility of the amine group is an important aspect for the interaction for this site in a similar fashion to the template. Dye G exhibits the greatest level of interaction with the EPH-MAA 1:3 imprint site. This feature excludes its use as a potential indicator for the selective displacement by ATS analogues as displacement of the indicator upon approach of the template or a related molecule is unlikely due to the estimated difference in interaction magnitudes.

The CHARMM investigation comparatively produces slightly different results. Dyes C and E are observed to exhibit a lower level of interaction with the imprint site than EPH. Dyes C and E are observed to interact less strongly with the imprint site than EPH (Figure 3.16). Dye G is observed to exhibit the largest interaction in the
CHARMM forcefield, confirming its lack of appropriateness as an indicator for ATS in a synthetic ATS receptor. Both Dyes A and B present higher interactions that EPH; with Dye A more capable of interaction with the imprint site.

**Figure 3.16:** Comparison of interaction estimates from gas phase molecular screening experiments  
A) indicator molecules AM1, B) indicator molecules CHARMM, C) interaction magnitude relative to template AM1, D) interaction magnitude relative to template CHARMM. (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented by normalised values based on the interaction of the template.
It was hypothesised that the secondary amine (Dye B) would enable higher levels of interaction due to it being more accessible and less constrained by additional methylation than the tertiary amine of Dye A. It was expected that the secondary amine would behave more like the template for the imprint site, and thus interact with the imprint site more strongly, making it effective as an indicator of template binding. This is likely to be due to significantly greater interaction with the imprint site than the template and thus a lower propensity to be displaced. However, this hypothesis is not
supported by the results of these simulations (Figure 3.16). Although the primary amine of AMPH is seen to interact slightly more strongly than the secondary amine MAPMH, the same behaviour is not observed for the Dye A and Dye B methylation difference.

3.3.3 - Screening of MAA 1:3 cluster using GBSW method to implicitly model the solvent environment using CHARMM Molecular Mechanics Force Field (AccelrysDS).

3.3.3.1- Drug screening

In a similar fashion to the EPH- MAA 1:2, the increase of solvent dielectric constant is observed to generate a decrease in the level of interaction between screened molecule and the EPH- MAA 1:3 imprint site (Figure 3.18). The template relative interaction also exhibits a large amount of variation. In the most apolar environment investigated (toluene), the template interacts more strongly with the imprint site than the remainder of the ATS, by over 20% in the case of MDMA. In chloroform, all ATS interact more strongly with the imprint site, the greatest of these being approximately 20% above that of the template.

Increasing the dielectric constant results in an increasing level of interaction relative to the template, although the apparent magnitude of the interaction is clearly decreasing. The primary amine of AMPH appears to be more subject to the dielectric constant variation than the other ATS. As the dielectric constant environment is changed to represent water, the interaction of the other ATS is seen to remain constant, while the template’s interaction with the imprint site decreases, AMPH is observed to interact more strongly with the imprint site. Thus, the relative interaction of this ATS is observed to be approximately 130% of EPH.
Figure 3.18: Comparison of interaction estimates from molecular screening experiments using CHARMM A) Amphetamine Type Substances, B) Other drugs, C) ATS shown relative to the template (EPH), D) Other drug types shown as relative to the template (EPH). (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented by normalised values based on the interaction of the template.

The tropane alkaloid COC, exhibits a decreasing magnitude of interaction with the imprint site as the dielectric constant is increased. Relative to the template, COC
interacts more strongly with the imprint site as the solvent model becomes more polar, exhibiting the largest relative interaction of approximately 120% in water. The opioid molecules offer greater deviation from the behaviour of the template and the imprint site. The opioid molecules are observed to undergo a decreasing interaction as the dielectric constant increases amongst the organic solvents modelled. However, in the implicit water model, both HER and MOR are observed to participate in greater interaction with the imprint site than in MeCN. COD however, appears to exhibit the same level of interaction with the imprint site in both the MeCN and H2O environments.

Relative to the template, the opioid molecules exhibit a significant difference in their interaction (Figure 3.18). This relative interaction deviation is lower in the implicit water model, than in the organic solvents.

### 3.3.3.2 – Indicator screening.

The indicators involved in the screening protocol show that the increase of the solvent dielectric constant term results in decreasing levels of interaction in the case of the organic solvents modelled (Figure 3.19). Similarly, there is little change in the apparent magnitude of the binding strength between imprint site between the solvent model of MeCN and H2O. Dye G again is unlikely to be of use as a selectively displaced indicator due to the significantly higher interaction with the imprint site than the template and its analogues.

In toluene, all indicator molecules with the exception of Dye G display a lower level of interaction with the imprint site than EPH. Noticeably, Dyes D and F exhibit the least interaction with the imprint site at this dielectric constant. The interaction between imprint site and indicator molecules, although decreasing with the increasing polarity of the solvent term, is observed to increase relative to the template. However, as predicted before the gas phase calculations, Dye B is observed to interact with the imprint site more strongly than its analogue Dye A. The level of interaction between dyes and the imprint site increases as the dielectric constant increases, however the relationship is non-linear (Figure 3.19). Between MeCN and water, there appears to be little difference in the interactions between imprint site and indicator molecules.
3.4 - Screening of Drug Molecules against EPH- MAM 1:5 imprint cavity using AM1 Semi-Empirical Molecular Orbital Theory. (SPARTAN^04) and CHARMM Molecular Mechanics Force Field (AccelrysDS)

The EPH- MAM 1-5 imprint site involves the largest amount of intermolecular interaction with a bifurcated network of hydrogen bonds existing between the monomer units and between the amine and carboxyl functional groups of the monomer molecules. From the investigations carried out previously (Chapter 2), and from the work conducted by Yu and Mosbach, such a functional monomer is likely to introduce a significant hydrophobic character to the MIP.\(^{160}\) This is especially relevant given the large stoichiometric excess predicted in this case, and as a result, the amount of surface area available for hydrophobic interaction.
3.4.1 - Screening of Drug Molecules

The original simulations carried out in Chapter 2 demonstrated that there was a significant increase in the interaction between EPH and the MAM cluster when the stoichiometry was T: FM 1:5. This cluster orientation (Figure 3.20), shows a cleft in the structure in which the ephedrine molecule lies. The hetero atoms of the aliphatic tail interact with the imprint cluster while the phenyl group does not appear to interact with the imprint site. Thus, the hydrophobic interactions as predicted by Yu et al [163]. are clearly a key factor in the interaction between the phenyl group of the template and the pseudo-receptor site.

In the AM1 modelling experiments full entry into the binding cleft of this synthetic receptor site is not observed for the opioids (Figure 3.23) and may explain the lack of strong, favourable interaction with the imprint site. However HER is seen to occupy the cleft that is likely to be the cause of the strong interaction with the imprint site. COC is observed to interact slightly more favourably with the imprint site than EPH, and despite not fully entering the imprint cavity, the interaction between the acrylamide R-NH$_2$ and C=O and the acetate groups of the ligand COC is clear (Figure 3.23)
Figure 3.21: Comparison of interaction estimates from gas phase molecular screening experiments. A) drug molecules AM1, B) Drug molecules CHARMM, C) interaction magnitude relative to template (1) AM1, D) interaction magnitude relative to template CHARMM. (A and B) Lower values represent stronger interactions, while a stronger interaction than the template is represented by a normalised values based on the interaction of the template.
Reintroduction of EPH into the imprint site does not appear to result in an identical orientation of the template within the cluster. As a result, the template appears to interact slightly less favourably with the imprint site than the other ATS involved in the ligand screening in the case of the AM1 calculations (Figure 3.21). Despite this, there is good discrimination of the opioid substances except for HER, most likely due to the length and flexibility of the diacetoxy substitution at the 3’ and 6’ positions. In this case, though neither COD or MOR interacts strongly with the imprint site, COD interacts more strongly than does MOR – a reversal of the observed pattern in the MAA imprint site ligand screening.

The ATS exhibit stronger interaction than EPH in the gas phase AM1 calculations, due to their insertion deeper into the imprint site than EPH (Figure 3.23). Although AMPH does not fully venture into the cavity, the interaction in this case being the strongest of the class appears to be centred on interaction between the primary amine of AMPH and both functional group types of the monomer cavity.

In the CHARMM simulations, EPH is observed to interact more strongly than any other ATS molecule investigated (Figure 3.21). There appears to be much less class discrimination exhibited by this imprint site in the gas phase for the ATS family over the tropane COC and the opioids. In a deviation from the performance of the MAA imprint sites, MDMA and AMPH exhibit the weakest interaction with the imprint
site of any of the drug molecules investigated. COD is observed to participate in the strongest interaction of the non-template drug molecules.

Figure 3.23: Eph-MAM 1:5 cluster with inserted drug molecules A) MDMA, B) MAPMH, C) AMPH, D) COC, E) COD, F) HER. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green)

3.4.2 - Gas Phase Screening of Indicator Molecules

The screening of the indicator molecules in the AM1 level theory sees all dye molecules interact with the imprint site more strongly than EPH (Figure 3.24). Dye G is again observed to exhibit the strongest interaction and the strength of this interaction
is likely to be due to the observed molecule’s insertion into the imprint site (Figure 3.25) coupled with the interaction between the OH of Dye G in a network of interactions with charged sites on the imprint site.

Dye E exhibits a marginally stronger interaction with the imprint site than the template. Dye A exhibits a stronger interaction than its analogue Dye B. Its orientation is closer to that of the ATS (Figure 3.25) than Dye B, and while both substituted ends of the benzoxadiazole moiety appear to be in contact with the imprint cluster, the polar species of these substitutions are not sufficiently close to the HBA/HBD groups of the imprinted site. The insertion into the cavity appears to allow both non-specific interactions with the hydrophobic areas of the molecular surface, and with the HBA/HBD sites. Of the dye molecules analogous to the dye used by Shimizu and Greene\textsuperscript{[151]}, Dye C exhibits the strongest interaction with the EPH-MAM 1-5 imprint site. This indicator appears able to fully insert into the binding cleft, and the sulfamoyl group exists in close proximity to both NH and C=O functional groups of the imprint site (Figure 3.25).

In the CHARMM calculations (Figure 3.24), the dye molecules bind less strongly to the imprint site with the exception of Dye G. In considering the utility of the dyes for competitive displacement, Dyes A and B are observed to interact the most strongly with this imprint site during these calculations with Dye B participating in stronger interaction than Dye A. This is in agreement with the prediction that the terminal secondary amine should interact more strongly with the imprint site than the tertiary amine due to accessibility of the lone pairs for interaction with the functional monomers, and also in the case of Dye B the ability to act as a HBD. Dyes C and F are observed to exhibit the weakest interaction with the imprint site, while Dye E’s interaction with the imprint site is similar to that of Dye A. Despite the greater bulk of the aminopyrrolidino group (in comparison to the aliphatic group of A and B) it is apparent that the primary amine in this position is capable of similar levels of interaction.
Figure 3.24: Comparison of interaction estimates from gas phase molecular screening experiments
A) indicator molecules AM1, B) indicator molecules CHARMM, C) interaction magnitude relative to template AM1, D) interaction magnitude relative to template CHARMM. (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented by normalised values based on the interaction of the template.
Figure 3.25: AM1 displacement indicator insertion into the 1:5 Eph:MAM cluster geometry. A) Dye A, B) Dye B, C) Dye F, D) Dye D, E) Dye C, F) Dye G. Potential mapping shows areas of positive charge (blue), negative charge (red) and neutral charge (green).

3.4.3 Screening of EPH- MAM 1:5 cluster using GBSW method to implicitly model the solvent environment using CHARMM Molecular Mechanics Force Field (AccelrysDS).

While the incorporation of a solvent dielectric constant as an implicit model of increasing solvent polarity was observed to decrease the overall magnitude of the
interaction between all molecules and the MAA imprint sites, the previous experimental work of Yu and Mosbach suggests that the acrylamide functional monomer is capable of greater interaction than MAA through hydrogen bonds and hydrophobic interaction in aqueous solution.\textsuperscript{[163]} Therefore, it was expected that there would be an observable increase in the interaction between imprint site and all molecules as the dielectric constant term is increased towards an implicit representation of an aqueous environment. Due to the influence of the solvent polarity in this series of theoretical experiments, the calculations carried out were expected to produce an increase in estimated interaction for only those molecules that possessed similar electrostatic properties (eg dipole moment) as the template.

### 3.4.3.1 – Drug screening.

The MAM functional monomers were predicted to exhibit a different behaviour to the MAA FM. As the dielectric constant was increased, the interaction of the imprint site and ATS were observed to become increasingly favourable (Figure 3.26). In fact, both MAMPH and N-MPEA are observed to exhibit a larger increase in interaction than EPH between chloroform and acetonitrile.

While EPH is observed to exhibit increased interaction with the imprint site in water compared to MeCN, both MAMPH and N-MPEA exhibit a decrease in interaction between MeCN and water. AMPH, in another deviation from the previously observed trends in this chapter, is seen to exhibit a weaker magnitude of interaction with the imprint site than the N-methylated analogues. This suggests that the greater steric bulk of this group plays a role in either direct interaction with the imprint site, or that its hydrophobic or van der Waal interaction allows closer orientation the amine that results in a stronger interaction. In all cases, MDMA exhibits a slightly greater interaction with the imprint site than EPH, which may be due to the polarisation difference caused by the electron withdrawing methylene dioxy group attached to the phenyl group of the basic, phenylethylamine structure.
Figure 3.26: Comparison of interaction estimates from molecular screening experiments using CHARMM. A) Amphetamine Type Substances, B) Other types of drugs, C) Amphetamine Type Substances shown as relative to the template (EPH), D) Other drug types shown as relative to the template (EPH). (A and B) Lower values represent stronger interactions; (C and D) while a stronger interaction than the template is represented by normalised values based on the interaction of the template.

The behaviour of the opioid class of molecules displays a different pattern to that of the ATS. While COD and MOR are observed to increase in interaction between
ligand and imprint site as the polarity is shifted from toluene to acetonitrile, (though MOR exhibits little difference in interaction between the implicit chloroform environment and that of acetonitrile), the interaction of HER is observed to decrease between TOL and CHCl₃, and exhibit little variation between CHCl₃ and MeCN.

The behaviour of the opioids and the EPH- MAM 1-5 imprint site exhibits the greatest deviation from the previously observed behaviour of the ligands and the EPH imprinted site. Not only does this behaviour not fit with the behaviour of the previously conducted in silico screening, but it does not follow the same pattern as the ATS for this imprint site. While EPH and the ATS exhibit an increasing interaction as the polarity of the implicit solvent is changed to represent the aqueous environment, the same shift of dielectric constant results in the significant decrease in the strength of the interaction of the opioids with the EPH- MAM 1-5 cluster. This decrease makes the interaction of the opioids in toluene greater than in water, and the interaction is approximately 3 fold weaker than EPH in the same implicit solvent environment. COC exhibits little difference in interaction magnitude to EPH in any implicit solvent environment, though this interaction is marginally greater than EPH in all cases.

As the effect of hydrophobicity was considered to be a major factor in water by Yu and Mosbach[^163^], and also that the neutral acrylamide was shown to form stronger hydrogen bonds in more polar solutions than the carboxylic acid in the same solvent, the interaction between ligands and receptor site becomes stronger as the polarity of the recognition environment increases. The considerable weakening of the interaction between the imprint site and the opioid species as the polarity of the environment increases can play a major role in the discrimination shown by a MIP. It can be expected that in water, the methacrylamide MIP will exhibit recognition of the template and its analogues.

3.4.3.2 – Indicator screening.

The interaction of the indicator molecules with the EPH- MAM 1:5 imprint site is observed to follow the same general pattern for the ATS, in that increasing interaction magnitude is observed to occur with increasing polarity, though little further change is observed after the MeCN implicit solvent model. In all environments but CHCl₃, where
all dyes bind more favourably than the template, Dye F is observed to participate in weaker interactions with the imprint site than EPH. Dyes E and G exhibit the strongest interaction relative to the template in all solvent model calculations, and thus are unlikely to be suitable as displacement indicators.

Figure 3.27: Comparison of interaction estimates from molecular screening experiments using CHARMM A) Indicator substances, B) Indicator substances relative to the template (EPH). (A) Lower values represent stronger interactions, (B) a stronger interaction than the template is represented by normalised values based on the interaction of the template

Dyes A and B display interactions within 20% of the strength calculated for the template in all implicit solvent environments. The implicit MeCN environment generates the largest interaction between these dye molecules and the imprint site relative to the template and it is also observed in this case that Dye B interacts more strongly than Dye A, a reversal of the behaviour in the alternate implicit solvent environments.
3.5– Conclusions

In the gas phase, the two force fields used to investigate the energy of interaction (AM1 calculations in SPARTAN and CHARMM calculations in AccelrysDS) were observed to produce differing magnitudes of interaction. However, as a comparative tool, their utility lies not in predicting the absolute magnitude of interaction, but in the assessment of the behaviour of a variety of differing structural analogues and differing molecular classes. Although there is currently no method for the *in silico* screening of MIP systems in their entirety, the work of Wu and Li \(^{(187)}\) is indicative of the utility of such techniques in providing insight into the potential interactions that may dictate selectivity in the binding site. The behaviour of the ATS was quite similar, and their behaviour deviated significantly from the opioids, and to a lesser extent cocaine.

In the EPH-MAA 1:2 receptor model, the opioid class demonstrated an ability to interact more strongly with the imprint site than the template. This feature is likely to be related to the lack of size exclusion in the model cavity, allowing molecules exhibiting a larger molecular volume (than the template) to interact with the imprint site. The ability for interaction with the competing drug classes is not unexpected, as the major interactions between imprint site model and the ligands screened against it are likely to be electrostatic or hydrogen bonding. Hydrophobic and van der Waals interactions also play a significant role in the specificity of interaction between synthetic receptor cavity and the ligands. Such features are not included in these models due to the need for computational efficiency, but also the lack of a reliable model for the post-polymerisation structure of imprinted polymers.

The indicator molecules were generally observed to exhibit more interaction with the imprint site than the template or its associated analogues. The difference between the interaction magnitudes of the indicator substances were observed to exhibit less variation from the template as the implicit solvent model became more polar. The dye utilised by Shimizu and Greene (DYE A) was observed to interact more strongly with the template in the apolar organic solvent environments, whilst it exhibited a lower level of interaction in both the implicitly modelled MeCN and water environments.

More polar environments are best suited to the use of the Dye A, as well as its analogues Dye B and C for the indication of ATS recognition events. It is also possible
that in toluene, Dyes B, C and E would be useful indicator substances for the indirect monitoring of the recognition of ATS binding at the imprint site of EPH-MAA 1:2. Further to this, the addition of a solvent dielectric constant term was used to model the effect of the solvent upon the interactions between ligand and the functional monomer units of the imprint site. This allows screening through a potential range of solution phases for the eventual real world recognition of the ligands. In this case, the increase in solvent dielectric constant appears to generate a significant decrease in the magnitude of the interactions between the imprint cavity and all ligands screened.

The EPH-MAA 1:3 cluster demonstrates a similar ability to the EPH-MAA 1:2 cluster to discriminate between the different classes of drug molecules in the gas phase. The absolute magnitude of the calculated interactions is not the ultimate goal of these investigations, but the relative performance of the imprinted site geometries against a variety of different ligands.

In the gas phase, the ATS substances were observed to generate interactions that were in good agreement with that of the EPH and each other. Variation of the amine group (1°/2°) generated variation in the strength of the interaction, as did the methyl substitution of the phenylethylamine backbone.

The tropane alkaloid, COC is observed to participate in interactions of a similar magnitude to the template (AM1), or stronger (CHARMM) in the gas phase. This interaction appears to be an electrostatic interaction with the proximate tropane N and the O of the acetate group.

The opioid molecules were observed to participate in weaker interaction with the imprint site in the gas phase, with COD exhibiting the lowest magnitude of interaction, followed by MOR and then HER. In the AM1 calculations, it is likely that the acetoxy substitution of the 3’ and 6’ positions allow for the interaction of these functional groups with the imprint site, which as there is no size exclusion component of the calculations, has resulted in an interaction magnitude only marginally less than that of EPH. In the CHARMM calculation however, there is a much greater separation between the interaction of the template and imprint site and all opioid class molecules screened.

As the solvent dielectric constant term was varied to implicitly incorporate the effect of the solvent polarity in the interaction between the imprint site and screened
ligands, it was observed that the increasing polarity decreased the interaction between the imprint site and all ligands. However, the relative interaction of all ATS in comparison with the template is observed to become larger as the dielectric constant was increased. COC exhibited little observable difference in interaction with the imprint site than the template in toluene, however as the dielectric constant increases, the interaction of COC increased relative to the template. The opioid molecules are observed to participate in interactions with the imprint site that are significantly weaker than EPH. As the solvent dielectric constant is increased, the interaction of HER and the imprint site becomes significantly stronger in comparison to the other opioids. COD is affected the least by the variation in the dielectric constant. HER is the most affected opioid, through manipulation of the dielectric constant, and is observed to participate in stronger interactions with the imprint site than the template in water. MOR exhibits marginally weaker interactions with this imprint site in the same environment. It is possible that this is due to the greater presence of lone pairs of electrons on the acetoxy substituted positions of the opiate backbone.

Most indicator molecules exhibit a stronger interaction with the imprint site than the template in the gas phase, similar to the EPH-MAA 1:2 case. The AM1 calculations show Dyes E and F to exhibit weaker interactions with the imprint site than the template while in the CHARMM calculations, Dyes C and F exhibit weaker interaction. Presumably the difference between the interaction magnitude of the template and its analogues is caused by the significant difference in the polarity of the molecules, the indicator molecules being much more polarised than the ATS, thus their electrostatic interaction with the imprint site is stronger. Dye G is unlikely to be a useful indicator for the monitoring of ATS recognition by this imprint site due to its significantly higher calculated interaction with the imprint site. Dyes A and B are expected to be the most appropriate indicator molecules due to the observed similarities in the orientation of the molecules and the imprint site, as well as the largely similar interaction magnitudes as the template and its analogues.

In the implicit solvent screening, the indicator molecules with the exception of Dye G exhibit a weaker interaction with the imprint site than the template. However, as the dielectric constant is increased, this interaction is seen to be greater than the interaction of EPH in all cases.
It is likely that the inclusion of a method for the incorporation of size exclusion parameters for the screening of potential ligands through a range of imprinted sites could provide further discrimination ability to the computational method.

The behaviour of the MAM imprint site is significantly different to the EPH imprinted MAA sites. The re-introduction of the template molecule was observed to exhibit different interaction magnitudes dependent on the force field used for calculation. However, the CHARMM calculation displayed a significantly stronger interaction in comparison to the other drug and indicator molecules screened than in the semi-empirical AM1 calculations. However, the CHARMM calculations offer little in the way of selectivity between the molecules screened during the gas phase investigation. Significantly greater separation was observed between the molecules of the different classes in the AM1 calculations; specifically the opioid class was significantly hindered in its interaction with the imprint site. The failure of HER to interact with the imprint site in the same fashion as the other opioids appears to be due to the increased amount of flexibility and polarity induced by the di-acetoxy substitution.

The indicators display a similar divergence of interaction between the two forcefields in the gas phase as do the drug molecules. In AM1 calculation, the indicator molecules are observed to participate in greater interaction with the EPH imprinted cavity, but in the CHARMM simulations, the template displays greater interaction than the dye molecules with the exception of fluoroscein.

In accordance to the hypothesis that the increase in dielectric constant would result in an increase in the interaction between imprint site and the template (following the observations made by Yu and Mosbach regarding acrylamide functional monomers for aqueous phase recognition), the interaction between imprint site and the collection of ligands bearing similarity to the template is observed to increase as the dielectric constant increases. This linked increase appears to hold throughout the implicit modelling of the VOC solvents, however no such increase in interaction is observed as the value of the dielectric constant is increased to implicitly model water. It would appear however, that this pattern of interaction holds for molecules of a similar nature to the template, as this behaviour of the opioid class displays much difference to that of the ATS. These molecules are observed to exhibit a decreasing interaction with the imprint
site as the dielectric constant is increased, resulting in the greatest ability for the computational discrimination between molecules being present in aqueous solution.

Dyes A and B are observed to participate in similar relative interactions to the template (EPH) in all implicit solvent environments, suggesting that their interaction with the imprint site bears significant similarities to that of the template. Although the interaction is observed to be greater than that of either the template or its analogues, it is unlikely to be competitively displaced by these molecules. The published observations by Shimizu and Greene suggest that this is not the case. The segment of the MIP excluded from this computational scheme i.e. the size exclusion of molecular volume by the crosslinking monomer at the imprint site must play a significant role in the real world recognition of ligands by MIP synthetic receptors.

The variation in the behaviour of the MAM imprint site in comparison to the MAA imprint site is a factor of the different types of functional group present. While MAA is acidic, and therefore significantly ionisable, MAM is a neutral monomer that will be far less vulnerable to variation in the charge of the solvent environment in which it is found. Yu and Mosbach also postulate that the NH-O hydrogen bond is significantly stronger than the OH-O hydrogen bond, in aqueous environments.

The interaction between all species and the imprint site appears to increase in parallel with the increase in dielectric constant, which is observed to increase at every step including the aqueous environment. The EPH-MAA 1:3 cluster was observed to participate in weakening interactions with all screened ligand molecules as the dielectric constant was increased, however this reduction in interaction appeared to have little effect on the relative interaction of the molecular species with the imprint site relative to that of the template. The MAM cluster is observed to exhibit an increasing level of interaction with the ATS with increasing dielectric constant to MeCN, however little further increase is observed as the constant is increased to reflect the implicit aqueous environment. The opioid molecules however, are observed to decrease in interaction with increasing dielectric constant, with the weakest interaction being observed in water. Thus, the greatest separation between template class and the other molecular species is observed in the implicit water environment.
Chapter 4: *In vitro* application of *in silico* predictions of monomer association and stoichiometry.

Although the interactions predicted to generate the most favourable template – functional monomer associations in the *in silico* screening protocol were expected to exist in the pre-synthetic solution, further investigation is necessary to confirm these predictions.

This has been commonly carried out via spectrophotometry or spectroscopy of the template environment in the presence of varying concentrations of a functional monomer present in the solution. The stoichiometry of the transient clusters present in the pre-polymerisation solution is commonly assessed via the application of a Job plot, also known as the method of continuous variation. In this method the total solution concentration is maintained at a constant value, and the molar fractions made up by the component template and functional monomer molecules are varied. The chemical shift, in ppm (parts per million), is multiplied by the mole fraction and plotted against the mole fraction.

When a plot of $(\Delta \sigma_{(\text{ppm})} \times \text{mole fraction})$ vs. mole fraction is constructed (where $\Delta \sigma_{(\text{ppm})}$ is chemical shift of a $^1\text{H}$ or $^{13}\text{C}$ resonance in the case of these NMR studies), the stoichiometry that is related to the most energetically favourable stoichiometry will be represented by the highest point on this chart. The Job plot reflects the environment where the formation of the predominant, stable complex is presented as the highest point of the plot. Where the complexation is non-equilibrium, the maximum of the Job plot is related to the total consumption of the limiting reagent by the chemical reaction.

Recognition of natural ligands by natural receptors occurs almost exclusively in aqueous solution. Conserved water molecules have been identified in ligand binding sites of GPCRs via crystal structures. Thus, for aqueous recognition of neurotransmitter type molecules the ability of the functional monomer and template to interact with the presence of $\text{H}_2\text{O}$ molecules in solution is likely to be a necessity. As EPH crystallises as EPH hemi-hydrate, no further attempt was made to remove these waters of crystallisation, though the NMR solvents were dried before use. If the clusters that form in the solution are transient, non-covalent interactions the observed shift related to
the shielding or deshielding of the nuclei involved in the interaction may be present at some stoichiometries but not at others.

Additional variation in the shift observed for labile protons could be due to exchange with these waters of crystallisation. This effect was not considered by Ansell et al. in their detailed investigation of the pre polymerisation interactions, as they took great care to exclude any water molecules from their solutions used in the NMR analysis.\textsuperscript{[171-173]}

4.1 Job plot and titration analysis.

4.1.1 Previously performed NMR investigations.

Ansell et al. have performed extensive NMR analysis of the EPH - MAA association in organic solution (CHCl\textsubscript{3}, toluene and MeCN) that involved detailed analysis of the chemical shift associated with the methyl group protons and the carbons to which the hetero atoms of EPH are bonded.\textsuperscript{[171-173]} Such analysis was deemed necessary as the NH/OH groups are observed as a single peak and was impossible to fit to the model. The model used considered the interactions shown in Figure 4.1 and consist of the (FM)\textsubscript{2} (MAA dimer), (FM)\textsubscript{T} chelating complex (Involving both the NH and OH groups of the template and the carboxylic acid group), the (FM)\textsubscript{2}T and the (FM)\textsubscript{3}T in addition to (FM)XLM (the functional monomer, cross linking monomer species). The geometry of these interactions do not appear to exhibit a similarity to either the structures predicted by the \textit{in silico} modelling reported previously in Chapter 2 and 3, and also by Piletsky et al.\textsuperscript{[175]} and Dong et al.\textsuperscript{[212]}. The chelating interaction was postulated to require the \textit{gauche} conformation of the vicinal hetero atom groups of EPH (as opposed to the \textit{anti}-conformation modelled by these groups). These conformations describe the relative stereochemistry around a \textit{sp}\textsuperscript{3} hybridised, carbon-carbon $\sigma$ bond, and describe the orientation of the vicinal substituents of these carbon atoms. The \textit{gauche}- conformer refers to an observed torsion angle of $\pm 60^\circ$ between the O and N atoms (in the case of EPH) while the \textit{anti} - conformer refers to the torsion angle being between $\pm 90^\circ$ and $180^\circ$.\textsuperscript{[209]}

No constraints were placed on the individual molecular conformations, and each molecule was minimised using the MMFF force field before inclusion in the cluster
model. There is an apparent match between the predicted structures of the (FM)$_3$T complex, however, the (FM)$_2$T and (FM)T complexes do not agree with the structures postulated by Ansell et al. (Figure 4.1). The cooperation of the FM units in the (FM)$_3$T complex to interact both with the other FM units and the template is evident in this model structure. The proposed chelating interaction was not predicted in either the (FM)T or (FM)$_2$T cluster.

![Figure 4.1: Ansell et al. (171-173) postulated interactions for EPH and MAA. (Left) and AM1 models for the same complexes from this work](image)

The conformation of the vicinal HBA/HBD groups of the EPH molecule in the calculated global energy minimum is observed to be in the anti- state for (FM)$_2$T. However for the (FM)T and (FM)$_3$T, the conformation of these vicinal groups is gauche. Despite this proposed necessity for the chelating interaction to take place, no such interaction was predicted in the gas phase modelling. It is possible however, that
the micro-environment created by the presence of solvent molecules could induce this chelating interaction to take place.

![Figure 4.2: NMR data for EPH and MAA in CDCl₃ as reported by Ansell et al.](image)

The continuous variation plots generated by Ansell et al. were constructed using the shifts of the methyl protons of EPH, and the associated carbon atoms, while ignoring the exchangeable protons due to their combined signal. The sharp peak at mole fraction of 0.5 is indicative of the (FM)T complex being the principal association species in the solution, as observed after plotting the data for ¹H atoms, while a similar pattern was observed for the ¹³C atoms (Figure 4.2). The carbon atoms geminal to the exchangeable protons (Figure 4.2c and d) showed the greatest rate of change until there is an equivalent amount of both template and functional monomer in solution. Although this is the greatest rate of change for the association, the hydroxy connected carbon’s association is observed to increase to the (FM)₂T stoichiometry, and little change between (FM)₂T and (FM)₃T.
Continued addition of monomer units to the solution was observed to result in the aliphatic carbon continuing to shift upfield, although by 3:1 solution stoichiometry, little further change was observed. The association constants (in CDCl₃) were calculated to be $K_{(FM)T} = 10000 \text{ M}^{-1}$, $K_{(FM)2T} = 80 \text{ M}^{-1}$ when the data was fitted by eye in Ansell 2005, and adjusted to $K_{(FM)T} = 6830 \pm 480 \text{ M}^{-1}$, $K_{(FM)2T} = 240000 \pm 50000 \text{ M}^{-1}$ when their NMR data was calculated using hypNMR in Ansell et al. 2008. In comparison, the published association constants for MAA with other templates were observed to be significantly lower, between MAA and phenylalanine anilide $30 \text{ M}^{-1}$ in acetonitrile-$d_3$ (Sellegren et al. [210]), and between acetic acid-nicotine in CDCl₃ $8 \text{ M}^{-1}$ by Svenson et al. [211]. Reasons given for this greater association were the extra basicity of the secondary amine and chelating nature of the MAA–EPH interaction could be the cause of the observed complex stability. In addition, the calculations of Ansell et al. take account of MAA dimerisation, which was predicted to effectively lower the free monomer concentration approximately 50-fold in the region of interest. The calculated association constants for the dimerisation of the MAA molecules to form the $(FM)_2$ complex were calculated to be $330 \text{ M}^{-1}$ in CDCl₃, $18 \text{ M}^{-1}$ in TOL-$d_8$ and $0.305 \text{ M}^{-1}$ in MeCN-$d_3$. This self association was observed to be approximately $\frac{1}{4}$ of the magnitude of previously determined self association of $(FM)_2 = 1200 \text{ M}^{-1}$ (in CDCl₃) reported by this group through the fitting of the carboxyl carbon only. In all cases, the association of all species was determined to be largest in CHCl₃, slightly lower in TOL and significantly lower in MeCN, which is consistent with the relative polarities of these solvents. The predictions of speciation in the polymers synthesised in each of the respective solvents suggested that the largest proportion of $(FM)T$ site formation was present in CHCl₃ while this complex stoichiometry was predicted to be lowest in TOL.

Although the association in MeCN for $(FM)T$ is weaker than in TOL, the MAA dimerisation is also significantly lower, which was predicted to result in increased formation of the stoichiometric cluster. In the 1-4 polymers, the dominant species in CHCl₃ is $(FM)T$, in TOL it is $(FM)_2T$ but a significant amount of $(FM)_3T$ is formed, and in MeCN it is $(FM)_2T$ with a significant amount of $(FM)T$ with little $(FM)_3T$ formation predicted by Ansell et al. Ansell et al. identified that the stoichiometric 1:1 polymer was the least capable polymer for strong association with reintroduced EPH. For their purpose of chiral preparative separations, there is a necessity for desorption of the
ligand from each active site in an energetically favourable fashion. Thus, their goal was to create a MIP for the specific purpose of separating enantiomers or analogues in a post-reaction solution. This influenced not only their desire to have the system operating in a volatile organic solvent (for ease of recovery) but also a weaker interaction between ligand and receptor site to prevent peak tailing in the HPLC analysis of the separation. However, other goals such as depletion of the mobile phase through the immobilisation of the template in strong binding sites where the chance of the template bleeding out over time is minimal, the weaker, and less geometrically organised 1:1 imprint sites are not likely to be the most useful of the imprint cavities. It is likely that the approach of using different surface (or ligand) modifiers to modulate the recognition environment will create significant opportunities to “tune” the recognition environment to the desired mode of application.

The use of both amine (basic) and acetic acid modified mobile phases by Ansell et al. showed suppression of differing imprint site stoichiometries. The amine modifier reduced binding to all binding sites proportionally, but the acid modifier reduced binding to the medium and weak sites more than it affects the strong binding sites. Hence, for retention (and separation) in DCM–AcOH mobile phase in HPLC applications, it is the sites arising from (FM)$_2$T and (FM)$_3$T complexes that are important. Following this conclusion reached by Ansell et al., the use of a mobile phase component to modify the accessibility of the imprinted sites, or to modulate the ligand to promote or detrimentally affect the interaction through modulation of the structure of the ligand was considered an important tool for utilisation of the most selective imprint sites created in the imprinting process.

4.1.2 EPH - MAA NMR investigations conducted in this scheme.

The similarities between the investigations carried out by Ansell et al. and the results of the Job plot shown in Figure 4.3 show that there are only minor differences between the different experiments. There is clearly a peak at mole fraction of 0.5, indicating the (FM)T complex being a major component of the association. EH$_a$ and EH$_b$ are observed to show a shoulder in this peak at mol fraction = 0.2-0.4, suggesting
that the formation of the (FM)$_3$T and (FM)$_2$T complexes are prevalent in solutions containing an excess of FM.

Figure 4.3: $^1$H EPH / MAA NMR continuous variation plot showing mole fraction of EPH v $\Delta\sigma$(ppm) x mole fraction

There is a significantly different shape observed in the Job plot to that observed by Ansell et al. 0.4-0.6 mole fraction for EC$_a$, EC$_b$, EC$_c$, EC$_f$, exhibits a flattened peak, on either side of which is observed a repeated drop in value. There appears to be little change in the plot of EC$_c$ and EC$_a$ between 0.2 and 0.3, while EC$_b$ and EC$_f$ are observed to increase slightly through this mole fraction ratios. As the difference between these two systems is the absence of water in the experiments conducted by Ansell et al., the strict speciation at 1:1 stoichiometry is likely to be hindered by the presence of water in
the solutions, resulting in the formation of clusters of higher FM equivalence, and differing stoichiometries.

4.2 Methacrylamide (MAM) NMR experiments performed in this scheme.

Fundamentally, the behaviour of the MAM monomer and EPH template suggest a similar interaction would be possible, as the monomer possesses both a HBA and HBD ability. Thus, it is possible that a chelating interaction could exist between the secondary amine and hydroxyl groups of the template with the acrylamide functional group. However, such an interaction was not predicted during the modelling experiments, and was not observed in the Job plot or the titrations carried out using MAM and EPH.

Figure 4.4: $^1$H EPH / MAM continuous variation plot showing mole fraction of EPH v ($\Delta\sigma$(ppm) x mole fraction)
Figure 4.5: $^{13}$C NMR EPH - MAM continuous variation plot. mole fraction of EPH vs $(\Delta\sigma \text{ (ppm)} \times \text{mole fraction})$

The continuous variation plots for the EPH - MAM (Figure 4.4 and 4.5) system shows that a variety of cluster stoichiometries exist in solution due to the difference in observed shape exhibited for the different protons plotted. The observed chemical shift is observed to be of an order of magnitude lower than that observed in the EPH - MAA system above, and those observed by Dong\textsuperscript{[212]} and Ansell.\textsuperscript{[171-173]} The observed shape of the Job’s plot is also significantly different to the case observed for the MAA system, suggesting weaker interaction between the monomer units and the template.

The $^1$H Job plot shows that there is no major peak around a mole fraction of 0.5, suggesting that a single FM monomer is not the only interaction between T and FM. As the observed Job plots do not exhibit smooth functions that reach a single peak, it is likely that the interaction between FM and T is likely to be a combination of multiple competing equilibria. A large peak is observed at mole fraction 0.1 and is maintained at 0.2 for EHc, the aliphatic methyl substitution, while at 0.2, there is a smaller peak exhibited for EHs. The $^{13}$C plot of the continuous variation shows a different, more
defined outcome. The majority of the resonances reach a peak at a mole fraction of 0.5 suggesting the presence of a weak 1:1 complex. The two methyl group carbons, EC<sub>g</sub> and EC<sub>h</sub> displaying a skewed maximum towards higher ephedrine mole fractions, suggest stoichiometries lower than 1:1.

It is apparent that the MAA participates in stronger, presynthesis interactions with EPH than MAA due to the significantly greater chemical shift observed in the case of the MAA Job plots conducted both here, and by Ansell et al. The behaviour of these monomers is expected to be different due to the different functional groups present, and the ability for the MAA to act as a HBD and HBA. Both demonstrate an association with EPH in the pre polymerisation solution, at stoichiometries other than 1:1.

4.2.1 - NMR Titrations

The sequential addition of molar equivalencies of functional monomer (relative to template) provides additional insight into the pre-polymerisation solution association when viewed in tandem with the Job plot data. Ansell et al. have performed exhaustive experiments and determined that the association between MAA and EPH exists mostly as a 1:1 and 1:2 complexes. As the stoichiometry is raised, the incorporation of the additional monomer units to the (FM)<sub>n</sub>T should continue to produce additional shifts in the EPH atom resonances if the additional monomer units are associated with the template cluster. If a plateau is observed in the chemical shift as additional molar equivalent aliquots are added, it is indicative of no further addition to the (FM)<sub>n</sub>T clusters.

The significantly lower chemical shift observed in the EPH- MAM Job plots suggested that the NMR titrations would not produce similar outcomes to the MAA experiments as conducted earlier, or by Ansell et al. The observations of the stronger MAA association in CHCl<sub>3</sub> are evident in the shape of the titration plots, and the significantly larger chemical shifts observed through these associations. Although the titrations carried out by Ansell et al. show a significant shift upon the addition of one molar equivalent aliquot of MAA, a plateau in the observed shift is not observed at this point, but establishes at a 1:2 stoichiometry. In some cases, it was observed that continued addition of monomer resulted in the reversal of the chemical shift, suggesting
that there were fewer functional monomer units associated with the template at higher stoichiometric ratios. The modelling conducted in Chapter 2 supports this result, with the 1:2 and 1:3 stoichiometries producing larger estimated interactions with EPH than the models at higher stoichiometries or the 1-1 model.

Figure 4.6: Plot of EPH vs. MAM $^1$H titration. Stoichiometric ratio of FM: T plotted on x axis, and the $\Delta \sigma$ (ppm) observed plotted on the vertical axis.

MAM was not expected to generate a similar pattern to MAA in this regard. As the stoichiometry that is predicted to exhibit the largest interaction with EPH occurs at 1:5, little shift was expected below this value. The maximum shifts were also expected to be significantly lower than the MAA case, as this was observed in the Job plot experiments.
The $^1$H spectra of the titrations (*Figure 4.6*) show that the largest shifts are observed for the protons attached to the carbon atoms to which the vicinal hetero atoms are bonded, EH$_a$ and EH$_b$. Smaller shifts are observed for the methyl protons EH$_c$ and EH$_d$, which is in agreement with the structures of the modelling, predicting greater interactions with the EC$_e$ and EC$_f$ regions of the molecule. The $\Delta \sigma$ (ppm) observed at 1.5 equivalents of MAM added is related to the 3:2 FM:T ratio observed in the Job plot (*Figure 4.4 and 4.5*). Little change is observed between this addition, and 5 equivalents of MAM added, except an increase in the shift for EH$_a$ between 2 and 3 equivalents added. Upon the addition of the $5^{th}$ molar equivalent of MAM, the largest $\Delta \sigma$ (ppm) is observed. This shift is greatest for EH$_a$, and decreases with distance away from the hydroxyl group. Little observable shift can be seen in the $^{13}$C plot, there appears to be an interaction observed at FM:T 3:2, and a shift observed at 5:1.

![Figure 4.7: Plot of EPH - MAM $^{13}$C titration. Stoichiometric ratio of FM: T plotted on x axis, and the $\Delta \sigma$(ppm) observed plotted on the vertical axis.](image)

Some observable association at the predicted 1:5 T:FM stoichiometry is observed despite the much lower shifts in comparison to the EPH-MAA system. It is
more observable in the proton plot than the $^{13}$C. In combination with the titrations performed by Ansell et al., and the construction of the *in silico* models detailed in earlier chapters, EPH - MAA 1:2 and EPH - MAM 1:5, along with, and EPH - MAA 1:4 and EPH - MAM 1:4 polymers (to represent the commonly utilised stoichiometry in the synthesis of MIPs).

The different interactions between EPH and MAA and MAM are most pronounced here. Although the interaction between MAA and EPH appears to be stronger than that between MAM, with the saturation of the template occurring at a FM:T ratio of 2:1 in the case of MAA, and no observable; saturation occurring in the MAM case, however a comparatively large shift was observed at (FM)$_5$T.

### 4.3 Re-evaluation of the Molecular Modelling results.

The construction of the molecular models to assist in the prediction of interaction between the template and potential functional monomers identified several monomers and cluster stoichiometries as interacting favourably with EPH. Attempts to validate the existence of these stoichiometries in solution were undertaken through a combination of literature review and NMR titrations, resulting in the construction and analysis of Job plots and NMR titrations, combining the observations of Ansell et al.\cite{171-173} and Dong et al.\cite{212} to explain the *in silico* predictions.

It was observed that the absolute magnitude of these solution based interactions was not predicted through the *in silico* experiments. The *in silico* screening failed to achieve correlated results with the practical experimentation carried out using the NMR techniques. In addition, the *in silico* investigations did not identify the proposed chelating interaction identified by Ansell et al.\cite{171-173}. Despite this, the modelling has indicated that there are observable interactions at the stoichiometries suggested as from the NMR investigations. It is possible that this may be associated with the gas phase nature of the initial screening process, which may not be the best descriptor for the solution phase association of the pre polymerisation clusters.

To determine if the *in silico* modelling process could be manipulated to provide interaction magnitudes closer to obtained from NMR investigations, the FM identified in the AM1 screening were resubmitted at higher levels theory, both a second semi-
empirical description, PM3, and also under \textit{ab initio} conditions using Hartree-Fock calculations at 6-31G theory.

**Table 4.1:** Re-submission of AM1 SEMO molecular modelling, verification of estimated interaction magnitude results after NMR investigations using PM3 and HF6-31G calculations.

<table>
<thead>
<tr>
<th></th>
<th>AM1 (kJ/mol)</th>
<th>PM3 (kJ/mol)</th>
<th>HF 6-31G (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA 1:1</td>
<td>-16.22</td>
<td>-10.31</td>
<td>-15.7409</td>
</tr>
<tr>
<td>MAA 1:2</td>
<td>-21.02</td>
<td>-7.58</td>
<td>-13.3681</td>
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<td>-7.79</td>
<td>-15.5885</td>
</tr>
<tr>
<td>MAM 1:5</td>
<td>-57.87</td>
<td>-178.95</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.8:** Original AM1 geometries from the EPH templating functional monomer investigations.

PM3, (used by Dong \textit{et al.},\textsuperscript{[212]}) shows that the predictions of interaction are lower for all clusters except for the MAM 1:5 imprint site (\textit{Table 4.1}). The EPH- MAA
1-2 cluster is observed to exhibit a lower level of interaction with the template than both the 1-1 and 1-3 sites. The only one of these models in which a hydrogen bond is predicted between the monomer cluster and template is the 1:5 EPH- MAM (Figure 4.9). The EPH- MAA 1:3 interaction from the PM3 simulations is predicted to be very similar to that in the AM1 simulations (Figure 3.13), despite the variation in the reported value for the interaction. The similarity in the SE-MO used for the calculation of these interactions is evident. Although the magnitude of the calculated interaction shows clear difference between the estimated interactions, there appears to be little observable difference in the orientation of the association clusters (Figures 4.8 and 4.9).

![Figure 4.9: PM3 model geometries A) EPH-MAA 1:1, B) EPH-MAA 1:2, C) EPH-MAA 1:3. D) EPH MAM 1:5. Hydrogen bonds are shown as an orange line.](image)

This feature is also noted for the ab initio HF 6-31G calculations however the calculated interaction magnitudes are stronger than the PM3 calculations. Despite the
higher level of theory, calculation in the gas phase of what will be in practice a solution phase phenomena can only be of indicative application.

Direct comparison or correlation of these estimated interaction magnitudes between force fields or to experimentally determined enthalpies of formation is not appropriate, although they may allow prediction of an interaction being more or less favourable than that of a molecular structure being used as a reference. In this case the comparison of interactions between the pseudo-receptor site and template molecule and the strain that this association generates in the molecular structures of the species involved. The suggestion of these results at higher level is that the differences in association as the cluster stoichiometry increases are quite small, especially in the gas phase. This finding tends to agree with the work of Ansell. It remains unclear if the explicit incorporation of solvent molecules in the pre synthesis screening would enhance the ability to direct the choice of functional monomer, porogen and other components of the MIP synthesis regime.

The cooperative interaction resulting in the formation of a hydrogen bond between the carboxyl group of a MAM monomer and the hydroxyl group of the template is the only obvious explanation for this significantly larger interaction between monomer cluster and template in the EPH-MAM 1:5 cluster. Numerous hydrogen bonds exist within the monomer cluster in addition to that between the template and monomer, which suggests that the monomers are observed to be playing some sort of stabilising role in the global energy minimum conformation in Figure 4.9.

Figure 4.10 shows the molecular geometries from the output of the HF 6-31G* models constructed. There is a difference observed in the EPH - MAA 1:2 cluster geometry in Figure 4.10B and the AM1 and PM3 imprint site models from Figure 4.8 and Figure 4.9. There is significantly less separation between the functional monomer cluster units themselves in the ab initio model than in the SE-MO models. The strongest interaction between the template and the cluster appears to be an OH-N hydrogen bond, which is predicted in both models. As the cut-off levels for the H-bonds are set within the software package, not the force field, the higher prediction of hydrogen bonds in the higher level theory clearly shows a reduced separation between the modelled species. The interaction between the second functional monomer is no longer in the plane of the aromatic ring, but perpendicular to this functional group.
Figure 4.10: Molecular geometries from HF 6-31G calculations in SPARTAN software package. Hydrogen bonds are shown as an orange line. A) EPH-MAA 1:1, B) EPH-MAA 1:2, C) EPH-MAA 1:3.

It is possible that the position of the functional monomer is stabilised both by interaction of the oxygen lone pairs and the delocalised electrons of the π system, and an electrostatic interaction between the NH group of the template and the C=O of the second functional monomer. This is supported by the findings of Suzuki et al., in their investigation of the interaction between benzene and water molecules, in which they found that benzene forms hydrogen bonds with water molecules in MP2 6-31G** \textit{ab initio} calculations.\cite{213}

This apparent divergence from structures predicted by the SE-MO models constructed suggests that some of the abbreviations made to the atomic and subatomic properties of the molecules make a significant contribution to the final structure predicted in these models. As the \textit{ab initio} methods deal with individual electrons, it is
perhaps unsurprising that the location of the functional monomer predicted to interact with the phenyl ring changes, while the interaction with the tail of the template molecule does not. This may be a result of full definition of the electrons within the aromatic groups. Strangely, the chelating interaction postulated by Ansell et al. is again not predicted by either of the SE-MO or ab initio calculation methods, despite the nature of these models exhibiting the gauche conformation suggested as being necessary for the chelating interaction to occur.\textsuperscript{[171-173]} It is possible that the second MAA unit from the SE-MO studies is optimised able to interact with the template’s phenyl ring in both positions.

The EPH:MAA 1:3 cluster shows significantly less variation in the relative position of the functional monomer units. In both the PM3, and HF 6-31G* models, two functional monomers appear to interact directly with the template, while a third appears to act in a stabilising role, interacting with both of these functional monomer molecules. As was suggested in Chapter 2, the absence of a predicted hydrogen bond in the modelled clusters at lower levels of theory is largely dependent on the cut-off values, and although such strong intermolecular non-bonding interactions may be overlooked by the software package, such features may exist in real systems and be functions of the abbreviations of molecular properties made for fast, efficient calculation. The implication of this is that the less monomer units involved in the interaction between imprint site and the template, the less conserved the position of the monomer units around the template.

Of note is the successful use of the abbreviated atomic descriptions in the SE-MO and MM techniques\textsuperscript{[167, 174, 175, 180,186, 208, 212, 214, 216-219, 221]} to predict the most appropriate functional monomer and the stoichiometry of the imprint cluster. An improvement beyond using a single force field approach for the prediction of imprint site properties is to follow an iterative process of model refinement, where the initial screening process can be done at lower levels of theory, with further geometry optimisation being performed at higher levels of theory on the systems exhibiting promise.

The need for such further optimisation may be unnecessary given the documented performance of the SE-MO methods by Dong et al. in predicting the selectivity of the modelled imprint site.\textsuperscript{[208]} It is likely that such a refinement may produce more reliable interaction when the ab initio site is returned to a lower theory
level for screening of selectivity. Although the associations in gas phase may be used as an indicative tool, their eventual association in solution phase will also depend on other features of the pre-polymerisation solution.

The completion of ab initio calculations at HF 6-31G for the EPH: MAM 1:5 cluster was unable to be completed due to computational limitations. After 4 months of calculation, no result was returned.

4.4 Polymer Synthesis approach.

Polymers were synthesised to further investigate the application of the molecular modelling scheme undertaken in previous chapters. Polymer compositions were selected from the functional monomer and stoichiometry screening previously conducted in the previous chapters, in tandem with their non-imprinted counterparts. These selections were MAA at a ratio of 1:2, and MAM at a ratio of 1:5. Polymers were also using an arbitrary selection of stoichiometry 1:4, employed by the groups of Chianella et al., Cormack et al., and Liu et al. Investigations comparing the two monomers should provide insight into the utility of screening the stoichiometry of the template/functional monomer ratio prior to the synthesis of the polymers. It should also allow a determination of the favourable application of functional monomer self association, in creating a network of cooperative interactions with the template, rather than relying on one or two stronger but still non-covalent interactions.

Both the imprinted polymers and their matching non-imprinted partners described in Table 4.2 (all components included except the presence of the template molecules, excluded from table to prevent overload of data) were synthesised using thermally induced (60 °C), radical polymerisation through the decomposition of AIBN (azo-iso-butyronitrile) for 24 hours. After the polymerisation was complete, the polymers were progressively ground, by hand using a mortar and pestle. The polymers were wet sieved in methanol, to suppress the generation of polymer dust, followed by the collection of the fraction of particles between 38-45µm. This particle range was selected as preliminary experiments were hampered by the physical handling issues caused by finer particles.
Although smaller particles present the fewer kinetic barriers to the analyte finding its way to the imprinted cavities, the particles caused severe filtration and sampling issues for the experimental procedures used. As a result, a slightly larger particle size was selected to reduce the problems associated with handling such fine, particulate samples. The polymers were sieved in an iterative process, until the available polymer mass was between 38 - 45 µm. The particulate solution was then filtered using a sintered funnel and placed in a cellulose extraction thimble, before being added to a soxhlet extraction apparatus containing an approximately 10% v/v acetic acid in methanol solution.

Table 4.2: Polymer synthesis recipes for the MIP involved in this project. NIP partners follow the same recipe; however they do not include template molecules in the synthesis solution. 15mL of porogen was used in each case.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Mass/µmol/ratio</th>
<th>FM</th>
<th>Mass/µmol/ratio</th>
<th>XL</th>
<th>Mass/µmol/ratio</th>
<th>Porogen</th>
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<td>0.95832/484/20</td>
<td>TOL</td>
</tr>
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<td>0.95832/484/20</td>
<td>MeCN</td>
</tr>
</tbody>
</table>

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The extraction was allowed to continue for 48 hours before the solution was changed and replaced with methanol before another 48 hour extraction was carried out to remove any acetate anions from the polymer network. The polymer particles were then dried in an oven at 60 °C for 24 hours, before being transferred into glass sample vials, and further dried under high vacuum. Before the characterisation of the binding abilities of the synthesised imprinted polymers was carried out, an investigation of their behaviour in the porogens used and also in aqueous solution was conducted following Spivak, Kempe, Yu and Mosbach, Qing-Zhi, Umpleby II et al., as well as Sharma and Borovik (225-230) who previously concluded that MIPs exhibit optimal rebinding ability in the polymerisation solvent. This phenomenon is postulated to arise as a result of the maintenance of the imprinted cavity volumes that optimises the positioning and distance of functional monomer groups relative to the template. By investigating the swelling behaviours, an indication of binding performance in solvents of varied polarity may be gained.

The impact of porogen character on a polymer designed for use in aqueous environments was highlighted by Piletska et al., [186]. The paper concluded that acidic monomers are more effective than basic monomers in forming ionic interactions with a range of templates (cocaine, methadone and deoxyephedrine). In particular, it was noted that:

- To maximize the role of ionic interactions in MIPs, polymers should be prepared using strongly acidic functional monomers such as acrylamido-2-methyl-1-propanesulfonic acid (AMPSA) or 2-(trifluoromethyl) acrylic acid (TFMAA).
- The concentration of the crosslinker is incidental in determining the monomer’s ability for ionisation.
- To maximise the incorporation of the ionised monomer/template complexes in the polymer network, the porogen should have the maximum available hydrophobicity.

An imprinting factor (I= [B_{MIP}] / [B_{NIP}]) of 2.6 for cocaine and 1.4 for methadone and deoxyephedrine was determined from this study. In contrast, the work of Ansell et al. determined that TFMAA performed worse for the imprinting of EPH due to the
observed precipitation of the EPH:TFMAA.\textsuperscript{[171-173]} Covalent bonding was also observed between both the TFMAA and EPH molecules via NMR spectrometry.

The use of functional monomers that are stronger acids was not suggested to be a useful feature from the previously conducted modelling. The stronger acids exhibited a higher prevalence for self association than for association with the template. This may be a case where, similar to natural receptors, the combination of multiple, weaker interactions are able to still provide high levels of interaction despite not possessing strong single point interactions.

Ansell \textit{et al.}\textsuperscript{[171-173]} suggests that the best performing polymers may not be those whose imprint sites are very strong, and their conclusion was that the polymers that performed best were those which exhibited the largest percentage of weak, homogeneous interaction sites. It was determined here that the most significant interaction with the template was observed with a stoichiometric ratio of T:FM. Although it is not explicitly suggested in their series of papers, if the number of reciprocal interactions between template and functional monomer are reduced, it follows that the contribution of non-specific interactions such as hydrophobic and van der Waals interactions to the total interaction between imprint site and ligand must be greater.

Imprinting of a polymer specific for EPH was also carried out by Piletsky \textit{et al.} using hydroxy ethyl methacrylate\textsuperscript{[231]} after an \textit{in silico} screening process.\textsuperscript{[175]} This work observed a greater enantioseparation of the racemate of the template in a polymer constructed using 10 fold excess of functional monomer to template. Although the predicted interaction between HEMA and EPH was smaller than the acidic monomers in previous studies carried out by Piletsky’s group, it was used as an indication that monomers other than those containing acidic functionality can be used successfully to create functional MIPs.

4.5 \textbf{Swelling of polymers in a variety of solvents. The effect of porogen on the structure of the imprinted polymer.}

The ability for each polymer to swell was studied by placing 50mg of each polymer (dried under high vacuum) in a tared SPE cartridge. The polymer inside was
sequestered between two polyethylene frits and the appropriate solvent was added to each polymer sample. 24 hours later, each SPE cartridge had the supernatant removed via the use of a vacuum manifold (preppy SPE) and the samples were again weighed. It is incorrect to compare the mass based swelling of a polymer without taking into account the differing specific gravity values for the solvents used. Therefore, to allow a comparison between solvents of differing densities and the volumetric expansion of the polymer in different solvents, the specific gravity of the solvent must be accounted for. SI data values were utilised for the specific gravity of each solvent. The amount of swelling was assessed using the following equation;

\[ \text{Polymer swelling} \% = \frac{\Delta M}{S_{(sg)}} \times 0.05 \times 100 \]  
\( \text{Equation 4.1} \)

Where \( \Delta M = (M_{\text{swollen}} - M_{\text{unswollen}}) \)

If the volume of the polymer before and after swelling behaviour is recorded, not only will the interstitial space be included, but also any void spaces not visible to the eye. The effect of the porogen on a given polymer system, the effect of varying the cross linking monomer, the effect of differing functional monomers and the effect of variation in the stoichiometric ratio between template and functional monomer are all available avenues for comparison of the synthesised polymers.

The nature and properties of the porogen used in any investigation of MIP interaction play a large role in the physical behaviour of the synthesised polymer, but the introduction of dried polymer particles to solvents possessing different physical and chemical properties can significantly influence the strength and nature of interactions between the template and imprinted polymer cavities.
While it remains somewhat unclear precisely how polymer swelling contributes to analyte binding selectivity, the widely held belief maintains that replication of porogen characteristics (the solvent in which the polymer was synthesised) generates the greatest imprinted polymer performance \[^{225-230}\]. Such solvents replicate interactions between adsorbent phase and the analyte present during the imprinting process.

As the cross linker is the major component of the polymer (approximately 80%), the behaviour of these polymers without the addition of functional monomers can help to elucidate the role of the functional monomer, and the crosslinking monomer on the behaviour of the imprinted polymer in a variety of porogens.

It remains unclear what features of the polymer swelling are required to maintain “good” binding ability. Given the established belief that the “best” solvent for rebinding is the one in which the polymer was synthesised, it follows that the highest level of interaction will most likely be found in solvents that may be able to modulate the interaction between adsorbent phase and the analyte while swelling the polymer to a similar level as the porogen.

The experiments in this section were designed to allow insight into whether the presence of template or functional monomer affects the swelling relative not only to the cross link only polymers but to the type of functional monomer and the stoichiometry used. The physical structure differences in the polymers after synthesis in different porogens should also be highlighted by this study.

A major role that the solvent plays in the swelling of the polymer is to maintain the three-dimensional structure of the imprinted cavities so that the locations of the reciprocal point interactions are located in a manner similar to the porogen, it is likely that even in polymers that are not in the presence of the porogen during the adsorption process that similar levels of selectivity and analyte adsorption could take place so long as the swelling of the polymers in the solvent used for the binding study allows the polymer to replicate the spatial arrangement of the reciprocal interaction sites.

Thus, it is plausible that should these functional groups remain in similar locations to those experienced in the native porogen, that the remaining non-specific interactions could be modulated through manipulation of the solvent properties. Of specific importance is that as an aqueous environment for detection is desired (to mimic the environment of the native GPCR receptors as much as possible), the investigation
should enable a determination of the most appropriate polymer synthesis porogen and composition for this task.

### 4.5.1 Swelling of Cross-linker Reference Polymers

To understand the effect of different porogens and solvents on the structure of the synthesised polymers it is necessary to investigate how the principal component (the cross linking monomer *ca.* 80 mole % of the polymer matrices) behaves in a variety of different solvents after being polymerised in a range of solvents. These experiments should also highlight the effect on the physical structures of the co-polymers synthesised containing both functional and cross linking monomers. It is likely that the crosslinking monomer occupies more than 80% of the eventual polymer network due to the size difference between the functional monomers and the crosslinking monomers, and the number of moles present in the synthesis solutions.

![Swelling of Cross-linker Reference Polymers](image)

*Figure 4.12: Reference polymer % swelling results. Individual bars represent the % swelling in the introduced solvent whilst the x axis identifies the synthesis porogen. Error bars represent maximum observed variance from the sample mean swelling value. (Number of repetitions=3)*

High swelling of the EDGMA monoliths prepared in acetonitrile (≥500%) was observed for all solvents tested (*Figure 4.12*). By comparison, the expansion of this polymer when prepared in chloroform and toluene was much more limited. The behaviour suggests the presence of a more open polymer network possessing greater voids and channels, although the exact behaviour of the solvent in generating this result is unclear.
EDGMA cross linked polymers were observed to possess greater flexibility than their DVB counterparts when prepared in the same solvent, as observed by their greater propensity to swell in different solvents. MeCN as a porogen for EDGMA polymers appears to promote significant swelling in the presence of all solvents.

Swelling was more uniform across the range of porogens for DVB, suggesting that the presence of the \( sp^2 \) hybridised aromatic ring, and the short alkane chains joining these aromatic groups in the final polymer’s cross linked structure significantly reduces flexibility in the resultant polymer in comparison to EGDMA. Swelling in water is not observed for the DVB polymer prepared in acetonitrile, again suggesting that both greater rigidity and hydrophobicity are influencing factors in this case.

There was little variation in the amount of solvent absorbed by the DVB reference polymer regardless of the porogen used in its synthesis (Figure 4.12) except for the DVB polymer synthesised in MeCN and swollen in its porogen. CHCl\(_3\) and TOL exhibited higher abilities to swell the crosslinking polymers in all cases except for the DVB reference polymer that was synthesised in MeCN, where in an MeCN environment the polymer absorbed a similar level of MeCN as TOL. In all reference cases, DVB showed significantly lower swelling in aqueous environments, due to the hydrophobic nature of this monomer creating a significantly non-polar environment within the polymer matrix.

### 4.5.2 Swelling of EPH imprinted MAA and MAM, EGDMA and DVB co-polymers prepared in toluene.

Incorporation of MAA with the two cross-linking monomers into the polymer formulation (both MIP and NIP) resulted in increased polymer swelling in all solvents (Figure 4.13 A and B), indicating that the functional monomer has a significant effect on polymer architecture. The effect was more pronounced in the case of the DVB polymers suggesting that incorporated carboxyl units improve flexibility.

The co-polymerisation of MAA and the different cross linking monomers in TOL shows a large change in the ability of the copolymers to swell upon the introduction of all organic solvents when TOL was utilised as the porogen (Figure 4.13A and B). There was an observed increase in swelling between the reference
polymers and the imprinted and non-imprinted MAA copolymers. When synthesised in a non-polar organic solvent, the DVB polymers are capable of higher levels of swelling than the EGDMA polymers synthesised in the same porogen. However, the increasing polarity of the solvent is seen to be associated with a lower amount of observed swelling, including a very weak swelling response in aqueous environments (Figure 4.13).

The MAA-co-EGDMA (TOL) polymers were observed to demonstrate higher levels of swelling than the reference polymers in all cases. The greater ability of these polymers (than the MAA-co-DVB polymers) to swell in aqueous environments was predicted. The incorporation of the functional monomers into the imprinted matrix, and the non-imprinted matrix enabled a much larger volume of liquid to be absorbed in all cases; however the effect is less significant in the 1:4 case.

The non-imprinted polymers appear to swell more than the imprinted partner. Although the 1:2 MAA – co – EGDMA polymer swells more than its non imprinted pair, the remaining polymers in Figure 4.13 demonstrate that the imprinted polymer is less capable of solvent absorption. A comparison of the DVB crosslinked polymers display this feature, including binding from aqueous solution. Although the swelling is minimal in aqueous environment, the NIPs swell more than the MIPs exhibiting an identical similar concentration of reagents. This is potentially because of the random location of the imprint clusters within the synthesised polymer network results in interruption of networks of high swelling through the incorporation of voids in the structure associated with the imprint cavities.

It was expected that if the incorporation of the functional monomer units into the co-polymer allowed greater swelling to take place, the effect would be greater in circumstances where the % composition was higher. This was not observed to be the outcome where EGDMA was the XL, but there was no decrease in the observed level of swelling when the XL was DVB and the concentration of MAA was increased.

The variation of the functional monomer from the MAA to MAM shows a significant effect on the ability of the polymer network to swell upon the introduction of solvent after polymerisation is complete. While the EGDMA polymers exhibit similar levels of swelling in all solvents when synthesised in TOL (Figure 4.13), differing from the cross link reference polymers to a minor amount the DVB XL polymers show a
significantly higher magnitude of swelling exhibited in relation to the EGDMA polymers, but also in relation to the reference polymers.

Despite the ability of these polymers to swell in organic solvents, their ability to swell when suspended in an aqueous solution is still severely impaired by the hydrophobic nature of the DVB scaffold of the polymer.

When the crosslinker is DVB, it appears that an increased presence of MAM is associated with an increase in the level of swelling possible in organic solvent. Although the 1:4 polymer when swollen in H₂O demonstrates a 2 fold increase over the reference polymer and the remaining polymers synthesised in TOL, the DVB polymers again seem to be unsuitable for performance in H₂O. However, the polymers constructed with EGDMA exhibit swelling behaviour very similar to that of the organic solvents after synthesis in TOL.

The non imprinted polymer with ratio 0:5 is the exception to this pattern, and shows a significantly higher level of swelling that the remaining polymers in this set that all exhibit similar behaviour to the reference polymer when treated with an aqueous solution. The incorporation of further stoichiometric units to the polymer resulted in an increase in the magnitude of swelling for TOL (Figure 4.13). No such increase was observed for the remaining polymers.
Figure 4.13: EPH polymers (TOL porogen) % swelling results. Individual bars represent the % swelling in the introduced solvent whilst the x axis identifies the synthesis porogen. Error bars represent maximum observed deviance from the sample mean swelling value. (Number of repetitions=3).
4.5.3 - **Swelling of EPH imprinted MAA and MAM, EGDMA and DVB co-polymers prepared in CHCl$_3$.**

When synthesised in CHCl$_3$, the MAA – co-polymers exhibit some variation in the level of the swelling observed. It was observed (*Figure 4.14*) that all MAA – co – EGDMA and MAA– co –DVB polymers displayed higher levels of swelling than the reference polymers, and that the highest amounts of swelling was observed upon the introduction of the non-polar solvents (TOL and CHCl$_3$).

The addition of more functional monomer units into the polymer is seen to have an effect of lowering the amount of solvent able to be absorbed. The DVB polymers exhibit no such variation, although the amount of swelling observed in the 1:4 MAA polymers, (co-polymerised with both EGDMA and DVB) when swollen with MeCN is much lower than the extent observed in the other solvents.

Unlike the MAA co-polymers synthesised in TOL, there appears to be no relationship between the identity of a polymer as imprinted or non-imprinted and the relative level of swelling when the same polymers compositions were synthesised in CHCl$_3$. Synthesis of the MAM-co-EGDMA polymers in CHCl$_3$ (*Figure 4.14*) showed an overall increase in the level of swelling in comparison to those synthesised in TOL, including the reference polymers. The reference polymers and the EGDMA based polymers showed similar magnitudes in the amount of swelling observed. Although both non-imprinted polymers display greater capacity for swelling than do the imprinted polymers possessing the same ratio of composition in most cases (MeCN being the exception), the porogen swells these polymers significantly more than the H$_2$O.

The MAM-co-DVB copolymers synthesised in CHCl$_3$ showed a decrease in the level of swelling in comparison to the polymers synthesised in TOL, however the behaviour when treated with an aqueous solution is not improved. The 1:5 MAM-co-DVB polymer shows greater swelling in H$_2$O than the other polymers synthesised containing MAM.
Figure 4.14: EPH imprinted polymers (CHCl₃ porogen) % swelling results. Individual bars represent the % swelling in the introduced solvent whilst the x axis identifies the synthesis porogen. Error bars represent maximum observed deviance from the sample mean swelling value. (Number of repetitions=3).
4.5.4 - **Swelling of EPH imprinted MAA and MAM, EGDMA and DVB co-polymers prepared in MeCN.**

In a polar, aprotic porogen, (MeCN) the observed behaviour of all EGDMA polymers is reversed. The cross linked reference polymer exhibits significantly higher swelling in all solvents than any containing MAA. The solvent that swells the polymers the most, despite their synthesis in MeCN is CHCl₃. This feature is observed in each case (*Figure 4.15*).

The MAA-co-EGDMA (MeCN) 1:4 and 0:4 polymers display similar levels of swelling in MeCN and H₂O to those polymerised in the apolar solvents, however there is a reduction in the level of swelling observed in CHCl₃ and TOL when compared to the same polymers.

The most significant difference (between cross linking polymer and functional co-polymer) was observed to occur with the DVB polymers and the reintroduced organic solvents. In the case of the MAA – co – XL systems, the incorporation of polar surface groups into the polymer network produced a significant increase in the level of swelling observed when the polymers were synthesised in non-polar porogen.

The polymers of the same composition when synthesised in MeCN were observed to be capable of significantly reduced swelling in comparison to the reference XL polymers, in the case of the EGDMA polymers. The DVB polymers showed an increasing ability for solvent uptake with increasing levels of functional monomer incorporation (*Figure 4.15*).

Synthesis of the MAA – co - DVB polymers in MeCN enables these polymers to demonstrate significant levels of swelling in an aqueous environment (*Figure 4.15*). The amount of functional monomer does not appear to play any role in the governance of the level of swelling between polar and apolar solvents in this case. When synthesised in MeCN, the EGDMA reference polymers show significant solvent uptake.

The 1:5 MAM-co-EGDMA shows the overall greatest level of solvent uptake other than the reference polymer for all solvents. This polymer also shows a significantly higher level of swelling in H₂O than the other polymers polymerised in MeCN. Strangely, the synthesis of these MIPs in MeCN does not appear to be
associated with an ability to absorb this solvent more readily than the other examined solvents.

The DVB cross linked polymers showed a surprising level of swelling in H₂O given the ability to swell demonstrated by the polymers synthesised in the nonpolar porogens. It is clear that the porogen plays a significant role in the organisation of the functional monomer sites within the polymer cross linking scaffold, and therefore the flexibility of the imprinted sites.

The MAA polymers appear to show a greater propensity for swelling in all solvents than do the MAM polymers when the porogen used is non-polar. In MeCN, the level of swelling observed between the two differing functional monomer systems is quite similar at 1:4. However, both of the designed systems (1:2 MAA and 1:5 MAM) appear to demonstrate higher levels of swelling than the 1:4 polymers. Higher swelling is also observed for the MIP when compared to the NIP in these cases. It is likely that the void spaces left by the removal of the template plays some role in allowing more area for the solvent to fill.

A comparison of the effect of EGDMA and DVB as a cross linker shows a great affect of the porogen on the ability of the polymer to swell, indicating a significant structural organisation effect of the porogen. When the polymers are synthesised in non-polar media, the DVB polymers show a significantly larger amount of swelling in organic solvents than the EGDMA polymers, though it is not repeated with water. The swelling is greater in the MAA polymers in comparison to the MAM polymers, when swollen in organic solvents. When a polar porogen is used, the level of swelling observed in the EGDMA polymers is significantly lower than the cross link reference polymer. The DVB polymers exhibit higher swelling than their reference cross linked polymer.

The addition of more MAA functional monomer units to the EGDMA polymers generally results in slightly higher levels of swelling for the organic non-polar solvents, while the swelling is generally seen to be lower for MeCN and water. The DVB MAA polymers are not observed to follow this pattern, with little difference in the polymer swelling regardless of functional monomer composition. The MAM polymers generally show no observable differences in the swelling behaviour due to the amount of functional monomer in the polymer.
Figure 4.15: EPH imprinted polymers (McCN porogen) % swelling results. Individual bars represent the % swelling in the introduced solvent whilst the x axis identifies the synthesis porogen. Error bars represent maximum observed variance from the sample mean swelling value. (Number of repetitions=3).
When synthesised in MeCN, there is a general trend towards greater swelling in polymers containing more functional monomer. Little swelling is observed in water for the DVB polymers after synthesis in the hydrophobic porogens.

4.6 Conclusions.

For the direction of the design and synthesis of a MIP for aqueous environments, following the assumptions made in the literature about conservation of the imprinted recognitions sites, the DVB polymers appear to be unsuitable for this purpose. Their ability to swell significantly when suspended in organic solvents is not replicated in aqueous solutions, except when the porogen is MeCN. This observation of the swelling of these polymers may be one of the causes of the observation by Ansell et al. that DVB polymers perform significantly worse for the adsorption of EPH than those polymers made using EGDMA as the cross linking monomer for the imprinting of EPH specific polymers. \[171-173\]

The assumption that MIPs work best in their porogen because of the conservation of the steric separation of the reciprocal interactions between monomers and template also suggests that the performance of MIPs may be improved if non-bonding interactions can be increased or decreased without significant alteration of the imprint site. The MAM 1:5 polymer synthesised in toluene shows very similar swelling magnitudes between its porogen and water, whose effect is likely to increase the interaction between MIP and analyte. The MAA polymers exhibit greater swelling, generally, and less difference between the magnitude of the swelling of the porogen and water when the porogen is hydrophobic. Thus, it is likely that the selective interaction of EPH with MAA-co-EGDMA and MAM-co-EGDMA imprinted polymers synthesised in both toluene and chloroform can be improved through performing the adsorption in water as these environments enable better hydrophobic interactions than the associated organic solvents, and the level of swelling being as close as possible to that observed for the porogen in each case. The level of swelling is not an exact match in most of these cases; however it is possible that further improvement to the adsorption characteristics could be made should further tailoring of the recognition environment be possible.
Chapter 5: **Binding analysis of polymer performance in the porogen:**

Solid phase extraction (SPE) was selected as a technique for the assessment of the binding performance of imprinted and control polymers. SPE offers advantages over traditional equilibrium rebinding techniques because of its rapid throughput and particle sequestration. In SPE, the influence of mobile phase flow past the polymer stationary phase is to significantly increase the rate of mass transfer.\(^{[233]}\) This feature is similar to two commonly observed daily phenomena: the stirring of a cup of tea containing sugar to increase the mass transfer of the solid phase sugar crystals into the solution phase; and the increase in the speed of drying wet hair by passing warm air past the surface using a hair dryer. A review of this effect was conducted by Pangakar et al., focussing on the mass transfer rates in stirred tank reactors where it was observed that increased mass transfer was observed for circumstances of increased agitation rate.\(^{[234]}\)

The higher energy of the interaction between imprint site and the template-like molecules in comparison to the non-specific binding sites is linked also the the ease of desorption from these sites. If the adsorption to a non-imprinted site is of a lower interaction magnitude (as MIP theory suggests), flow past this surface during the experiment may produce an associated increase in desorption at only the non-specific interaction sites. This was observed by Ansell et al.,\(^{[171-173]}\) Due to a combination of strong and weak heterogeneous imprint sites, the variable adsorption behaviour caused a significant level of peak tailing in their chromatograms. They overcame this problem through the creation of polymers that had more imprint sites, with lower levels of interaction with the analyte. It was noted that the weaker imprint sites exhibited lower retention – due presumably to the lower level of interaction between imprint site and template, but also less tailing. For this to occur, desorption from the imprint site must occur more freely with the flow of the mobile phase past the column surface. Chianella et al.\(^{[235]}\) and Turner et al.\(^{[140, 141]}\) have both successfully utilised SPE to assess MIPs for the adsorption of a variety of targets including the environmental toxin myocystin L-R and caffeine.
5.1 - Saturation binding and performance of analysis of binding capability.

The study of the binding between the template molecule or analyte with the imprinted polymer is generally undertaken through the construction of an adsorption isotherm that displays concentration dependent recognition behaviour. Manipulation of the ratio of bound vs. free analyte concentrations provides information on the nature and distribution of binding sites present.

Equilibrium binding isotherms are typically constructed in one of two ways; (i) by varying analyte concentration against a constant polymer mass, or (ii) varying polymer mass against a constant analyte concentration. In both cases, the concentration range examined needs to be sufficiently wide (often several orders of magnitude) to achieve saturation binding. While binding isotherms provide a visual comparison of the relative binding properties of MIPs, specific information relating to binding site density and affinity must be derived through the application of an appropriate binding model. A number of these have been documented in MIP literature.

The Langmuir isotherm describes the adsorption of the target to the adsorbent where binding is homogeneous (only one type of interaction site). Rearrangement of the Langmuir isotherm to a linear form (otherwise known as the Scatchard equation) allows the calculation of the association constant (K) and the number of available binding sites (N). The Langmuir isotherm for analyte adsorption can be expressed as follows:

\[
B = N \left( \frac{(KF)}{1 + (KF)} \right) \quad \text{Equation 5.1}
\]

Where

- \( B \) = the concentration of bound analyte
- \( F \) = the concentration of analyte remaining in the depleted solution
- \( N \) = the maximum number of binding sites present in the adsorbent
- \( K \) = the association constant
Rearrangement gives the Scatchard equation:

\[
\frac{B}{F} = K(N - B)
\]  \hspace{1cm} \text{Equation 5.2}

This allows the extraction of \(K\) and \(N\) from a linear equation of the form:

\[y = mx + b;\]

where \(m = -K\) and \(b = KN\)

Because MIP binding sites typically exist as a continuum of site affinities (high to low), a non-linear Scatchard plot results that is usually interpreted as a bimodal analysis of high- and low affinity sites.

A more appropriate model of the binding sites may be obtained by using the heterogeneous Freundlich binding model, detailed in Rushton et al. \[237\] and given below:

\[
B = aF^m
\]  \hspace{1cm} \text{Equation 5.3}

\[
\log_{10} B = m\log_{10} F + \log_{10} a
\]  \hspace{1cm} \text{Equation 5.4}

Where \(B\) = amount of analyte bound (\(\mu\)mol), \(m\) = heterogeneity index, \(F\) = concentration of analyte in solution (mM), and \(a\) = fitting parameter.

Typical methods of investigating the association of the template molecule (or analyte) with the imprinted polymer rely on the existence of the system at equilibrium. As polymer mass is sequentially added to an analyte solution of set concentration, leading to adsorption until equilibrium binding is reached. Analyte binding occurs with functional monomer components present both in the high affinity imprint sites and at non-specific or low affinity sites on the remainder of the polymer surface.

Use of the Freundlich isotherm allows for construction of the association distribution for the polymer species, using the fitting parameters obtained through the
logarithmic plot of the bound and free analyte populations and calculation of the
number of binding sites present \((N)\) that have the association constant \((K)\):

\[
N_i = 2.3am(1 - m^2)K_i^{-m} \tag{5.5}
\]

After this, the number of binding sites, \(N_{(k1-k2)}\) between the limits \(k1\) and \(k2\) can be
calculated using:

\[
K_{\text{avg}(k_1-k_2)} = \left( \frac{m}{1-m} \right) \frac{K_1^{1-m} - K_2^{1-m}}{K_1^{-m} - K_2^{-m}} \tag{5.6}
\]

This affinity distribution is valid only within the limits of the calculation, and thus
to compare between polymers using this scheme, the same limits must be applied to
each analysis. The limits are defined by:

\[
k_1 = \frac{1}{F_{\text{max}}} \tag{5.7}
\]

\[
k_2 = \frac{1}{F_{\text{min}}} \tag{5.8}
\]

Where \(F_{\text{max}}\) and \(F_{\text{min}}\) are the maximum and minimum free populations of the
analyte respectively.

The heterogeneity index ‘\(m\)’ is a measure of the relative populations of high and
low affinity sites within the polymer matrix. A value of \(m\) approaching 1 is quite
homogeneous, while a value of \(m\) that approaches 0 indicates a polymer whose
interaction sites are very heterogeneous. ‘\(a\)’ is a fitting parameter used to calculate the
\(N\) and \(K\) values for a specific polymer.

The occurrence of non-specific adsorption in a MIP may be observed by plotting
the total amount of analyte bound (that is a function of the polymer mass present in each
adsorption) against the mass of analyte bound per gram of polymer present. Although
the SPE experiments are not conducted at equilibrium, an investigation of the behaviour
of the polymers using SPE was carried out. The obtained values may present some
variation from the equilibrium values that could be obtained from typical isotherm
collection, however as the benefits of SPE for application in a field testing regime
outweigh the equilibrium based use of MIPs the use of SPE to attempt a similar
characterisation was undertaken.

5.2 Adsorption behaviour of reference polymers (cross
linking monomer only) conducted in porogen.

All polymers were evaluated using 1 mL of 4 mM ephedrine solution. In each
case the eluent was the porogen used to prepare the respective polymers (acetonitrile,
chloroform and toluene). The postulation of many groups is that the polymers exhibit
their optimal performance in their respective porogens due to maintenance of the steric
location of the reciprocal functional group placement relative to the template molecule.

The DVB reference polymer synthesised in MeCN exhibited the greatest amount
of template uptake, with the maximum adsorption reached with 20 mg of polymer held
in the cartridge (Figure 5.1).

The DVB reference polymer synthesised in CHCl$_3$ exhibits a similar saturation
point, requiring the presence of 20-30 mg of polymer. There is a difference in the
maximum amount of adsorption observed, with the 50 mg sample of the CHCl$_3$ polymer
removing a maximum of 2.5 µmol from the eluent solution. When synthesised in TOL,
the adsorption by the DVB polymer demonstrates an increase in the amount of analyte
depleted, which does not exhibit the saturation observed in the MeCN and CHCl$_3$
polymers, the maximum amount of depleted analyte observed between 40 and 50mg of
polymer.

The EGDMA reference polymers were observed to exhibit similarities to the
DVB reference polymers in relation to the solvent used, but not the magnitude of EPH
adsorption. The MeCN synthesised polymer again demonstrates the largest adsorption
of the eluent solution, the maximum (ca. 1 µmol) amount adsorbed observed to occur
between at 50mg of polymer per mL of eluent solution.

The polymer synthesised in TOL is observed to reach its maximum amount of
bound analyte between 40 and 50mg of polymer (ca. 1 µmol). The CHCl$_3$ EGDMA
polymer system demonstrates a lower adsorption than the polymers synthesised in
MeCN, however the observed error of the TOL and CHCl₃ series make discrimination between these two systems impossible.

**Figure 5.1:** Maximum binding plot for DVB and EGDMA reference polymers synthesised in various porogens towards ephedrine. Binding as a function of polymer loading (1 mL of 4 mM ephedrine solution in toluene, chloroform and acetonitrile respectively) evaluated by SPE. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).

**Figure 5.2:** Isotherm binding plot for DVB and EGDMA reference polymers evaluated by SPE. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3). To remove the influence of changing polymer loading, both free and bound amounts are made independent of polymer mass to allow meaningful comparison.
The data shown in Figure 5.1 was then transposed to generate binding isotherms (Bound v Free) for each of the reference polymers (Figure 5.2). These, along with the Freundlich isotherm regression plot (Figure 5.4) and the affinity distribution based on the Freundlich isotherm highlighted the influence of porogen character on analyte binding affinity. The behaviour of the EGDMA polymers synthesised in non-polar solvents is unable to be separated, and although the separation between the performances in different solvents is less here, it is clear that higher analyte affinity is observed in MeCN in comparison to TOL and CHCl₃.

There is a clear effect on the performance of the DVB reference polymer depending on the porogen used for its synthesis and rebinding. The MeCN synthesised polymers show the greatest affinity for EPH, followed by the polymer synthesised in CHCl₃ and finally TOL, while both the EGDMA polymers synthesised in TOL and CHCl₃ show little variation in their affinity for the EPH.

The level of adsorption by the DVB polymer observed in relation to the EGDMA polymer, in combination with the observations made during the swelling experiments, and those made by Ansell et al. [171-173] (that the DVB polymers perform worse than those synthesised using an EGDMA cross linking monomer) mean that achieving the goal of creating a selective, synthetic receptor capable of operating in an aqueous environment, using DVB as a cross linker is unlikely. Swelling results (Chapter 4) suggest that DVB cross linked polymers are far more sensitive to solvent effects than the EGDMA cross linked species. Higher levels of sorption under polar solvent conditions further suggest that non-polar interactions between the DVB unit and the aromatic head group of the EPH dominate the binding event. There may also be an association with the amount of swelling possessed by these polymers, and the potential for the matrix itself being responsible for the clearly non-specific binding.

Porogen character is observed to play a significant role in analyte sorption. The aromatic character of TOL appears to disrupt binding leading to lower levels of ephedrine sorption compared to CHCl₃ and MeCN. Molecular size of the solvent may be a cause of this feature, with the EPH molecules being trapped by the network of the DVB polymer in pores created by the solvent molecules. As TOL is a larger species, it is likely that its use as a porogen may result in larger pore diameters.
The DVB reference polymers possess a much higher affinity for the template in all solvents investigated than do the EGDMA reference polymers. The reference polymers prepared in MeCN show a higher affinity for EPH compared with identical polymer formulations prepared in CHCl₃ or TOL. Their N values (Table 5.1) are higher in almost every case, except for the chloroform polymers, which suggests that non-selective surface adsorption by the DVB polymer is significantly greater than the EGDMA formulations for each solvent/porogen system.

**Figure 5.3:** Binding stability plot for DVB and EGDMA reference polymers towards ephedrine, synthesised in various porogens. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).

The greater non-specific binding of the DVB polymers in comparison to the EGDMA polymers is clearly shown by plotting the amount of analyte bound as a function of polymer loading vs. the total amount of analyte bound (Figure 5.3). The CHCl₃ and TOL EGDMA polymer systems show a more stable association, and also lower non-specific interactions with EPH than the same polymer composition synthesised in MeCN and all of the DVB polymers. All polymers exhibit a decrease in the amount of analyte bound g⁻¹ as more total analyte is bound, suggesting that this interaction is not located at well defined and conserved sites. This feature was expected due to the absence of imprinted sites within these polymers. If the polymer adsorbs EPH only at the higher affinity imprinted sites, it would be expected that a constant amount of EPH would be adsorbed per gram of polymer. As it is known that flow past
a surface increases the kinetic rate of adsorption and desorption, it is reasonable to assume that desorption at the imprinted cavities would be hindered due to higher affinity for the template, while the desorption of the template from non-specific sites would be less hindered.

**Figure 5.4:** Freundlich isotherm regression analysis for DVB and EGDMA reference polymers evaluated by SPE. To remove the influence of changing polymer loading, both free and bound amounts are made independent of polymer mass to allow meaningful comparison. (Number of repetitions=3).

The plotted Freundlich isotherm regression analysis (*Figure 5.3*) and the fitting parameters (*Table 5.1*) show a higher homogeneity of interaction sites for the polymers synthesised in MeCN for both cross linking agents. It is also clear that the EGDMA polymers exhibit a lower affinity for the analyte (EPH) than the DVB polymers. This is due to the hydrophobicity of the DVB polymers in comparison to the EGDMA polymers, and also the ability to participate in π-π stacking interactions with EPH. Thus, to reduce the level of non-specific interaction, DVB is unlikely to be viable as a crosslinking monomer for the selective adsorption of EPH under aqueous conditions.
Figure 5.5: Affinity distribution plot showing Log K vs. N for DVB and EGDMA reference polymers towards ephedrine, synthesised in various porogens. The porogen was used as the analyte solvent in each case. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. (Number of repetitions=3)

<table>
<thead>
<tr>
<th>Polymer and porogen</th>
<th>m</th>
<th>a</th>
<th>$N_{(k_1-k_2)}$</th>
<th>$K_{(avg(k_1-k_2))}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGDMA CHCl$_3$</td>
<td>0.234</td>
<td>42.209</td>
<td>17.464</td>
<td>0.028</td>
</tr>
<tr>
<td>EGDMA Tol</td>
<td>0.179</td>
<td>43.481</td>
<td>12.967</td>
<td>0.016</td>
</tr>
<tr>
<td>EGDMA MeCN</td>
<td>0.547</td>
<td>133.937</td>
<td>42.431</td>
<td>0.410</td>
</tr>
<tr>
<td>DVB CHCl$_3$</td>
<td>0.651</td>
<td>664.355</td>
<td>168.565</td>
<td>1.045</td>
</tr>
<tr>
<td>DVB Tol</td>
<td>0.695</td>
<td>231.953</td>
<td>52.364</td>
<td>1.625</td>
</tr>
<tr>
<td>DVB MeCN</td>
<td>0.347</td>
<td>1126.160</td>
<td>419.477</td>
<td>0.077</td>
</tr>
</tbody>
</table>

Table 5.1: Freundlich isotherm linear regression fitting parameters for polymers containing only crosslinking monomer. $m$= heterogeneity index, $a$= fitting parameter. The affinity distributions are calculated using Equations 5.3-5.8. $k_1$=log0.4 and $k_2$= log1.5

The polymers synthesised in MeCN show an increased affinity for EPH in comparison to polymer counterparts prepared in the more hydrophobic solvents, suggesting synthesis in this porogen generates a polymer that exhibits higher levels of non-specific EPH binding compared with polymers synthesised in toluene and chloroform.

It is apparent from the interaction between the reference polymers and ephedrine (cross-linking monomer only) that the lowest level of non-specific interaction occurs when the polymerisation is conducted in toluene (Figure 5.5). The calculated values for the sites of interactions show that this polymer exhibits different behavior from the rest.
of the cohort (Table 5.1). Thus it should be possible to reduce the level of non specific interaction with the crosslinked structure of these polymers, which is the major component of the structures, by synthesizing in toluene. Although fewer sites are formed, the average association constant for these sites and EPH is significantly greater than for polymers synthesized using the same monomer in a different porogen. This feature is likely to be due to the size of this solvent molecule creating pores that the analyte is able to pass through. Thus, less sites are created that are amenable to EPH interaction in comparison to the MeCN and CHCl₃ using the DVB crosslinker polymers, although these are sites with higher affinity for EPH. However, in comparison to the EGDMA polymers in the same solvent system, more sites are created in the DVB polymers (Table 5.1). It is likely that the aromatic nature of toluene generates spaces that the aromatic head of EPH can pass between benzene units of the cross linker, resulting in π-π stacking interactions and consequently a greater number of sites available for EPH binding.

As a result of this feature, and the conclusions reached after the swelling experiments the DVB polymers were excluded from the remainder of the analysis of MIP performance. Not only is the level of non-specific interaction significantly higher for the DVB reference polymers, but the lack of ability for swelling in aqueous environments suggest that the imprint sites may be inaccessible to ligands.

5.2.1 MAA MIP loading isotherms conducted in MIP porogen.

The binding of the EPH imprinted MAA polymers synthesised with a template to monomer ratio of 1:4 (Figure 5.6) showed that the MeCN polymer demonstrates the highest ability to deplete the template from the eluent solution (ca 3 μmol). There appears to be a plateau in the amount of analyte adsorbed at around 50mg.

The plot of bound analyte concentration .per gram vs. total bound analyte concentration for the EPH-MAA-co-EGDMA polymers (Figure 5.7) highlights the areas of the isotherm defined by concentration of analyte vs polymer mass in which the non-specific binding of the analyte is highest. This level of non-specific interaction is indicated by a peak in the amount of bound analyte.g⁻¹ of polymer. The polymers which
expressed the highest level of non-specific interaction were thus considered less suitable candidates for the optimisation of selective and specific interaction. In these cases, the magnitude of the non-specific interactions means that the level of selective adsorption would need to be at least an order of magnitude higher to provide a selective and specific response.

The observed downward trend for all polymers as the amount of bound EPH increases suggests that there is a significant proportion of non-specific interaction in all polymers. The lowest of these trends is observed for the polymer treated in toluene, while MeCN appears to allow for the greatest level of template adsorption, though it is clearly associated with high levels of non-specific binding. It is likely that the observed non-specific interactions are created by functional monomer units that are not associated with the template upon polymerisation. As the most polar porogen, and also a hydrogen bond acceptor itself, the association between MeCN molecules and residual FM and XLM molecules must play a role in making these sites accessible to the analyte, contributing to the increased levels of the non-specific binding observed.

Figure 5.6: Maximum binding plot for an EPH imprinted, MAA-co-EGDMA 1:4, synthesised in various porogens. Binding as a function of polymer loading (1 mL of 4 mM ephedrine dissolved in acetonitrile, toluene and chloroform solution) evaluated by SPE. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).
Figure 5.7: Binding stability plot for EPH 1:4 MAA EGDMA polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).

The Freundlich isotherms (Figure 5.12) suggest that the 1:4 CHCl₃ polymer exhibits the greatest homogeneity of imprint sites in the arbitrary polymer stoichiometry group, whilst the polymer synthesised in toluene is the most heterogeneous of the arbitrary stoichiometry MAA polymers.

Figure 5.8: Binding plot for EPH 1:4 MAA EGDMA polymers evaluated by SPE. The porogen was used as the analyte solvent for the analysis in each case. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).
Figure 5.9: Maximum binding plot for an EPH imprinted, MAA-co-EGDMA 1:2, synthesised in various porogens. Binding as a function of polymer loading (1 mL of 4 mM ephedrine solution) evaluated by SPE. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).

The analysis of the computer modelling optimised stoichiometry of EPH and MAA (1:2) is plotted and like the polymers synthesised in TOL in the 1:4 stoichiometry, the polymers synthesised in the non-polar porogens exhibit a continued, almost linear rise in the amount of analyte depleted during the solid phase extractions (Figure 5.9). The TOL polymer exhibits the second highest maximum binding at the highest polymer loading observed, and it significantly out performs the polymers synthesised and bound in other solvents in extracting analyte from the eluent solution. The polymer synthesised in CHCl₃ depletes the analyte from eluent the least through the entire range of polymer loading.

The EPH- MAA 1:2 MIP synthesised in MeCN exhibits little cohesion in its isotherm results (Figure 5.10), suggesting that there is a significant level of random adsorption behaviour in the adsorption of EPH using SPE. A second possibility is that the EPH molecules are able to become trapped within the MIP matrix in some cases, despite not interacting directly with the imprinted sites. Synthesis of this polymer composition in MeCN is suspected to have generated a polymer surface that exhibits many areas amenable to non-specific interaction with the template molecule. Thus, the fact that this polymer exhibits the largest amount of analyte adsorbed is of secondary
importance as the evidence suggests that the major component of the adsorption is non-specific, non-cavity located adsorption.

![Figure 5.10: Isotherm binding plot for EPH 1:2 MAA EGDMA imprinted polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation of results from the sample mean. (Number of repetitions=3).](image)

When the binding stability analysis was performed on the polymers synthesised in the hydrophobic solvents (Figure 5.11), there was a significantly lower observed level of non-specific interaction between MIP and template. The polymer synthesised in toluene appears to exhibit a slightly greater level of non specific interaction to the polymer synthesised in chloroform, however it is also observed to exhibit an adsorption of approx 60 µmol.g⁻¹ in comparison to that of the chloroform polymer at 40 µmol.g⁻¹.

The designed MAA polymers clearly out perform the polymers synthesised using arbitrary 1:4 stoichiometry with specific reference to the amount of EPH adsorbed per gram of polymer (Figure 5.7 and 5.11). The stable adsorption of more analyte per gram in the designed polymers than those constructed using the arbitrary stoichiometry of 1:4 suggests that not only are the imprint sites more ordered, but that the additional functional monomer units present in the 1:4 polymer actually hinder the formation of reliable imprint sites. The magnitude of obvious non-specific bonding events in these polymers is significantly lower in the designed polymers than those synthesised using the arbitrary stoichiometry. The MIP synthesised in TOL at 1:4 displays the interaction...
that appears to have the smallest non-specific binding component, despite exhibiting the least amount of analyte binding.

Although there is a non-specific binding component present in the 1:2 TOL series (Figure 5.11), the magnitude of the adsorption, $g^{-1}$ of polymer, in addition to the lack of a large non-specific binding component suggests that this polymer is a good candidate for further investigation.

Of the MAA 1:2 polymers, the CHCl$_3$ polymer bound in CHCl$_3$ exhibits little observable non-specific binding. Although the magnitude of the binding is lower than the TOL series, it is likely that this polymer is also a good candidate for further study. The stable adsorption per gram with increasing load suggests that the isotherm may not fit the model possibly due to insufficient range of concentration of analyte binding per gram. It is also likely that this polymer is a viable candidate for further investigation, if only to provide a comparison with its toluene synthesised analogue, as the observed lowest non-specific binding were observed in the cross linker only reference polymer when the EGDMA monoliths were synthesised and treated in CHCl$_3$ and toluene.

![Figure 5.11: Binding stability plot for EPH 1:2 MAA EGDMA polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).](image)

The performance of the two different MAA polymers synthesised in chloroform and toluene suggests that although they contain differing amounts of monomer, that there is little difference in the total amount of analyte adsorbed in the range of experiments carried out. It is likely that the porogen being hydrophobic has played a
role in directing non-complexed MAA units present in the 1:4 stoichiometry polymer to self associate (dimerise) and must be included in the polymer network in such a manner that their functional groups are not available for interaction with the re introduced analyte.

Table 5.2: Freundlich isotherm linear regression fitting parameters for EPH-MAA co EGDMA polymers. m= heterogeneity index, a= fitting parameter. The affinity distributions are calculated using Equations 5.5-5.8. k1= log0.4 and k2= log1.5

<table>
<thead>
<tr>
<th>Polymer</th>
<th>m</th>
<th>a</th>
<th>N(k1-k2)</th>
<th>Kavg(k1-k2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA 1:4 CHCl3 EGDMA</td>
<td>0.492</td>
<td>179.432</td>
<td>61.426</td>
<td>0.257</td>
</tr>
<tr>
<td>MAA 1:4 toluene EGDMA</td>
<td>0.255</td>
<td>102.612</td>
<td>50.828</td>
<td>0.034</td>
</tr>
<tr>
<td>MAA 1:4 MeCN EGDMA</td>
<td>0.332</td>
<td>165.730</td>
<td>61.571</td>
<td>0.067</td>
</tr>
<tr>
<td>MAA 1:2 CHCl3 EGDMA</td>
<td>-0.153</td>
<td>21.193</td>
<td>-11.281</td>
<td>-0.001</td>
</tr>
<tr>
<td>MAA 1:2 toluene EGDMA</td>
<td>0.264</td>
<td>21.414</td>
<td>7.585</td>
<td>0.037</td>
</tr>
<tr>
<td>MAA 1:2 MeCN EGDMA</td>
<td>0.091</td>
<td>59.676</td>
<td>28.507</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Figure 5.12: Freundlich isotherm regression analysis for EPH-MAA co EGDMA polymers evaluated by SPE. To remove the influence of changing polymer loading, both free and bound amounts are made independent of polymer mass to allow meaningful comparison. (Number of repetitions=3).
Figure 5.13: Affinity distribution plot showing Log K vs. N for EPH- MAA co EGDMA polymers towards ephedrine based on Freundlich Isotherm parameters (Table 5.2), synthesised in various porogens. The porogen was used as the analyte solvent for the analysis in each case. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. (Number of repetitions=3)

This contrasts with the performance of MeCN, where such behaviour was neither predicted nor observed. The 1:4 MAA polymer is observed to adsorb almost twice as much analyte as the designed 1:2 MAA polymer (200 μmol.g\(^{-1}\) vs. 100 μmol.g\(^{-1}\)). This result suggests that MeCN as a porogen may interfere with intermolecular interactions in the pre-synthesis solution, limiting FM dimerisation and also weakening T:FM interactions. No observable trend suggests that a similar behaviour is exhibited when the polymer is synthesised in hydrophobic organic solvents.

The fitting parameters of the Freundlich isotherm (Figure 5.12) suggest that the MAA polymers produced display greatest homogeneity of sites when synthesised and bound in chloroform, as opposed to the same polymer formulations in both toluene and MeCN (Table 5.2). The majority of the MIP systems display a heterogeneous distribution of adsorption sites in the current experiments, because the majority of the polymer matrix is composed of cross linking monomer without the capacity for specific adsorption. In this case, the MAA 1:2 polymers treated in CHCl3 and MeCN did not fit the model well, however the reason for this is unclear. The negative heterogeneity index and number of calculated imprint sites suggest that the Freundlich model does fit this data series well. Greater homogeneity is typically a sign of poor imprinting but in
this case the lower stoichiometry may result in a lower proportion of free MAA units that leads to improved homogeneity.

There appears to be no consistent trend as to the observed number of imprint sites generated in each system based on the number of functional monomer equivalencies, or the porogen in which they were polymerised.

5.2.2 - MAM MIP loading isotherms conducted in MIP porogen.

The variation of functional monomer according to the in silico modelling (Chapter 2) was predicted to create a strong interaction at 1:5 and a lower interaction with the 1:4 polymers as there was no significant favourable interaction predicted at this stoichiometry. Although only 2 hydrogen bond donor sites exist on the template molecule, the modelling predicted the cooperative action of the functional monomer units, resulting in a single strong interaction at this stoichiometry. The NMR investigations predict a strong interaction at between template and functional monomer at 1:5 T:FM.

In the 1:4 EPH:MAM system (Figure 5.14), the polymer synthesised and treated in MeCN is able to bind a greater amount of the analyte than the polymers synthesised in toluene and chloroform. There is no observed plateau of binding associated with the increase in polymer loading. The chloroform system is observed to result in the lowest amount of analyte binding at all polymer loadings, and this is pronounced at lower polymer loadings (Figure 5.14).
Figure 5.14: Maximum binding plot for an EPH imprinted, MAM-co-EGDMA 1:4, synthesised in various porogens. Binding as a function of polymer loading (1 mL of 4 mM ephedrine solution) evaluated by SPE. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).

The plotted isotherm of the TOL polymer exhibits a maximum binding ability of 70 µmol.g\(^{-1}\). A plateau is observed in the polymer loading plot, at approximately 45mg of polymer (Figure 5.14). The plot of bound analyte.g\(^{-1}\) vs. total bound (Figure 5.15) analyte indicates that there is a large uncertainty in the amount of analyte bound/mg also suggests that the distribution of the imprinted sites may be unstable throughout the polymer structure.

The CHCl\(_3\) polymer exhibits a maximum bound quantity of polymer of 35 µmol.g\(^{-1}\). A plateau is observed in the polymer loading plot, at a point similar to that observed in the TOL system, approximately 45mg of polymer (Figure 5.14). This polymer exhibits a stable magnitude of bound EPH.g\(^{-1}\) of polymer (Figure 5.15).

Although the predicted interaction between MAM and the template was significantly lower in magnitude, (Chapter 4) than that of MAA, when synthesised into an imprinted polymer there is only a slight difference in the amount of bound analyte at all polymer loadings, for all solvent systems investigated. (Figures 5.6-5.21)
Figure 5.15: Binding stability plot for EPH 1:4 MAM EGDMA polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).

The designed polymer composition, EPH:MAM 1:5 sees the MeCN polymer adsorb the greatest amount of EPH from solution (Figure 5.17). A possible plateau in the amount of analyte bound from solution is observed to exist between 35 and 60 mg of polymer load. It is also seen to exhibit the greatest level of error in the observed values recorded in the adsorption experiments. It also exhibits the largest amount of analyte bound, 140 μmol.g⁻¹ polymer (Figure 5.18). The profile of the amount of analyte bound.g⁻¹ shows that there is non-specific binding involved in the behaviour of all MIPs at this composition ratio. The toluene polymer is observed to exhibit the lowest amount of analyte bound of all 1:5 MAM polymers. It is possible that this is due to a higher level of functional monomer inclusion in the imprint cavities that is inherently related to there being a lower number of cavities.
Figure 5.16: Isotherm binding plot for EPH 1:4 MAM co EGDMA imprinted polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation of results from the sample mean. (Number of repetitions=3).

Presumably, the predicted interaction from Chapter 2 that 5 MAM units are required to form the cooperative network of interactions required for interaction with EPH are not able to be formed with only 4 molar equivalencies of MAM in the 1:4 polymers.

The amount of non-specific binding observed in the MAM 1-5 polymers (Figure 5.18) is significantly less than in any of the previously observed polymers when synthesised in all porogens. Although there is clearly a non-specific portion of the interaction when synthesised in MeCN, the maximum amount of analyte bound is approx 140 μmol.g⁻¹ and an apparent plateau at 80 μmol.g⁻¹, before a final drop off at higher polymer load. The second observed deviation may be caused by the plateau being caused by a combination of specific and non-specific interaction. Little observable non specific interaction is observed for the polymers synthesised in chloroform and toluene, in agreement with the suggestions of Yu and Mosbach,¹⁶⁰ that hydrophobic porogens may force the polar imprint sites into the active polymerisation globule, resulting in better performing and more uniform polymers than achieved in polar porogens. The heterogeneity of the calculated Freundlich isotherms (Table 5.3,
Figure 5.20) suggest that the polymers synthesised in apolar media exhibit an equal or greater homogeneity than those synthesised in polar MeCN.

Figure 5.17: Binding plot for EPH 1:5 MAM co EGDMA imprinted polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation of results from the sample mean. (Number of repetitions=3).

Figure 5.18: Binding stability plot for EPH 1:5 MAM EGDMA polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation from the sample mean of results (Number of repetitions=3).
Figure 5.19: Isotherm binding plot for EPH 1:5 MAM co EGDMA imprinted polymers evaluated by SPE. All polymers were exposed to ephedrine solution (1 mL, 4 mM) for an equal amount of time. Error bars represent the maximum observed variation of results from the sample mean. (Number of repetitions=3).

The calculated number of interaction sites $N_{(k1-k2)}$ suggests that the designed polymers have followed the behaviour observed by Yu and Mosbach, and have resulted in a higher population of more ordered sites due to the sequestration of the polar groups in similar areas during the polymerisation in the most hydrophobic solvent available (Table 5.3). Strangely, a higher heterogeneity index does not necessarily correlate with a lower number of sites as demonstrated by the 1:4 imprint sites from the toluene series.

Overall, the MAM polymers appear to generate more homogeneous polymers than the MAA polymers, with the greatest homogeneity of sites observed for the EPH-MAM 1:5 co EGDMA polymer synthesised and treated in toluene. This suggests that the MAA yields more binding cavities – presumably due to the higher levels of H bonding and electrostatic interactions that are potentially available.
Figure 5.20: Freundlich isotherm regression analysis for EPH-MAM co EGDMA polymers evaluated by SPE. To remove the influence of changing polymer loading, both free and bound amounts are made independent of polymer mass to allow meaningful comparison. (Number of repetitions=3).

Table 5.3: Freundlich isotherm linear regression fitting parameters for EPH imprinted MAM-co-EGDMA polymers. \( m \) = heterogeneity index, \( a \) = fitting parameter. \( k_1 = \log 0.4 \) and \( k_2 = \log 1.5 \)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>( m )</th>
<th>( a )</th>
<th>( N_{(k1-k2)} )</th>
<th>( K_{avg(k1-k2)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAM 1:4 CHCl3 EGDMA</td>
<td>0.373</td>
<td>86.179</td>
<td>30.296</td>
<td>0.103</td>
</tr>
<tr>
<td>MAM 1:4 toluene EGDMA</td>
<td>0.528</td>
<td>215.477</td>
<td>70.394</td>
<td>0.346</td>
</tr>
<tr>
<td>MAM 1:4 MeCN EGDMA</td>
<td>0.287</td>
<td>145.144</td>
<td>52.650</td>
<td>0.046</td>
</tr>
<tr>
<td>MAM 1:5 CHCl3 EGDMA</td>
<td>0.393</td>
<td>221.871</td>
<td>82.137</td>
<td>0.112</td>
</tr>
<tr>
<td>MAM 1:5 toluene EGDMA</td>
<td>0.560</td>
<td>248.428</td>
<td>76.972</td>
<td>0.460</td>
</tr>
<tr>
<td>MAM 1:5 MeCN EGDMA</td>
<td>0.417</td>
<td>251.768</td>
<td>91.928</td>
<td>0.138</td>
</tr>
</tbody>
</table>

The polymers designed in the modelling scheme of the previous chapters appear to perform better than those utilising a standard 1-4 stoichiometry. They appear to perform better when synthesised in non-polar solvents, presumably due to the forced proximity of the polar groups in the presynthesis solution. The combination of this performance and the observed swelling behaviour (Chapter 4) suggest that the EPH imprinted DVB polymers are unsuitable for use in aqueous environment due to the observed swelling differences, and high level of non-specific binding events regardless of porogen or polymer stoichiometry.
5.3 **Solid Phase Extraction of EPH from varying eluent solutions.**

An investigation of the effect of solvent variation on the polymers used above in the rebinding of EPH was carried out using CHCl₃, TOL, MeCN and water. The template was added at identical concentrations to samples of MIP of identical composition except for their respective porogens. These tests were expected to provide correlation with the *in silico* models constructed in Chapter 3, and to assist in the selection of the best performing systems for creation of MIP systems for systematic aqueous discrimination of compounds.

For the design of biomimetic, pseudo-neurotransmitter receptors, it was expected that the use of a non-polar porogen, followed by the use of a highly polar solvent for recognition would closely mimic the natural environment of the endogenous GPCRs. Thus allowing the use of the shape selective and hydrophobic components of the imprinted cavity in conjunction with the electrostatic interactions possible at the template based reciprocal functional group sites. As the active site of the GPCR family exists in a hydrophobic region of the receptor,⁵²,²⁴⁸ and the receptors themselves are
located across cell membranes (made up of lipid bilayers), the attempt to mimic this feature of the natural receptors for ATS was expected to generate better performance when synthesised in hydrophobic organic solvents. When synthesised in the most hydrophobic solvent available, there should be significant partitioning of the hydrophobic and hydrophilic groups within the polymerising globule, as postulated by Yu et al. \cite{yu168}, producing functional mimicry of GPCR recognition.

Water is capable of significant networks of hydrogen bonding, and it was expected that increases in the specificity of the imprinted polymers may be observed due to the suppression of non-specific interaction. The use of water was also expected to reduce the total amount of adsorption observed through the interruption of some cavity located recognition, although the increase of hydrophobic interaction due to the nature of the majority of the polymer, analyte, and mobile phase is also a possible outcome. If the non-specific or low affinity interaction sites on the polymer are able to be occupied by water molecules, it is unlikely that arrival of the template will displace the water molecules from these potential sites for interaction. In contrast, the higher order sites may provide anchor points for the template to “evict” the solvent molecules that have occupied the vacant imprint site by utilising the multiple interaction sites produced by the imprint sites with higher stoichiometries of (FM)T. It was also a possible outcome that the incorporation of H$_2$O into the specific cavities would result in the cooperative interaction between analyte and cavity, as shown in the natural neurotransmitter receptors.\cite{chianella235} The work of Chianella et al. \cite{chianella235} suggests that aqueous environments may actually play a role in the increasing of the interaction between imprint site and template. Although this conclusion was drawn regarding the recovery via elution of microcystin L-R from a SPE cartridge that had been loaded with this toxin, the suggestion that interactions such as electrostatic and hydrophobic interactions would be stronger in this solvent was played a significant role in the successful recognition of microcystin-LR in aqueous solutions.

In this case however, it was necessary to desorb the analyte from the surface in an aqueous environment. If immobilisation of the analyte at the polymer surface is the primary goal, SPE coupled with an aqueous recognition environment should not affect the ability of imprint sites to selectively bind the template so long as polymer swelling behaviour is similar to that in its porogen.
When synthesised in TOL, the MAA-co-EGDMA polymers exhibit a divergent performance in their ability to rebind EPH from a variety of solvents (Figure 5.22). The 1:4 T:FM polymer shows little selectivity compared to its NIP in any eluent. The amount of the eluent adsorbed does exhibit significant variation between the porogen (TOL) depleting ca. 80% of the present analyte, to MeCN being able to bind ca. 20% of the available analyte.

By contrast, the MAA 1:2 MIP formulation exhibited selectivity in all solvents except chloroform, with the greatest amount of analyte binding observed in the porogen toluene (ca 80%) and water (95%). Levels of non-specific analyte binding in the control polymer were similar for both solvents. Analyte binding was significantly lower in acetonitrile (~30%), discrimination between the MIP and NIP remained at similar levels with I= 2.5.
Figure 5.23: EPH – MAM-co-EGDMA polymers at 1:4 and 1:5, synthesised in TOL. Adsorption experiments with solvent identified on x axis. (Number of repetitions=3). Error bars represent maximum observed variation from the sample mean.

MAM polymers prepared in toluene exhibited a similar binding profile to the MAA polymers for the generic 1:4 and computer optimised 1:5 formulations (Figure 5.23). In chloroform analyte binding was uniformly high (> 80%) for all polymers (MIP and NIP), however no binding selectivity was observed. The 1:4 MAM MIP was observed to posses significant reproducibility errors in both chloroform and water and showed poor recognition in all solvents except MeCN (that had only low level binding). These findings suggest this polymer possesses only poorly defined template cavities within its structure.

MeCN is observed to produce the lowest adsorption levels of the polymers synthesised in TOL (Figure 5.17 and 5.18), presumably due to the blocking of site of potential interaction by the solvent molecules that prevent the bridging of interactions by a molecule like H2O as identified by Pardo et al. Increased adsorption was observed in the aqueous environment for the 1:5 polymer in relation to the porogen (TOL). It is likely that the increase in both the MIP and NIP adsorption in this case is related to additional hydrophobic interaction in water as opposed to toluene.

Both functional monomers show increased imprinting factors at the computationally derived stoichiometry in comparison with the arbitrary stoichiometry.
When synthesised in CHCl$_3$ (Figure 5.24) the 1:4 MAA polymer displays moderate ($I = \text{ca. 1-1.5}$) selectivity in water, however in toluene, the observed variation obscures the true extent of this binding. Both the CHCl$_3$ and MeCN polymers show no selectivity when compared to the NIP. Little analyte was bound in MeCN, while the largest amount was bound by the polymer synthesised in TOL (ca. 55%).

The 1:2 MAA polymers display slight selectivity in CHCl$_3$ ($I=1.3$) and TOL ($I=1.6$) but in water the MIP shows a significantly higher selective adsorption of the template (ca. $I=3.1$), coupled with significant depletion (ca. 95%) of the template during the elution. In both TOL and CHCl$_3$, the analyte is removed from solution to a significant level (ca. 80%) however the NIP also adsorbed a large amount of the analyte from solution. When the stoichiometry was 1:4, no selectivity was observed in any solvent. The high polarity of water appears to improve the performance of the 1:2 MAA polymers synthesised in CHCl$_3$ that may be due to the increase in the favourable combination of electrostatic and hydrophobic interactions. Toluene may assist in the definition of the imprint site by limiting the hydrophobic interactions during the polymerisation in this solvent. It appears that in water, the non-specific portion of the binding (shown by the behaviour of the NIP) is reduced, as expected.
The largest observed lack of precision (Figure 5.24) is observed during the rebinding of the analyte in TOL, to the 0:4 polymer of almost 40% of the total analyte.

The MAM –co- EGDMA CHCl₃ (Figure 5.25) polymers show little selectivity in any solvent, the highest performing of which is the 1:4 polymer in TOL at ca. 1.5. The amount of adsorbed analyte is lowest in H₂O for both stoichiometries, while in MeCN the 1:5 polymer binds close to 60% more template than the 1:4 (90% and 30% respectively). In both the TOL and CHCl₃ solutions, the stationary phase adsorbent MIPs were able to deplete the template solution by 85-95%. Large observed variation of results was recorded for both stoichiometries in CHCl₃, with no observed selectivity (compared to the associated NIP) for the template in any eluent.
The polymers synthesised in MeCN (Figure 5.26) show a differing pattern of interaction to the non-polar porogens. The 1:4 polymer displays moderate selectivity in MeCN (ca 1.5), though the observed error suggests little weight can be placed on this factor, and little selectivity for the template in H₂O and CHCl₃ (ca. I=1), with both of these systems displaying an error that does not allow discrimination between the imprinted and non-imprinted polymers.

In TOL however, the MeCN synthesised 1:4 polymer displays ca. 2.3 selectivity for the template, which is also associated with the largest level of template adsorption (ca. 75%) from the eluent solution. No obscuration of the selectivity is observed, with the errors for this system observed for these polymers not overlapping. This is not repeated by the 1:2 polymer, which exhibits no selectivity for the template in any solvent other than H₂O (ca. 2.5) and the largest adsorption of the template from solution (ca. 95%).
Figure 5.27: EPH – MAM-co-EGDMA polymers at 1:4 and 1:5, synthesised in MeCN. Adsorption experiments, solvent identified on x axis. (Number of repetitions=3). Error bars represent maximum observed variation from the sample mean for these experiments.

When synthesised in MeCN the MAM polymers display little affinity for the template in any solvent, with neither the MIP nor the NIP successfully adsorbing more than 50% of the analyte. No selectivity is observed in any system, suggesting that because the individual interactions between EPH and MAM are weaker than those of MAA, MeCN is responsible for the interruption of these interactions in the pre-polymerisation solution, which has resulted in poorly defined cavities. It is also possible that the global structure of these polymers is such that the imprint sites are unavailable for template interaction.

5.4 Conclusions

As SPE is not an equilibrium technique, the experiments described herein are intentionally not performed at equilibrium. The isotherms constructed are not performed at equilibrium intentionally and the information they provide is related directly to the technique later used to assess the selectivity of the polymers. The technique of SPE has been utilised in previous studies of MIP behaviour, and although the information gathered by equilibrium studies of MIP behaviour is useful, its direct application to the SPE technique is unclear. Experiments have been conducted to
mimic the general techniques, instead using SPE as the vehicle rather than equilibrium based tests.

The comparison of imprinted polymers to other imprinted polymers rather than the NIP pair is of utility in determining the effect of the porogen and eluent on the MIP itself. Identification of non-specific binding components in the imprinted polymers interactions alone, as opposed to the assumption that non-imprinted polymers will exhibit the same binding ability due to similar structures is not supported by the obvious differences in swelling ability and performance of different polymers in different environments. Subsequent application of the imprinted polymers into a scheme investigating the selectivity of the MIP vs. NIP is also necessary; however the specific behaviour of the MIP cavity and non-specific surface is of importance.

The difference in the behaviour of the polymers synthesised in MeCN in the pseudo-isotherm and solvent variation experiments was an unexpected outcome. This may be potentially explained by the packing of the polymer particles in the SPE tubes for this section and the sealing of the particles between two frits that were lightly compressed after the initial swelling process. This was not performed in the pseudo isotherm experiments that may explain the obvious difference in performance of these polymers. Due to the irregularity of the shape of the polymer particles, a result of the mechanical grinding process, control of the void space within the packed column was difficult. The error observed in some experiments may have been significantly contributed to by these irregularities.

As the elution is carried out under vacuum, the polymer particles are likely to experience significant pressure effects, and a more compressible polymer structure is likely to be more susceptible to these forces. During the mechanical grinding of the polymers the MeCN polymers were significantly easier to grind to size, suggesting that their physical structure may be less rigid than the same polymers synthesised in apolar media and therefore more susceptible to effects such as these. Such irregularities are known to present issues relating to the performance of chromatographic columns using a variety of stationary phases. It is also possible that this compression resulted in the generation of channels within the packed tubes that allowed the direct passage of solvent without the carriage by the imprinted and non-imprinted sites of these polymers. Such uneven flow is a plausible explanation for the lower observed adsorption in these
experiments. If the path of the analyte molecules through the packed bed does not pass all active sites, no adsorption can occur at these sites. As all polymers were treated identically in this section, this may be evidence of clear physical differences between the polymers synthesised in MeCN and the hydrophobic organic solvents. Similar behaviour was not noted for the toluene or chloroform polymers. Coupled with the physical swelling behaviour differences observed in different solvents identified (Chapter 4), this effect may be responsible for the marked differences in the performance of the MeCN synthesised polymers in this section. A high level of selectivity was still observed in water (I=2.5) for the MeCN synthesised MAA 1:2 polymer, suggesting that selectivity is still able to be generated in a polar solvent, however its magnitude is lower than those polymers synthesised in apolar media. The content of water in the organic phase can also play a significant role in the variation in retention observed.\[241\]

The conclusions of Piletska et al.\[220\] are consistent with the findings presented that polymer performance is improved under aqueous rebinding for MIPs synthesised in an apolar porogen. If the porogen is less polar than the component functional monomer and template, then these “more” polar molecules will be located inside the active polymerisation globule. Thus, the imprint sites should be better defined when the MIP is synthesised in apolar solvents, resulting in better selectivity and higher adsorption of the template analyte. The proposition that this association is driven by phase separation appears to be justified by the observed increases in adsorption in water compared to the porogens. The authors make a secondary conclusion that the polymer morphology cannot be solely responsible for the observed differences in polymers between polar and apolar solvents due to the accessibility of the functional monomers due to variation in polymer porosity.

The better performance of these polymers synthesised in the porogens exhibiting the lower dielectric constants and polarities suggest that not only does the stoichiometry of the solution play a role, but the environment in which it is synthesised. This feature is more evident in the polymers synthesised in MeCN. If the porogen is less polar than the component monomers and template, the polar components will aggregate inside the active polymerisation globule. The effect of this will likely be imprint sites
incorporating higher numbers of functional monomer than may be possible in more polar porogens.

The polymers synthesised in TOL display the greatest selectivity for the template molecule of all polymers studied, regardless of the functional monomer used in the synthesis. The MAA-co-EGDMA (TOL) polymer at 1:2 exhibits the greatest selectivity in both TOL and water, with significant adsorption of analyte from the eluent solution. There was little observed selectivity when the polymers were treated with CHCl₃, despite significant solvent adsorption.

When this polymer is synthesised in CHCl₃, selectivity is observed in TOL, CHCl₃, and highest in water (I=3.1), with no observation of selectivity in MeCN. The MAA-co-EGDMA polymer exhibits slight selectivity only when synthesised in CHCl₃, and rebound in H₂O. There may be selectivity possible in CHCl₃ and TOL, but observed error obscures the exact favourability of adsorption of template to the MIP over the NIP.

The MAM-co-EGDMA polymers are observed to show a large ability for template adsorption when synthesised in apolar media. Little selectivity was observed in any of these polymers with the exception of the toluene prepared 1:5 polymer. In MeCN, no selectivity and much lower adsorption was observed for both the arbitrarily selected and computationally designed stoichiometry in all solvents.

Differences in the binding affinities of the MAA and MAM formulations suggest that interactions between template and polymers differ. The MAA polymers are likely to involve both hydrogen bonding and ionic interactions, in combination with the hydrophobic effects generated by both the aromatic head of the EPH molecule and the hydrophobic bulk of the crosslinker. This is supported by the emergence of selectivity in the TOL synthesised, 1:5 MAM-co-EGDMA polymer eluted with TOL. The hydrophobic solvent must be responsible for the lower observed level of interaction with both the imprinted and non-imprinted polymer, suppressing significant non-selective adsorption. That imprinted cavities do exist in this polymer at the modelled ratio and not at the arbitrary stoichiometry is proven by the observation of selectivity in TOL.

Such a feature is supported by the emergence of selectivity in the TOL synthesised, 1:5 MAM-co-EGDMA polymer eluted with TOL. The hydrophobic
solvent molecule must be responsible for the lower observed level of interaction with both the imprinted and non-imprinted polymer, suppressing much of the non-selective component of the adsorption. That imprinted cavities do exist in this polymer at the modelled ratio and not at the arbitrary stoichiometry is proven by the observation of selectivity in TOL.

A similar outcome was noted for polymers synthesised with MAA or MAM with EGDMA in chloroform using SPE as the analysis vehicle (MAM polymers performing better than MAA polymers for the selective extraction of indomethacin from water) by Yang et al. \[242\]

As the interaction between the polymers synthesised using different functional monomers exhibit clearly different behaviours based upon differing interactions, it is likely that optimisation of their specificity would aim to reach the same goal – suppression of the non-specific binding – but would approach the goal from different vectors.

Selectivity has been observed for both modelled polymers, with a greater selectivity observed in the stoichiometries predicted in the modelling investigations than those polymers synthesised using the arbitrary stoichiometry. This was observed for both of the EPH modelled stoichiometries, the MAA-co-EGDMA 1:-2 using the apolar porogens and MAM-co-EGDMA polymers 1:5 utilising the TOL porogen and they are suggested as good candidates for optimisation of the recognition environments. Optimisation of the recognition environment is required to suppress the non-specific binding events that generate lower selectivity in the imprinted vs. non-imprinted system and thus increase the selective binding of the template molecules.
Chapter 6: Implementation of protocols optimising MIP performance in aqueous environments.

In water, the ability of hydrogen bonds to dominate the direct interaction between receptor and ligand is significantly reduced due to the ability for water itself to participate in large networks of hydrogen bonds. In the case of the MAA-co-EGDMA polymer and any ATS, the potential ionic interactions between the EPH amine unit and the carboxyl group will be affected by solution pH, and ionic strength. In the case of the MAM-co-EGDMA polymer that possesses no ionisable groups, the interplay of pH and ionic strength will not affect the structure of the polymer, but will only impact the EPH molecule at higher pH.

Pardo et al. demonstrated through molecular modelling that not only does water play a significant role in the solvation of both ligand and receptor, but that highly conserved water molecules can play a critical role in the structure and activation of these critical physiological receptors. These critical water molecules are located in the binding site, in the central, intracellular regions of the receptor.

Paradoxically, Renzoni et al. noted that in certain cases, bound water molecules can alter the surface of a binding site to the detriment of the ligand binding process, while in other circumstances, water serves as a critical determinant of receptor selectivity. The first of these two roles is described by Tame et al., in which an oligopeptide (OppA) (Figure 6.1) is able to bind small amino acid chains that exhibit almost any structural organisation of functional groups due to water-mediated binding processes. The second is described by Chung et al., where water molecules form a network in the active protein-binding site of tyrosine kinase, Src.

A combination of these effects was published by Quiocho et al., where X-ray diffraction of the crystalline L-arabinose (Figure 6.2) binding protein from bacteria showed the critical role played by water in filling a void that exists in the bonding of the endogenous ligand L-arabinose, but is not present in the binding of structurally similar sugars. In all cases, the orientation of the ligands and the number of direct interactions between receptor and ligand (9 H-bonds) is identical. But without these assisting water molecules, the binding constant is calculated to be at least an order of magnitude smaller than the endogenous ligand.
MIPs are not a precisely analogous environment to natural world enzymes and receptors. As the binding pockets of many natural receptors and enzymes are largely hydrophobic in character, they still incorporate water molecules and molecules solvated in aqueous environments. It is therefore not unrealistic to consider that similar behaviour may prevail in the cavities of polymers that are largely of hydrophobic character.

One of the goals of MIP technology is to produce synthetic, biomimetic receptors. Assumptions based upon the behaviour and structure receptors that bind to chemically similar ligands are therefore not out of place. A second key goal of a functional MIP technology is the ability to selectively sequester the imprinted species from solution. Finding middle ground between these two goals is essential in advancing this technology towards its potential practical applications.

If recognition based upon hydrogen bonding interaction is largely negated in the aqueous environment, the prominence of ionic, orbital and dipole/dipole electrostatic based interactions increase in importance. As a result, manipulation of the presence of ionic species in solution especially for ionisable polymer surfaces (both anionic and cationic), should tailor the recognition environment to reduce the non-specific surface binding interactions. Increased performance of MIPs has been observed in cases where a surfactant is added to the rebinding solution to manipulate the properties of the MIP surface.\textsuperscript{186}

A second approach to suppressing direct hydrogen bonding between receptor and ligand in aqueous solution is to use interactions that are essential to the biological performance of the natural world’s small molecule receptors – hydrophobic interactions. The GPCR family of receptors are transmembrane proteins that contain large areas of hydrophobicity and hydrophilicity. The proposed binding sites have been determined to exist in the hydrophobic area by homology modelling of the \textit{ala}-adrenergic receptor.
based on bovine rhodopsin, and is supported by the published crystal structure of the human $\beta_2$-adrenergic receptor.\textsuperscript{52, 240}

![Figure 6.2: Active site of L-arabinose binding protein showing the void filling and bridging water molecules. Red and green show the first and second binding-site shells\textsuperscript{240}]

Important to the production of an effective neurotransmitter-like receptor, (especially relevant for designing a synthetic, bio mimetic, amphetamine type receptor) is the powerful role that hydrophobic interactions clearly play under aqueous conditions that allows the appropriate translation of the molecule to achieve maximum binding efficiency.

Yu \textit{et al.} showed that a selective and specific MIP could be produced using neutral acrylamide as the functional monomer in an EGDMA matrix to selectively detect chiral amino acids in combined aqueous/organic liquids.\textsuperscript{168} For amides and carboxylic acids, a number of different hydrogen-bonding complexes have been studied in the gas phase where it was shown that that O-H···O and N-H···O hydrogen bonds are the strongest among all single hydrogen bond systems. Although the carboxyl group can form strong ionic interactions with basic functional groups, the hydrogen-bonding ability of this functional group is weak in polar solvents. MIPs prepared in polar porogens, using carboxylic acid based functional monomers and print molecules
capable of forming hydrogen bond interactions have often exhibited weak recognition or no recognition at all.

Unlike the carboxyl group the amide group (a non-ionisable species), may or may not be advantageous for recognition under aqueous conditions. Large differences exist in the dielectric constants and dipole moments of the amide and carboxyl groups (acetic acid: $\varepsilon = 6.20$, $\mu = 1.70$ D; acetamide: $\varepsilon = 67.6$, $\mu = 3.76$ D), indicating that the amide group may form stronger hydrogen bonds in water. As the MAM and MAA monomers differ in their ionisation abilities, manipulation of the pH of the rebinding solution is unlikely to produce an observable effect on template - MAM interactions, but may affect template - MAA polymer binding.

6.1 Optimisation of aqueous conditions for effective extraction of EPH by SPE.

6.1.1 Optimisation of MAA-co-EDGMA (1:2) copolymer prepared in chloroform.

Ionisable functional groups within an imprinted polymer are subject to physical modulation by their environment such as temperature, pressure, pH, ionic strength etc. As a result, the optimal state for their action can be influenced. The use of pH to probe the recognition characteristics of a propranolol imprinted MAA co EGDMA polymer was performed by Andersson et al. using a sodium citrate buffer. A pH range of 5-8 and ionic concentrations of above 5mM were shown to provide similar performance to the same experiments carried out in organic solvent. Addition of NaCl(s) degraded the ability of the polymer to adsorb the analyte, suggesting that the major factor in the binding events of polymers containing acidic monomers is an ionic attraction.

Examination of the effects of ionic strength on template recognition have also been investigated by Turner et al., and Chianella et al., who utilised SPE to optimise a MAA-co-EGDMA (1-2) binding for caffeine and microcystin L-R, and by Andersson et al who optimised the binding of a MAA-co-EGDMA polymer for the binding of propranolol. Because the MAM functional group is not affected by pH
change, they were excluded from these experiments, and a different method to decrease the effect of non-specific adsorption in these polymers is discussed later.

EPH-MAA 1:2 polymers were shown in previous studies to demonstrate superior analyte binding compared with the 1:4 MAA MIP formulation. As a consequence, only the 1:2 formulation was evaluated in this series of experiments. Furthermore, MIPs produced in hydrophobic porogens have also been observed to outperform those prepared in MeCN, hence MeCN polymers have been excluded from this scheme.

Potassium hydrogen phosphate solutions were prepared and calibrated using a pH meter. Across the pH range examined, the MAA carboxyl units are most likely to exist in a deprotonated state, as the pKa of the polymerised and non-polymerised MAA units is reported to be 6.7 – 6.8 and 4.65[252] - 4.66[253] respectively.[186] Similarly, the pKa of the ephedrine amino group has been reported to be 9.56[254], suggesting that it will exist almost exclusively in the protonated state at lower pH values (< pH 8).

The dominant form recognition between template and MIP is predicted to be a combination of ion pairing and hydrophobic interactions, both of which may be influenced by buffer pH and ionic strength.

Figure 6.3: pH profile of the aqueous binding ability of EPH-MAA 1:2 –co-EGDMA polymers synthesised in chloroform, at 50mM H₃PO₄[1-5p] in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).
In the most concentrated ionic solution, little template selectivity is observed (Figure 6.3). At pH 9, analyte adsorption is approximately 65% and shows the highest level of selectivity (I= 1.5). It is possible that this increase is due to the higher proportion of un-ionised template that may increase hydrophobic cavity binding interactions. Little or no selectivity was observed below pH 8, suggesting that the ability of the polymer to associate with the analyte is limited at high ionic strength.

Increased levels of template binding is noted with a reduction in buffer concentration to 25 mM (Figure 6.4), most notably at pH 9 where template removal peaks at 90%. The selectivity profile however remains broadly similar to the results observed in 50 mM buffer, with little selectivity observed below pH 8.

The general increase in template sorption across the pH range is consistent with improved ion pairing interactions in the weaker buffer solution. As the concentration of the ionic species in solution decreases, there must be a lower proportion of the potential sites for electrostatic interaction between template and polymer surface blocked by competing ionic species.
Figure 6.5: pH profile of the aqueous binding ability of EPH: MAA 1:2 –co-EGDMA polymers synthesised in chloroform at 10mM $H_3PO_4^{(x-3)^-}$ in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).

Template sorption is observed to further increase across the entire pH range as buffer ionic strength is further lowered to 10 mM and 5 mM respectively. Template selectivity is however similarly observed to diminish across the pH range (Figures 6.5 and 6.6).

Figure 6.6: pH profile of the aqueous binding ability of EPH: MAA 1:2 –co-EGDMA polymers synthesised in chloroform at 5mM $H_3PO_4^{(x-3)^-}$ in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).
It is unclear why the levels of template selectivity observed in earlier unbuffered trials were not observed under buffered conditions. Potentially the structure of the polymer is such that the incorporation of charged species creates significant swelling changes that prevent the binding of the analyte to the imprinted cavities, or at basic pH, the monolayer adsorption is replaced by multilayer adsorption onto the polymer surface. Cavity formation also potentially results in structures that are easily blocked by the presence of charged species in solution.

Modest imprinting effects were only observed at high pH and high buffer concentrations. The loss of imprinting effect with diminishing ionic strength at high pH suggests that recognition based on hydrophobic interactions may make existing ion pairing interactions become less effective. Higher amounts of analyte depleted above pH 6.5 must be related to higher proportions of deprotonated template present in solution as the pKa of the amine is approached.

Figure 6.7: pH profile of the aqueous binding ability of EPH: MAA 1:2 –co-EGDMA polymers synthesised in toluene at 50mM H₃PO₄(3)- in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).

The binding profiles generated by the EPH imprinted MAA-co-EGDMA polymer (toluene porogen) in 50 mM and 25 mM phosphate were found to be almost identical to
those generated by the same MIP prepared in chloroform. (*Figure 6.7 and Figure 6.8*). Little template selectivity is observed across the pH range, with template sorption increasing substantially at pH 9, again suggesting increased participation of hydrophobic binding interactions at high ionic strength.

In the toluene prepared MIP, a reduction in buffer concentration from 50mM to 25mM did not realise the significant increase (~50%) in the level of template sorption experienced by the chloroform MIP, suggesting that ion pairing interactions may be less important as a template recognition feature – particularly at low pH.

At 10mM buffer concentration (*Figure 6.9*), differences in polymeric structure become apparent, with selectivity improving across all pH values. At lower pH values, the non-specific binding is reduced below 20% of total potential analyte concentration suggesting that the ion pairing interactions are lower at these values. This result compares favourably with the chloroform MIP, where non-specific binding remains relatively high (approximately 40%).

![Figure 6.8: pH profile of the aqueous binding ability of EPH: MAA 1:2 –co-EGDMA polymers synthesised in toluene at 25mM H₃PO₄(x-3)- in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).](image)

This result suggests that polymerisation in toluene may result in the possible exclusion of non-associated functional monomer units such that non-specific interactions with analytes are reduced. Apolar conditions may promote dimerisation of
non-associated FMs, leading to a comparatively lower number of free carboxyl units in the resultant MIP.

It is also possible that the non-specific sites are located in such a way that they are suppressed by the ionic concentration at these pH values, while the cavities are able to interact more strongly with the reintroduced template. Although the experiments carried out under basic conditions continue to deplete the template from solution, the selectivity observed in these alkaline solutions is not as pronounced as it is under acidic conditions in 10 mM PO$_4^{3-}$ buffer. Interestingly, the selectivity is observed to decrease as the level of binding is increased. In this series, the greatest selectivity is observed at pH 7, with I = 2.3, which subsequently fall to 1.6 and 1.19 at pH 8 and pH 9 respectively. These results suggest that simply having the strongest interaction between analyte and template may not be sufficient to produce the highest level of selectivity possible, and that weaker interactions may promote higher rates of cavity binding rather than non-specific surface binding interactions.

![Figure 6.9: pH profile of the aqueous binding ability of EPH : MAA 1:2 –co-EGDMA polymers synthesised in toluene at 10mM H$_3$PO$_4$ (x=3) in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).](image)

In the 5mM buffer, binding selectivity is seen to further improve, reaching optimal selectivity (I = 3.7) at pH 6. The trend of increasing analyte adsorption with increasing solution pH is again repeated. It is of relevance to note that optimal binding
occurs in buffer conditions of low ionic strength at pH values that are close to the accepted pKa values of the MAA polymer. When solution pH is below the pKa of the polymerised MAA groups, a majority of the carboxyl groups remain protonated. As pH rises, the population of ionised carboxyl units increases, which sees only a marginal increase in MIP binding relative to NIP uptake. The low ionic strength of the 5 mM buffer suggests that ion pairing plays a role in MIP recognition. Adsorption of the template from solution increases as ionic strength is reduced (50 mM to 5 mM), suggesting that ion pairing interactions are replaced by hydrophobic interactions in a highly ionic solvent conditions.

Figure 6.10: pH profile of the aqueous binding ability of EPH: MAA 1:2 –co-EGDMA polymers synthesised in toluene at 5mM H₃PO₄[(x-3)]-in aqueous solution. Eluent solution is 1mL of 4mM EPH solution at the salt concentration and pH annotated. Error bars represent maximum observed variation from the sample mean for these experiments. (Number of repetitions=3).

The differing performance between the chloroform and toluene MIPs is an interesting highlight to the influence of porogen character on MIP performance. Previous studies have investigated the effect of porogen polarity and hydrophobicity on MIP character. The apolar nature of toluene in particular may promote greater aggregation of polar groups in the pre-polymer mix leading to improved FM : template interactions, while limiting low affinity site formation through dimer formation.

The work of Ansell et al. suggests that mobile phase modifiers can affect the different interaction sites in different ways. Acid modified mobile phases were
shown to increase the effectiveness of high affinity imprint sites, while low affinity binding was favoured in the case of amine modified mobile phases.

Combining this observation with that of Yu and Mosbach regarding the incorporation of higher T:FM stoichiometries inside the polymerisation globule via the use of the most hydrophobic solvent available, the divergent performance of identical polymers except for the identity of the porogen may explain why there is such a distinct difference in the performance of the polymers studied above.\textsuperscript{166} It is clear that the toluene synthesised polymer is able to interact better with the template at lower pH conditions, suggesting that a greater proportion of high affinity sites are present in the polymer. Binding affinity is however also linked to buffer concentration that indicates ion pairing is a contributing recognition factor.

\textbf{6.2. Optimised aqueous phase applied to template and non-template binding, recognition and selective adsorption.}

As the MIP synthesised in toluene demonstrates better performance in the aqueous system optimised above (pH 6, 5mM), the selectivity for the template species against other drugs of this polymer was investigated. It was expected that modification of the mobile phase would result in a much higher selectivity towards the template, and the amelioration of interaction between the non-template substances and the imprinted polymer.

The selectivity of the EPH-MAA 1:2 and EPH-MAM 1:5 polymers was assessed in 5mM aqueous H\textsubscript{3}PO\textsubscript{4}\textsuperscript{3-} at pH6. As the MAM polymer would not have been ionised it this scheme, nor would EPH have existed in a deprotonated state, the MAM polymers were excluded from the optimisation scheme due to their chemical nature. They were returned to the experimental scheme here, to investigate their competitive selectivity.

Following the elutions, UV-VIS spectrometry was used to quantify the amount of analyte remaining in the eluent solution, and thus the quantity of analyte adsorbed in each experiment. Table 6.1 shows the results of these adsorption experiments. The dangers of solely relying on the I values for MIP vs. NIP binding in assessing the performance of imprint systems is evident, for while the EPH-MAA system shows high
selectivity for the template when compared to the I values for the other drug classes, both MDMA and HER adsorb more strongly to the MIP than does the template. As the use of the NIP is simply a suggestion of the level of non-specific adsorption in the MIP, it is actually the competitive behaviour of the MIP that is important, and not the arbitrary I values calculated by comparison of MIP and NIP polymers. In both of these cases, there is a large associated adsorption to the non-specific polymer in the optimised aqueous phase, which produces I values showing that the polymer is selective only for the ATS. If however, the quantity of analyte adsorbed by the MIP in each case is used to directly compare the adsorption of the drug species to the MIP, it is clear that the MAA polymers do not adsorb only the template species.

Large levels of non-specific interaction are apparent between the NIP and MIP with MDMA, COC and HER (Table 6.1). Although the optimisation of the mobile phase specific to the interaction of EPH showed significantly decreased non-specific interaction at 5mM and pH6, the same is clearly not true for these other compounds. The variation in functional groups present on the drug molecules, in addition to the variation in the size and surface areas of the different molecules screened is likely to be the cause of the different adsorption behaviours observed, especially the non-specific adsorption. Although the non-specific adsorption of EPH is slightly decreased at pH6 in the 5mM ionic solution, it is obvious that this environment does not decrease the non-specific adsorption of either COC, HER or MDMA to the EPH-MAA 1-2 polymer.

The observed adsorption behaviour of the EPH-MAA 1-2 polymer is similar to that predicted by the AM1 in silico screening of the pseudo receptor cavities, which demonstrated that HER, COD and MOR display similar estimated interaction energies. EPH appears to interact with the MIP least out of the screened compounds. The AM1 screening results suggest that MDMA, and opiate species interact more strongly with the functional monomer cluster. Table 6.1 shows that the EPH-MAA 1:2 polymer exhibits highest I value (calculated from Equation 1.1) however it does not demonstrate the highest affinity for the MIP. MDMA shows greater affinity for the MIP than the template, while COC exhibits a lower magnitude of interaction with the MIP, as predicted by the AM1 models. Lower I selectivity values are due to significantly greater NIP binding magnitudes for the non-template analytes. The relationship shown in this table highlights clearly that the utility of the computational scheme for
investigating the potential for MIP discrimination of the template over related and unrelated molecules. The template clearly has a shape effect on the polymer structure, as although HER and COC adsorb to the MIP, they show an equal or greater affinity (to that exhibited between the MIP and these non-template molecules) for the non-imprinted control.

It would appear that the AM1 level of theory is a better model than the CHARMM simulation approach in this case as COC is predicted by this calculation to exhibit the greatest interaction with the imprint site. However, the CHARMM simulations that involve the incorporation of the solvent dielectric constant to implicitly model the solvent environment (Figure 3.10), predict the stronger interaction of HER, MDMA and COC with the E-MAA 1:2 cluster in the aqueous phase. This outcome appears to suggest that although the use of molecular mechanics does not generate predictions of real world association in solution phase for cross selectivity, the incorporation of the solvent dielectric constant term increases the reliability of the fast screening technique. Although COC is predicted to exhibit a slightly stronger interaction with the imprint site, the lower adsorption magnitude may be indicative of a shape selective effect preventing this interaction taking place in the imprint cavity.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Analyte</th>
<th>[B].MIP (mM)</th>
<th>[B].NIP (mM)</th>
<th>$\frac{[B]<em>{MIP}}{[B]</em>{NIP}}$</th>
<th>MIPvNIP SI</th>
<th>MIP ONLY SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH- MAA 1:2</td>
<td>EPH</td>
<td>2.175</td>
<td>0.586</td>
<td>3.71</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>2.813</td>
<td>2.525</td>
<td>1.11</td>
<td>0.300</td>
<td>1.294</td>
</tr>
<tr>
<td></td>
<td>COC</td>
<td>1.796</td>
<td>1.927</td>
<td>0.93</td>
<td>0.251</td>
<td>0.826</td>
</tr>
<tr>
<td></td>
<td>HER</td>
<td>2.357</td>
<td>2.839</td>
<td>0.83</td>
<td>0.224</td>
<td>1.084</td>
</tr>
<tr>
<td>EPH- MAM 1:5</td>
<td>EPH</td>
<td>2.712</td>
<td>1.393</td>
<td>1.95</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>2.440</td>
<td>2.304</td>
<td>1.06</td>
<td>0.544</td>
<td>0.899</td>
</tr>
<tr>
<td></td>
<td>COC</td>
<td>1.225</td>
<td>0.763</td>
<td>1.61</td>
<td>0.825</td>
<td>0.452</td>
</tr>
<tr>
<td></td>
<td>HER</td>
<td>2.238</td>
<td>1.621</td>
<td>1.38</td>
<td>0.709</td>
<td>0.825</td>
</tr>
</tbody>
</table>

The MAM polymers are observed to exhibit a less specific MIP v NIP value for EPH selectivity (1.95 v 3.71) in the optimised phase. Although this value is smaller than for the EPH MAA 1-2 polymers, the value is reported as smaller due to greater non-specific interaction as larger amounts of template are adsorbed to both the MAM MIP and its associated NIP. For all analytes, the MAM 1:5 MIP adsorbs more of each
species than does its associated NIP, which results in I values of $\geq 1$ in all cases. The MIP only selectivity is however greater for the ATS, over COC and HER, however the amount of HER adsorbed is quite close to that of MDMA.

The AM1 predictions made in Figure 3.22 suggest that in the gas phase, the ATS will interact more strongly with the imprint cluster than the template, as will HER, while the interaction between COC and the MAM cluster will be largely similar to that of EPH. The CHARMM gas phase interaction estimations suggest that EPH will interact more strongly with the cluster than the remaining analytes.

Although there is a lack of crosslinker in the receptor cavity model, which may explain why the template displays a greater affinity for the MIP than the other analytes that are predicted to exhibit a greater interaction with the site. This shape selectivity for ATS may also explain why given that MDMA and HER are predicted to have similar interaction magnitudes, MDMA is adsorbed more readily than HER given its structural similarity to EPH.

The CHARMM calculation predicts greater interaction between EPH and the site than the remaining compounds; however MDMA is predicted to interact more weakly with the imprint site than HER and COC, which is not demonstrated by the wet experiments.

Comparison of the adsorption of drug species in aqueous solution and the predictions generated by the CHARMM calculations using GBSW method agree well with each other. MDMA is predicted to interact more strongly with the cavity in the computational investigation; however the size of the head group may be excluded from the cavity due to the cross linking monomer influence that is not included in the model. Similarly, the predicted interaction between the monomer imprint site and COC and EPH is virtually identical in magnitude, yet the physical outcome of the experiments shows that COC interacts with the EPH- MAM MIP least of the analytes compared. The only possible explanation for this is the size exclusion effect produced by the incorporation of the cross linking monomer. This was the issue identified in the in silico screening process, whereby although interaction is estimated between a ligand and the monomer cluster, the addition of cross linking monomers to the local environment of the imprint site may create additional size exclusion features that are undefined in the functional monomer cluster models created in Chapters 2 and 3.

Many applications of the principle of the indicator displacement assay technique have been realised and published in a variety of different areas. Initially, an indicator molecule is first allowed to reversibly bind to the receptor site. Upon the introduction of a competitive analyte to the receptor, the indicator molecule is displaced, which modulates the optical signal. This signal may be of a number of mechanistic types: electron energy transfer \[^{256, 257}\], photo-induced electron transfer \[^{258, 259}\], fluorescence resonance energy transfer \[^{260}\], or changes in the ionic concentration or pH \[^{261}\] of the environment in which the receptor exists.

Indicator displacement assays provide a number of advantages over the indicator-transducer-receptor type sensor. There is no need for the covalent linkage of the indicator to the receptor, and because of this, a number of different indicator molecules may be applied in conjunction with one receptor to provide a range of responses.\[^{262}\] Additionally, the technique is equally well suited to application in organic media and aqueous solution, and can be tailored to the sensing of a variety of compounds quickly and easily.\[^{263}\]

As far as biological applications of indicator displacement, a qualitative probe for the evaluation of ligand/RNA complexation utilised a substituted xanthenone substance to probe the behaviour of mRNA associated with HIV-1 replication and infection.\[^{264}\] Anslyn et al. have provided guidance in the creation of these types of systems, and the considerations required for their implementation, specifically the relative levels of interaction between indicator and host, as well as between analyte and host. Their study in this case related to the implementation of a colourimetric assay for the detection of citrate.\[^{265}\]

Indicator displacement assays have been applied in the quest for chiral chromatographic resolution in supramolecular systems \[^{266}\] which produced a colour change dependent on the recognition of the adroption of the enantiomers of phenylglycidol.
Shimizu and Greene published their use of Dye A (Figure 6.11) as a selectively displaced indicator of recognition by the specific sites of the imprinted polymer for a series of structurally related, small molecule amines in acetonitrile.\textsuperscript{[15]} Their analysis however required complex, linear discriminant analysis to determine the identity of these closely related species, which is of limited utility to a simple presumptive identification method. In addition, the personal safety issues associated with the use of organic solvent as the media for recognition does not remedy the current factors related to the safety of the colourimetric tests.\textsuperscript{[1]} While the method is unsuitable as a viable visual field test for structurally similar amines, its viability as a discriminatory presumptive test for the presence of ATS may be viable. Adaption to an aqueous test regime (from a batch rebinding method in MeCN) is appealing because of the elimination of organic solvents and the associated dangers of volatility, flammability and toxicity that is especially relevant given the metabolite of MeCN is the highly toxic cyanide anion.

This experimental scheme was intended to be a qualitative assessment of the capability of the designed system to discriminate between the template, analogous compounds and other potentially encountered substances in a realistic field setting. It was similar in intent to the work of Zhang and Umemoto in 2010 to indicate the binding of the ligand RNA complex.\textsuperscript{[264]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6_11.png}
\caption{Gas phase equilibrium geometry of A) N,N-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine (DYE A) and B) N,N'-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine (DYE B) showing the density potential surface, SPARTAN\textsuperscript{84}, AM1 level theory.}
\end{figure}

An analogue (Dye B) of the dye used by Shimizu and Greene (Dye A) was run in parallel to their dye in an attempt to determine the effect of increased interaction
between the dyes and imprint cavity. The effect this would have on the dyes ability to be displaced by the re-introduction of the template and its analogues (ATS), compared to other compound classes, cocaine and opiate type substances was of interest. It was predicted that greater interaction strength between the competitively displaced indicator and the synthetic receptor would result in a lower amount of dye displaced. It is likely that stronger binding interaction between dye and imprint cavity would result in a greater level of analyte discrimination, because only molecules with reciprocal functionality to the cavity will exhibit the high affinity interaction. Only molecular species exhibiting the high affinity interactions within the polymer cavity will be capable of displacing the dye from these imprint sites. Thus, the technique aims to utilise this approach to reduce the level of non-specific adsorption and within polymer cavities, limit competitive binding of analytes that do not share analogous structure with the template.

The analysis wavelength was not necessarily that which has the highest molar extinction coefficient (the lowest $\lambda$) due to the need to ensure that the absorption is not impeded by the drug molecules used.

Table 6.2: Calculated molar extinction coefficients for Dye A and Dye B. (Figures 8.2 and 8.3)

<table>
<thead>
<tr>
<th>Dye</th>
<th>Solubility limit</th>
<th>$\lambda$</th>
<th>Molar extinction Coefficient $\log_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediameine</td>
<td>127.2 mg.L$^{-1}$</td>
<td>214</td>
<td>3.93</td>
</tr>
<tr>
<td></td>
<td>5.062 x 10$^{-4}$M</td>
<td>350</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>459</td>
<td>3.00</td>
</tr>
<tr>
<td>DYE A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N,N’-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediameine</td>
<td>129 mg.L$^{-1}$</td>
<td>204</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>5.134 x 10$^{-4}$M</td>
<td>302</td>
<td>3.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>468</td>
<td>3.89</td>
</tr>
<tr>
<td>DYE B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incorporating the indicator displacement scheme into the SPE technique in the optimised environment (Table 6.2), shows the EPH- MAM 1:5 polymer outperforms the MAA polymer in discriminating between the ATS and the other drug classes. Although the MIP vs. NIP selectivity for the MAA 1:2 system demonstrates greater displacement for the ATS over the other drug classes (due to substantially greater displacement of dye from the NIP surface, the comparison of the MIP only values suggests that the
optimised aqueous environment produces greater association of all other molecules with the 1:2 MAA MIP than the template. The specific displacement portion of the MIP recognition of the template binding can be isolated by the following expression:

\[
\text{Disp}_{\text{specific}} = [d]_{\text{MIP}} - [d]_{\text{NIP}}
\]

\textbf{Equation 6.1}

Where \([d]_{\text{MIP}}\) is the amount of dye displaced from the MIP, and \([d]_{\text{NIP}}\) is the quantity of dye displaced from the NIP.

By further comparing the specifically displaced magnitudes of dye from each analyte, the EPH- MAA 1:2 system shows greater response to the recognition of MDMA than the template, minimal response to COC introduction, and the template outperforming HER by 2 fold.

The MAM polymers show slightly lower MIP vs. NIP selectivity than the MAA 1:2 polymers, however in a similar trend in the results for the simple adsorption experiments is observed with the MIP demonstrating preferential binding for the ATS over other drugs. This is evident both with a comparison of the \(d\) [MIP]/\(d\) [NIP] values, and also the displacement selectivity calculated based solely upon the behaviour of the displaced dye and the MIP alone.

\textbf{Table 6.3: Displacement of DYE A from toluene synthesised MIP in optimised buffer for EPH recognition. @350nm. (Number of repetitions=3)}

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Analyte</th>
<th>([d]) MIP ((\mu)M)</th>
<th>([d]) NIP ((\mu)M)</th>
<th>(\text{Disp}<em>{\text{spec}}) (\frac{[d</em>{\text{MIP}}]}{[d_{\text{NIP}}]}) ((\mu)M)</th>
<th>(\frac{I_{\text{MIP}}}{\text{NIP}})</th>
<th>MIP(\times)NIP SI</th>
<th>MIP SI</th>
<th>Cavity displaced SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA 1:2</td>
<td>EPH</td>
<td>13.35 ± 0.61</td>
<td>0.87 ± 0.73</td>
<td>12.48</td>
<td>15.31</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>MDMA</td>
<td>19.53 ± 0.24</td>
<td>0.51 ± 0.36</td>
<td>19.02</td>
<td>38.38</td>
<td>2.51</td>
<td>1.46</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td>COC</td>
<td>24.74 ± 1.94</td>
<td>54.42 ± 3.51</td>
<td>-29.68</td>
<td>0.46</td>
<td>0.03</td>
<td>1.85</td>
<td>-2.38</td>
<td></td>
</tr>
<tr>
<td>HER</td>
<td>48.24 ± 6.06</td>
<td>40.97 ± 10.30</td>
<td>7.269</td>
<td>1.18</td>
<td>0.08</td>
<td>3.61</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>MAM 1:5</td>
<td>EPH</td>
<td>58.66 ± 1.09</td>
<td>5.48 ± 2.30</td>
<td>53.19</td>
<td>10.71</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>MDMA</td>
<td>38.43 ± 0.61</td>
<td>8.14 ± 1.09</td>
<td>30.29</td>
<td>4.72</td>
<td>0.44</td>
<td>0.66</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>COC</td>
<td>6.809 ± 0.24</td>
<td>34.9 ± 4.48</td>
<td>-28.11</td>
<td>0.20</td>
<td>0.01</td>
<td>0.12</td>
<td>-0.53</td>
<td></td>
</tr>
<tr>
<td>HER</td>
<td>28.86 ± 5.45</td>
<td>24.50 ± 1.45</td>
<td>4.362</td>
<td>1.18</td>
<td>0.11</td>
<td>0.49</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>
Subjecting these values to EQN 5.1, the recognition of EPH results in the displacement of twice as much cavity bound dye than MDMA, and 10 fold more than HER. COC is not observed to displace any dye from the imprint cavities.

From this screening of the selectivity of the designed MIP system, it is clear that the EPH-MAM 1:5 polymers perform better than the similarly designed EPH-MAA 1:2 system. Although MIP v NIP selectivity was assessed to be greater for the template in previous experiments for the EPH-MAA 1:2 as opposed to the EPH-MAM system in both organic and aqueous environments, the ability of this polymer to adsorb not only the template but also other illicit drug molecules suggests that although the interactions are weaker in the pre-polymerisation solution between MAM and EPH, in aqueous solution, the discrimination ability of the MAM polymer is better. This feature is confirmed not only by the adsorption experiments, but also the pre-loaded dye displacement scheme. Although the EPH-MAA 1:2 polymer demonstrates the template and its analogues producing greater response from the displacement scheme in comparison to HER and COC, the magnitude of this differentiation is only 2 fold between EPH and HER. The EPH-MAM 1:5 polymer demonstrates a 10 fold larger specific displacement upon the introduction of the EPH in comparison to HER.

COC is observed to displace more DYE A from the surface of the NIP than the MIP. This may be due to the orientation of the dye molecules between the MIP and NIP. In the case of the MIP, the chromophore head of the dye molecules will be directed into the bulk solvent as the only available area for interaction with the imprint cavity due to the steric crowding (induced by the templating effect of ephedrine) is the aliphatic tail of the molecule. This will in turn effect the orientation of the dye molecules interacting non-specifically with the MIP surface. There may be some interactions (hydrophobic, Van-Der Waals’, π-π stacking) between these large and planar aromatic moieties that reduces the amount of non-specifically adsorbed dye molecules which interact with the MIP surface favourably. Thus there is less specifically adsorbed dye to be dispersed into the bulk solution through displacement by COC. In the NIP, no such orientation can possibly exist, as there are no cavities formed.
Table 6.4: Displacement of DYE B from toluene synthesised MIP in optimised buffer for EPH recognition. @302nm (Number of repetitions=3)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Analyte</th>
<th>[d] MIP (μM)</th>
<th>[dp] NIP (μM)</th>
<th>Disppspec</th>
<th>I MIP NIP</th>
<th>MIPvNIP SI</th>
<th>MIP SI</th>
<th>Cavity displaced SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA1:2</td>
<td>EPH</td>
<td>16.23 ± 2.97</td>
<td>12.60 ± 3.30</td>
<td>3.63</td>
<td>1.29</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>30.75 ± 8.25</td>
<td>13.26 ± 3.96</td>
<td>17.49</td>
<td>2.31</td>
<td>1.80</td>
<td>1.89</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>COC</td>
<td>7.99 ± 3.30</td>
<td>5.01 ± 4.29</td>
<td>2.97</td>
<td>1.59</td>
<td>1.24</td>
<td>0.49</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>HER</td>
<td>25.47 ± 15.51</td>
<td>56.82 ± 7.92</td>
<td>-31.35</td>
<td>0.45</td>
<td>0.35</td>
<td>1.57</td>
<td>-8.64</td>
</tr>
<tr>
<td>MAM 1:5</td>
<td>EPH</td>
<td>55.83 ± 4.95</td>
<td>16.56 ± 2.31</td>
<td>39.27</td>
<td>3.37</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>25.47 ± 2.64</td>
<td>14.58 ± 1.32</td>
<td>10.89</td>
<td>1.75</td>
<td>0.52</td>
<td>0.46</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>COC</td>
<td>5.68 ± 1.65</td>
<td>9.31 ± 0.99</td>
<td>-3.63</td>
<td>0.61</td>
<td>0.18</td>
<td>0.10</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td>HER</td>
<td>1.33 ± 2.97</td>
<td>24.48 ±1.65</td>
<td>-11.22</td>
<td>0.54</td>
<td>0.16</td>
<td>0.24</td>
<td>-0.29</td>
</tr>
</tbody>
</table>

Variation of the methylation position on the tail segment of the dye molecule, (Table 6.4) predicted to be the location of the interaction with the imprint site due to its similarity with the ATS phenylethylamine base structure showed similar displacement outcomes to the use of DYE A. The MAM MIP demonstrates better selectivity in relation to the reporting of ATS MIP than the MAA polymer. The relative displacement from the MIP by HER and MDMA is greater than that observed for EPH. Although the cavity specific displacement is higher for both ATS than the other two compounds, the MIP v NIP selectivity shows COC to be more favourable than the template.

The MAM polymers again show that not only is the template responsible for the displacement of larger amounts of dye than the other compounds, but that there is a significant class selectivity shown for this polymer, allowing less selective recognition of template analogue binding. Although the response is only half that of the template, it is still observed to be 2 fold greater for the template relative to the other drug classes examined.

Table 6.5: Estimated drug molecules affinities, relative to Dye A and Dye B for EPH imprint site models from Chapter 3.

<table>
<thead>
<tr>
<th>E-MAA 1:2 Dye A</th>
<th>E-MAA 1:2 Dye B</th>
<th>E-MAM 1:5 Dye A</th>
<th>E-MAM 1:5 Dye B</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH</td>
<td>0.027</td>
<td>0.012</td>
<td>0.010</td>
</tr>
<tr>
<td>MDMA</td>
<td>0.933</td>
<td>0.932</td>
<td>0.067</td>
</tr>
<tr>
<td>COC</td>
<td>0.182</td>
<td>0.170</td>
<td>0.097</td>
</tr>
<tr>
<td>HER</td>
<td>2.272</td>
<td>2.291</td>
<td>3.140</td>
</tr>
</tbody>
</table>
Comparison of the predicted interaction between the benzoxadiazole dye molecules and receptor site and the drugs screened show marked differences in the relative affinity for the imprint site. All drug molecules exhibit a greater estimated interaction with both of the modelled imprint cavities (Table 6.5). However the relationship displayed in Tables 6.3 and 6.4 shows a lack of agreement between the experimental values obtained for the dye displacement from both MIP systems involved and the simulations carried out. This result suggests that although direct interaction with the imprint site is predicted by the Wu and Li method, the potential for shape discrimination must be controlled by the crosslinking monomer that is not present in the model systems. Thus, until a good model of complete interaction sites including the effect of cross linker is developed, such modelling endeavours will be able to simply predict whether such molecules will be able to interact with the functional monomers of the imprint site.

There are unresolved experimental issues in the method utilised above. The amount of dye adsorbed to the surface of the MIP and NIP is uncontrolled and visually unequal between the MIP and NIP, for although exactly the same amount of dye solution was passed through each SPE cartridge, the MIP and NIP clearly exhibit different abilities to interact with every other species introduced. Although the amount of dye displaced during the elution may be calculated via its UV-VIS absorption, the initial amount of dye adsorbed to the surface may be different. The very small amount of dye released by the elution of the compounds through the loaded SPE cartridges presents some issues related to the determination of low magnitudes of displacement. If small deviations in the amount of dye non-specifically adsorbed to the polymers are present, such weakly adsorbed species may be easily displaced upon the introduction of any analyte, thereby confounding the presumptive identification of a molecule analogous to the template upon recognition by the MIP. Such situations may explain why the introduction of COC to the MIP and NIP results in more dye being released from the NIP than the MIP, which is not observed upon the introduction of any other analyte to the MIP/NIP pair.

Despite the optimisation of the pH and salt concentration of the aqueous environment for the recognition of EPH, the EPH- MAM MIP outperformed the MAA MIP in its ability to discriminate between template analogues and non-related species.
The high level of non-specific interaction particularly of HER with the MAA polymer means that the discrimination ability of this polymer is significantly lower than the MAM polymer.

**6.4 - Equilibrium based selective dye displacement technique**

In the environment tuned for the recognition of EPH by the 1:2 MAA MIP, the E-MAM 1:5 MIP was demonstrated to possess a better ability to discriminate between the adsorption of molecules analogous to the template’s structure from other common illicit drugs. Although the non-specific adsorption of EPH was significantly lower for the MAA 0:2 NIP, the associated lack of selectivity observed in the adsorption experiments suggested that the selective interactions between EPH and the MIP and NIP pair had been hindered by the ionic species in solution. The behaviour of the molecules that the polymer had been intended to discriminate between was far lower than originally expected given the reduction in the non-specific interaction achieved in the optimisation of the pH and salt concentration of the aqueous phase.

The MAM polymer demonstrated greater adsorption of the template, and was able to discriminate between the template and its analogues to a far greater extent, even though the non-specific binding observed was greater than the MAA 1:2 polymer. The indicator displacement protocol carried out using the SPE method generated far better discrimination of the template and its analogues using the E-MAM 1:5 polymer in comparison to the E-MAA 1:2 polymer.
To overcome the potential influence of variation in the amount of dye initially present in the polymer systems through the immobilisation method used above, a competitive equilibrium method was constructed. By controlling the amount of dye initially present in the system and ensuring that the concentration of dye in the analysis solution was above the limit of detection, it was possible to track the displacement of the dye from the surface.

As the preloading of the dye followed by removal of as much non-specifically adsorbed indicator left some uncertainty regarding the initial amount of dye present on the polymer surface, a more controlled experiment was desired. The amide polymer performed better than the MAA polymer in the SPE indicator displacement scheme, and further investigation of this behaviour was desired. Initial experiments using a UV-VIS instrument with a fibre optic “dip-probe” proved unsuccessful due to the polymer particles impinging upon the light path and creating obscuration of the analytical wavelength associated with the dye.
Cellulose dialysis sacks were prepared 24 hours prior to use, and the experiment was set up in triplicate according to the schematic displayed in Figure 6.12. As the pores in these sacks are designed to allow the diffusion of anything below 12000 Daltons, they offer no impediment to the gradient driven diffusion of the dye molecules or the analyte molecules added into the system. They effectively sequester the polymer particles from the supernatant – making the removal and re-addition of aliquots for UV absorption measurement a simple process. Both benzoxadiazole dyes demonstrated 3 absorbance maxima at similar wavelengths, all of which produced linear calibration functions (Figure 8.2, 8.3). The molar extinction coefficients for these wavelengths are displayed in Table 6.2, along with the experimentally derived solubility limits for these dye compounds A and B.

Elevated levels of non-specific binding are a direct result of increased levels of attraction between polymer and ligand. By allowing the indicator to come to equilibrium before it is displaced, the analyte is likely to either not adsorb to the non-specific sites due to saturation of the surface prior to the addition of the analyte, or to adsorb to the non-specific sites of the polymer in a multilayer over the adsorbed dye molecules without the need to displace them. Thus, the MIP may display a greater selectivity than may be possible with a virgin polymer surface. Through manipulation of a different feature of the polymer using a similar idea as the use of ionic species to mediate this non-specific interaction, presumptive identification of amphetamine type substances in aqueous media should be possible.

To overcome the unknown amount of dye involved in the system before the introduction of the analyte of interest, which was identified as a problem for the reliability of the displacement assay using SPE in 6.1, a known amount of indicator was added to a known amount of dry polymer in a measured total system volume. Due to overloading of the polymer surface by the dye that takes place before the addition of the analyte, a determination of the ability of sequential additions (Figure 6.13), and equilibration time after the addition was desired to determine the total amount of analyte displaced by a large excess of template (25mg, 1mg.mL^{-1} of solution). In this experiment, after the initial dye/polymer equilibrium is disturbed by the dissolution of template into the solution a second competitive equilibrium is established.
Figure 6.13: Sequential addition of EPH to Dye A and EPH imprinted 1:5 MAM-co-EGDMA polymer equilibrium.

Approximately $2.0 \times 10^{-5}$ mol is displaced by this addition, which presumably displaces all dye from the imprinted cavities as no further rapid displacement of indicator into the supernatant is observed following the repeated addition of similar quantities of template, confirming that the displacement of the dye occurs only from the imprinted cavities, and not the non-specific surface. Due to the incorporation of the dye into the cavity, and its subsequent displacement, and the disparity in size of the head groups of both the dye and template that prevents the interaction between imprinted cavity and this group, the interaction must involve the area of similarity between the molecules, the aliphatic amine group.
The absorbance of dye in solution, when the original equilibrium position is compared between MIP and NIP (Figure 6.14) shows a much lower concentration in solution for the MIP, which can be associated with greater adsorbance of the dye onto the polymer surface. This interaction must be associated with cavity-based uptake (in the MIP), as the only difference between the polymers is the presence of the template in
the MIP formulation. The variation of the methyl substitution on the amine tail of this molecule results in a different distribution of bound and unbound dye at equilibrium.

There remains a significant difference in the amount of dye displaced by the addition of the template (1mg.mL⁻¹), however there appears to be significantly greater competition between the template and the dye for the imprinted cavities (Figure 6.16). Reduced steric hindrance in the structure of Dye B (relative to Dye A) leads to stronger binding within the imprint site. This results in a higher level of interaction between dye molecule and imprint cavity, due to the accessibility of the charged site for interaction with the constrained receptor cavity. The I values for these dyes, displaced by EPH in aqueous solution are A = 4.71 and B = 3.37. As a result, Dye A performs better than Dye B for the recognition of EPH in water.

The ability of the indicator displacement assay to effectively discriminate between molecules of similar and dissimilar structure was assessed by investigating the response of the equilibrated dye system (MIP and NIP) system towards COC, MDMA and HER. The results of the displacement from the MIP and NIP (Figure 6.16-6.21) show that all compounds displace the dye from the surface of the polymer, with maximum template displacement observed for addition of MDMA to the system (Table 6.4). Calculation of the imprinting factor, in the same fashion as the previous experiments, results in an imprinting factor of 4.71 for EPH, and 4.7 for MDMA, in comparison to the imprinting factor for COC of 0.31, and 0.18 for HER (Table 6.6). The imprinting factors demonstrate that selectivity for ATS is an order of magnitude higher for amphetamines relative to other illicit substances. MDMA displaced greater quantities of dye from both the MIP and NIP polymers relative to EPH, indicating high affinity for the polymer surface. Displacement is likely attributed to the presence of the electron rich, oxygen containing methylenedioxy unit, which would be expected to associate closely with the likewise oxygen rich EDGMA crosslinker. The presence of this group would however, be expected to limit access of MDMA to the EPH cavities on the basis of greater steric bulk.
Table 6.6: Displacement of Dye A by drug molecules. (Number of repetitions=3)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$\Delta_{\text{disp}}$ MIP (µmol)</th>
<th>$\Delta_{\text{disp}}$ NIP (µmol)</th>
<th>$\Delta_{\text{d,MIP}}/\Delta_{\text{d,NIP}}$</th>
<th>$\text{SI}_{\text{disp}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH</td>
<td>11.99</td>
<td>2.54</td>
<td>4.71</td>
<td>1</td>
</tr>
<tr>
<td>MDMA</td>
<td>17.08</td>
<td>3.63</td>
<td>4.70</td>
<td>0.997</td>
</tr>
<tr>
<td>Cocaine</td>
<td>8.24</td>
<td>26.31</td>
<td>0.31</td>
<td>0.066</td>
</tr>
<tr>
<td>HER</td>
<td>6.18</td>
<td>34.31</td>
<td>0.18</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Figure 6.16: Concentration of Dye A in solution vs. time for Eph imprinted 1:5 MAM-co-EGDMA. Analyte spiked at 120 minutes. Absorbance values were read at 350nm to avoid obscuration of the absorbance spectra by the analyte. (Number of repetitions=3)
Figure 6.17: Concentration of Dye A in solution vs. time time for Eph imprinted 1:5 MAM-co-EGDMA. Analyte spiked at 120 minutes. Absorbance values were read at 350nm to avoid obscuration of the absorbance spectra by the analyte. (Number of repetitions=3)

Figure 6.18: Concentration of Dye A in solution vs. time time for Eph imprinted 1:5 MAM-co-EGDMA. Analyte spiked at 120 minutes. Absorbance values were read at 350nm to avoid obscuration of the absorbance spectra by the analyte. (Number of repetitions=3)
The template rapidly displaces the indicator from the cavities only, while the larger molecules with additional functional groups for interaction with the polymer surface are more able to displace the non-specifically adsorbed dye molecules. The selectivity of the polymers being used in this mode was assessed in the same fashion as the earlier experiments, where;

\[ I = \frac{B_{MIP}}{B_{NIP}} \]  

Equation 6.2

As the amount of dye displaced is a suitable indicator of the recognition/binding event that takes place, the ratio of the amount of indicator displaced from the MIP and the NIP will also generate a selectivity factor (Table 6.7) where:

\[ I = \frac{\Delta_d\text{.MIP}}{\Delta_d\text{.NIP}} \]  

Equation 6.3

Normalisation of this factor against the template shows a significant bias in detecting binding events within the imprinted cavities, by molecules that share a base structure with the template. This increased selectivity is not only far less time consuming to document, but the consumables and complexity associated with such assessments in comparison to HPLC quantification of binding are significantly reduced.

This disparity of a non-template molecule generating a larger amount of indicator than the template must be viewed in tandem with the displacement from the NIP, where the non-specific displacement by MDMA is also greater than that displayed by the template.

**Table 6.7: Displacement of Dye B by drug molecules. (Number of repetitions=3)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>( \Delta_{\text{disp}} \text{.MIP} \text{ µmol} )</th>
<th>( \Delta_{\text{disp}} \text{.NIP} \text{ µmol} )</th>
<th>( \Delta_{d,\text{MIP}}/\Delta_{d,\text{NIP}} )</th>
<th>SI_{\text{disp}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH</td>
<td>13.02</td>
<td>3.86</td>
<td>3.37</td>
<td>1</td>
</tr>
<tr>
<td>MDMA</td>
<td>13.92</td>
<td>9.31</td>
<td>1.50</td>
<td>0.444</td>
</tr>
<tr>
<td>HER</td>
<td>6.34</td>
<td>8.98</td>
<td>0.71</td>
<td>0.209</td>
</tr>
<tr>
<td>Cocaine</td>
<td>9.67</td>
<td>33.75</td>
<td>0.29</td>
<td>0.085</td>
</tr>
</tbody>
</table>

The variation of a single methyl group on the tail of Dye A produced a large change in the observed results of the competitive displacement scheme. It was expected that the variation of the terminal amine group on the tail of the indicator from a tertiary
amine to a secondary would result in stronger binding (due to no steric hindrance for entering the imprint cavity, and therefore closer alignment with the cavity functionalities). Initially, there appears to be a greater concentration of dye present in the solution. The equilibrium concentration of dye appears to differ between the different series in the experiment, and no plausible explanation for this could be determined. What remains important is the change in the concentration of free dye upon the addition of analyte to the system, which if the dye actually binds to the selective cavities, can be associated directly with the recognition of a molecule analogous to the template by the imprinted cavity (Table 6.7).

Figure 6.19: Concentration of Dye B in solution vs. time for Eph imprinted 1:5 MAM-co-EGDMA. Analyte spiked at 120 minutes. Absorbance values were read at 350nm to avoid obscuration of the absorbance spectra by the analyte. (Number of repetitions=3)
Figure 6.20: Concentration of Dye B in solution vs. time for Eph imprinted 1:5 MAM-co-EGDMA. Analyte spiked at 120 minutes. Absorbance values were read at 350nm to avoid obscuration of the absorbance spectra by the analyte. (Number of repetitions=3)

Figure 6.21: Concentration of Dye B in solution vs. time for Eph imprinted 1:5 MAM-co-EGDMA. Analyte spiked at 120 minutes. Absorbance values were read at 350nm to avoid obscuration of the absorbance spectra by the analyte. (Number of repetitions=3)
Although the initial equilibrium position sits at a higher value for the amphetamine type substances, the concentration of dye displaced from the imprinted polymers by the analytes is not significantly different from the concentrations displaced in the earlier investigation with one key difference. The displacement of Dye B by cocaine from the non-imprinted polymer (Figure 6.21) is greater than the displacement of the indicator from the MIP by the template. The reason for this increased displacement from a non-imprinted surface in this scenario and not the previous is unclear, as the larger opiate does not produce similar indicator displacement from the surface. However, even using this unexpected non-specific displacement value, the normalised selectivity indicates that DYE B is not as useful as its analogue DYE A for the indirect monitoring of recognition events at the imprinted sites.

It is likely that the variation of methylation position on the aliphatic tail of the indicator (Dye A to Dye B) has allowed greater interaction with the sites within the imprinted cavities due to reduced steric hindrance. This reduction in steric hindrance will make it more difficult for the template to displace the dye from the specific cavity, explaining the reduced selectivity for EPH by the same polymer. As MDMA possesses a larger head group than EPH, a larger interaction at the one point that the dye is involved would make the competitive displacement more difficult as the van-der Waals forces that are reciprocal for the template cannot participate fully with this template analogue.

6.5 - Conclusions.

The best solvent for the creation of imprinted polymers capable of aqueous template recognition is the most hydrophobic solvent that can dissolve the template and monomers. It is apparent that the solvent forces the polar components of the polymerisation solution into adjacent areas, and allows imprint clusters of higher stoichiometry to be formed. This solvent property was associated with solution stoichiometries of higher number by Ansell et al.\textsuperscript{[171-173]}, and Yu et al.\textsuperscript{[168]} proposed that a more hydrophobic solvent would incorporate more polar components inside the active polymerisation globule resulting in imprint sites with more functional monomer units participating.
Although it was possible to tailor the aqueous environment to create the most selective recognition of the template through manipulation of the pH and the concentration of ionic species in the mobile phase, this environment was did not reduce the non-specific interaction between the drug classes and the MIP and NIP pairs.

The use of acidic mobile phase appears to hinder the association with the less specific and weaker stoichiometric imprint cavities in agreement with the observations of Ansell et al. The divergent performance of the MAA polymers in the optimisation of the aqueous phase suggests that although their composition is identical except for the porogen used, the speciation of the final polymers is significantly different.

The sites with stronger association and more preorganisation are more useful than the weaker imprint sites associated with stoichiometric association, and fundamentally less likely to be involved in non-specific interaction with analytes other than the template. As the objective for these polymers is to immobilise the template and its analogues rather than perform a preparative separation of enantiomers, the higher functional monomer stoichiometry clusters provide stronger interaction and better discrimination between non-related compounds. The tailing of the HPLC separations outlined by Ansell et al. as a negative descriptor of performance are in this case a beneficial contributor to MIP performance. As the desorption of template analogues is not desired, use of the strongest interaction sites is preferential to using weaker, less preorganised sites.

The EPH- MAM polymers perform better in discrimination of competing drug classes from those of the template’s class. In addition, the displacement of DYE A from the polymer network upon the introduction of the template or its analogues to the loaded SPE cartridge performs better in the case of the MAM polymers than the MAA polymers. Although displacement from the imprint site is noticed in the MAA polymers only upon introduction of the template molecule or its analogue, the level of discrimination was significantly higher for the MAM polymers. Although the level of drug adsorption was relatively similar in the non-displacement examination, the addition of the indicator to the MIP system increased the selectivity of the system for the template and its analogues. It is apparent that the combination of individually weak interactions arranged in a specific orientation is capable of providing better aqueous
discrimination of a template that is soluble in water as opposed to stronger and less prevalent interactions.

During the SPE displacement investigations, the MAM polymer in combination with Dye A delivered $\delta$MIP vs $\delta$NIP selectivity of 10.71 for the template while Dye B gave 3.37.

Considering only the imprinted polymer, the MAM polymers significantly outperform the MAA polymers in providing greater adsorption capability for the template over the other drug classes.

For the discrimination of EPH type molecules using the equilibrium method, the dye used by Shimizu and Greene is a better performer than its analogue. Although both demonstrate an ability to act as a selectively displaced indicator of template analogue binding, the observed selectivity for these two dyes discriminating EPH are 4.71 and 3.37 respectively.

Although larger I values were obtained in the SPE investigations, a lack of ability to monitor the amount of dye initially adsorbed to the polymer surface, then to monitor the amount of dye that is washed from the polymer surface in the conditioning step leaves determination of the amount of dye available for displacement uncontrolled, and therefore may confound the observed quantitative results of these experiments. As the NIP adsorbs less dye than the MIP, it is expected that less will be able to be desorbed upon the elution of any of the analyte species. This difference between the MIP and the NIP is the fundamental reason why the equilibrium method was utilised after selective discrimination had been initially demonstrated.

A colourimetric sensor for ATS has been designed through utilisation of \textit{in silico} screening to determine the identity of the most useful functional monomers and the stoichiometry these associated clusters. It is clear that the values reported for these calculations are not the actual enthalpies of formation; however their utility as comparative tools remains.

The widely accepted statement that the best rebinding performance can be found in the porogen is disputed, as observation of the swelling behaviour in a variety of solvents allows the maintenance of the functional monomer groups in a similar position to the porogen while allowing an increase in the other non-specific interactions that participate in ligand/receptor interactions.
For designing bio-mimetic receptors, it is important to understand how these receptors function in the natural systems in which they are found. This enables manipulation of the environment to mimic the natural behaviour of the ligands and receptors involved in the biological systems most closely.
Chapter 7 – Conclusions and Future Directions

7.1 - Conclusions

7.1.1 - Modelling outcomes

A scheme of work has been developed to probe the implementation of a design strategy for the formulation of MIPs for specific targets. This includes consideration of the specific environment in which the discrimination between molecular entities takes place. This consisted of the construction of a library of potential functional monomers in silico, which was subsequently screened against the desired template, ephedrine, and a range of competitor illicit substances.

Ephedrine was selected as the target for a number of reasons. It is easy to obtain and is structurally similar to illicit amphetamine type substances (ATS). It is also an adrenergic receptor agonist. As the project aimed to create a biomimetic, synthetic receptor for ATS, it is appropriate to consider the endogenous receptor-ligand systems in which EPH and the ATS participate. EPH possesses more functional groups on the aliphatic tail for interaction with the functional monomer clusters than other members of the ATS class. This means that a polymer imprinted using EPH should not exclude an ATS from the cavity, as all others species possess a smaller molecular volume. Results showed that the best interacting clusters were EPH: MAA at 1:2, and 1:3, as well as EPH: MAM at 1:5. This screening also determined the propensity of many of these monomers to self-associate in preference to interacting with the template. Throughout this scheme, it was observed that acidic and neutral monomers were the most appropriate types of monomer for interaction with basic templates.

Counting the number of predicted hydrogen bonds from a computational scheme is an inappropriate technique to apply. This is due to the repeated failure of the SPARTAN molecular modelling package to successfully predict intermolecular hydrogen bonding interactions in many template-monomer simulations. No cause other than the arbitrary distance limits set within a software package could be attributed to this shortcoming. The finding suggests serious limitations exist for groups seeking to apply this computational approach to selecting functional monomers for MIP construction.

The MAA imprint cavities for EPH were comprised of a small number of stronger interactions, while the MAM imprint cavities exhibited a larger number of
weaker interactions. Although the strengths of the hydrogen bond interactions involved
(O-H••O= 5 kcal.mol⁻¹ while N-H••O= 2 kcal.mol⁻¹) differ in magnitude, it is likely
that the favourable results from the MAM system are the result of a
cooperative,bifurcated network of interactions.

Cooperative interaction between multiple functional monomer units and the
template was identified in the modelling of MAM and EPH, MDMA and NMPEA;
specifically at a T: FM of 1:5. In these cases, a large network of weak intermolecular
forces resulted in the higher interaction energies between EPH and the cluster relative to
the other modelled systems containing this monomer and the ATS templates. This is
observed due to smaller amounts of functional monomer self-association at this
stoichiometry. This can be explained by the presence of ATS driving this association to
exist in larger clusters through participation in a network of intermolecular interactions.
This results in the higher calculated interaction values between template and functional
monomer cluster.

The emergence of a network of hydrogen bonds between multiple functional
monomer units also suggests that calculating the interaction between a single monomer
molecule and a single template molecule, ranking the interaction magnitude is an overly
simplistic approach for biomimetic receptor design. Rather than using a single strong
interaction, biological recognition generally results from the combination of a number
of individually weak interactions. If producing a biomimetic receptor is the goal, then it
is best to apply a similar approach. The greater performance of the EPH: MAM 1:5
polymers in comparison to the EPH: MAA 1:2 polymer systems appear to support this
theory.

Although the active sites of many natural receptors and enzymes are largely
hydrophobic in character, they interact with, and incorporate water molecules and
solvated species. Similar behaviour prevails in the cavities of imprinted polymers where
much of the polymer is of hydrophobic character. Assumptions based upon the
behaviour and structures of natural receptors that bind to chemically similar ligands are
therefore not out of place.

While in silico screening in the gas phase is fast and easy to conduct, it is
questionable as to how closely this represents the behaviour of molecular species in
solution. However, exhaustive modelling of every single atom within the synthesis
solution is computationally expensive and presents so many degrees of freedom in the calculation of potential interactions (between template and functional monomer library) that it is currently unobtainable, even when utilising the lowest levels of theory. Due to the compromises in the definitions of many nuclear and electronic properties in the molecular mechanics method, and the extension to molecular dynamics simulations, the effectiveness of modelling these enormous systems is at the same time more efficient but less accurate.

Gas phase modelling most closely resembles polymer synthesis in hydrophobic solvents. The solvent molecules in this case are the least likely to interrupt or displace favourable interactions between functional monomer units and the template species, as such non-covalent interactions are dominated by attractive forces between polar functional groups. In apolar media, it is likely that polar groups will aggregate in close proximity to each other. To be dispersed throughout the solution would be thermodynamically unfavourable. This is supported by experimental observations that polymers exhibiting the greatest level of template adsorption were synthesised in the apolar solvent toluene. Selectivity for the imprint molecule was exhibited by these polymers when the adsorption is carried out in aqueous environments. This confirms that the hydrophobic character of the polymer and of the template plays an important role in the interaction between the template and polymer in polar environments. It was observed that in aqueous environments, this interaction was greater and more selective than in toluene.

Semi-empirical investigations have previously been explored by Wu and Li, who found that predicted interaction energies correlated favourably with experimentally observed differences in analyte retention times. In silico assessment of the MAA and MAM imprint cavities in different solvent environments presented some unexpected outcomes. Extending upon the technique of Wu and Li, the impact of solvent polarity on the nature of template:- functional monomer interactions were investigated by introducing a solvent dielectric parameter to mimic different solvent polarities. In the case of the cavities generated from acidic monomers; an increase in the dielectric constant value ‘ε’ decreased the magnitude of the interactions with the screened targets. In the case of imprint sites generated with neutral monomers; an increase in the magnitude of interaction was observed with increasing ‘ε’.
Some unexpected results were observed in this work which makes the application of this specific screening protocol difficult. The higher levels of interaction generated by non-template analytes are of greatest concern. If the computational rebinding scheme does not show explicit selectivity for the target, how can it be used to guide the design of real systems? There is no model of the cross-linked polymer structure that incorporates both high and low specificity sites. This means that due to the fact that the crosslinker is responsible for around 90% of the polymer network, 90% of the behaviour of the MIP cannot be effectively modelled. Without all components of the polymer formulation being incorporated into the computer model, only the relative magnitude of interactions between the frozen imprint site and the molecular target can be estimated. As all molecular species screened in the simulations possess similar functional groups arranged in a spatially similar manner, it is not unexpected that competitor molecules interact similarly with the frozen EPH imprint site. For this type of modelling approach to deliver meaningful results, an accurate model of the global polymer structure, incorporating all elements (i.e. functional monomer and crosslinker units) must be incorporated to allow steric hindrance to be accurately represented.

As the implicit solvent conditions change, it is possible to calculate the behaviour of the analyte species, and to predict their behaviour in real systems. In the case of the EPH imprinted MAA FM cluster, the increase of the dielectric constant value ‘ε’ is observed to generate a decrease in the magnitude of the interaction between the monomer cluster and the screened molecules. EPH did not generate the greatest interaction with the monomer cluster, the opioid class of compounds significantly outperformed EPH and the other ATS. The most dramatic example of this difference occurred in the implicit toluene environment (ε= 2.38) where MOR exhibited a binding interaction that was four-fold higher than EPH.

The neutral EPH: MAM 1:5 cluster showed that while both EPH and other ATS exhibited increasing binding with increasing dielectric constant, the opioids suffered a significant decrease in binding with the cluster. This decrease makes the interaction of the opioids in toluene greater than in water, and the interaction is approximately 3 fold weaker than EPH in the same implicit solvent environment. COC exhibits little difference in interaction energy relative to EPH across any implicit solvent environment.
Indicator dyes A and B are observed to participate in similar binding interactions to EPH across all implicit solvent environments modelled, suggesting that their interaction behaviour with the imprint site is largely unaffected by solvent character. The work of the Anslyn group [262,263,265] suggests that this behaviour may result in a higher level of discrimination in the displacement of molecular guests.

7.1.2 - Comparison between computational and experimental results

The conclusions of Piletska et al. [220], in relation to a correlation between computational modelling and experimental behaviour of resultant polymers, are largely consistent with the findings presented in this thesis (Chapters 3 and 5).

It was determined that polymer performance is improved under aqueous rebinding for MIPs synthesised in apolar porogen. If the porogen possesses a less polar character than the component functional monomer and template in the formulation, then the polar species will tend to aggregate into an active polymerisation globule. Thus, the imprint sites should be better defined when the MIP is synthesised in apolar solvents, resulting in better selectivity and higher adsorption of the template analyte. The proposition by Yu et al [163], that this association behaviour is driven by phase separation appears to be justified by the observed increases in adsorption when rebinding is undertaken in water relative to the porogen.

The improved binding performance under aqueous conditions, of polymers synthesised non-polar porogen suggests that not only does the stoichiometry of the interaction between monomers and template in solution play a role, but that the porogen also plays a role in the formation of imprint cavities during synthesis. The predictions made in Chapter 3, followed by the experimental findings presented in Chapter 5, show that this feature is evident in the polymers synthesised in acetonitrile, where lower adsorption performance was observed than in the polymers for chloroform or toluene porogens.

Chapter 4 shows that the swelling of these systems is significantly different in varied solvents. If the receptor cavities are to be maintained in an effective arrangement, the swelling of the polymer must be as close as possible to that exhibited in its porogen. There were differences in the performance of polymers synthesised in acetonitrile relative to those synthesised in chloroform and toluene. Coupled with the
physical swelling differences observed in multiple solvents, the effect of synthesis in polar solvent may be responsible for the relatively poor performance of the polymers synthesised in acetonitrile; by causing the imprint site to be in a similar relative geometry to the imprint site during synthesis. Selectivity was still observed in water (I=2.5) for the EPH imprinted MAA 1:2 polymer prepared in acetonitrile, suggesting that selectivity is still able to be achieved in a polar porogen however its adsorption magnitude is lower than those polymers having the same stoichiometric ratio of T:FM synthesised in apolar media. This may be due to a smaller number of the original imprint sites surviving the polymerisation conditions, or may be due to the changes in the relative orientation of the imprinted site monomers caused by the swelling of these polymers in different solvents.

Ephedrine imprinted polymers displayed the greatest selectivity when synthesised in toluene, regardless of the functional monomer employed in the synthesis. The MAA-co-EGDMA (synthesised in toluene) polymer at 1:2 displayed the greatest selectivity in both toluene and water and exhibited significant adsorption of analyte. When the formulation was prepared in chloroform, selectivity was observed in toluene, chloroform, and water (I=3.1), but selectivity was absent in acetonitrile. The MAA-co-EGDMA 1:4 (CHCl₃) polymer exhibited selectivity when rebound in H₂O. There may be selectivity possible in chloroform and toluene, but observed error obscures the exact favourability of adsorption of template to the MIP over the NIP.

There does not appear to be a link in this case between the ratio of template to functional monomer in the MIP, and final performance of the MIP systems in adsorption experiments. There may be cavity formation in both systems, however the observed error in the case of the higher stoichiometry (1-4 and 0-4) MAA polymers in Chapter 5 suggests that there is a larger proportion of unassociated monomer throughout the polymer structure which is capable of strong, non-specific interaction. In this case, higher pre-synthesis stoichiometry was not observed to result in higher selective MIP performance. This is in agreement with the in silico calculations for this monomer.

MAM-co-EGDMA polymers displayed high levels of template adsorption when synthesised in apolar media. Little selectivity was observed for any of these polymers with the exception of those prepared in toluene.
Differences in the binding affinities of the MAA and MAM formulations suggest that the nature of binding interactions between template and polymers differ fundamentally. The MAA polymers are likely to involve a combination of hydrogen bonding and ionic interactions in combination with the hydrophobic effects generated between the electron rich arene group of EPH and the EDGMA cross-linker. This is supported by the emergence of selectivity in the toluene synthesised, 1:2 MAA-co-EDGMA polymer eluted with toluene. The hydrophobic solvent must be responsible for the lower observed level of interaction with both the imprinted and non-imprinted polymer, suppressing significant non-selective adsorption.

This is supported by the observed selectivity of the 1:5 MAM-co-EDGMA polymers prepared in and eluted with toluene. The hydrophobic nature of this solvent must strongly influence binding interactions with both the imprinted and non-imprinted polymers by suppressing non-selective adsorption behaviour.

In aqueous environments the selective binding ability of the 1:5 MAM-co-EDGMA MIP (prepared in toluene) within the imprint cavities is seen to increase. At the same time, non-specific adsorption is seen to decrease. This is supported by the results of the EPH: MAM 1:5 adsorption experiments from both toluene and water presented in Figure 5.23. This was also noted to occur in the case of the EPH: MAA 1:2 polymer synthesised in toluene and shown in Figure 5.22. A similar outcome was noted by Yang et al.\textsuperscript{[242]} for polymers synthesised with MAA or MAM with EGDMA in chloroform using SPE as the analysis vehicle (MAM polymers performing better than MAA polymers for the extraction of indomethacin from water).

Not only does water not cause deleterious effects upon the cavity binding of the EPH to the MIP synthesised in toluene, it decreases the amount of non-specific binding with the NIP. It increases the importance of hydrophobic recognition elements within the cavity while at the same time interrupting polar non-selective binding interactions at the polymer surface. Within the cavity, the situation appears similar to the non-specific mediation of interaction between $OppA$ and structurally divergent amino acid chains by water molecules as demonstrated by Quiocho et al\textsuperscript{[248]} as well as in the active site of L-arabinose binding protein where void filling and bridging water molecules were observed.
7.1.3 - Tuning of the adsorption environment conditions

Although the carboxyl group is capable of forming strong ionic interactions with basic functional groups, this strength is weakened in polar solvents. MIPs prepared in polar porogens, utilising carboxylic acid based functional monomers and imprint molecules capable of forming hydrogen bond interactions have often exhibited weak recognition or no recognition at all.

Unlike the carboxyl group, the amide group is a neutral, non-ionisable species. It was initially unclear whether it may or may not be advantageous for recognition under aqueous conditions. Large differences exist between the values of dielectric constant and dipole moment of the amide and carboxyl groups (acetic acid: $\varepsilon = 6.20$, $\mu = 1.70$ D; acetamide: $\varepsilon = 67.6$, $\mu = 3.76$ D), indicating that the amide unit may form stronger hydrogen bonds in water.$^{[250]}$ Given the neutral nature of the amide group, manipulation of the pH of the rebinding solution is unlikely to produce an observable effect on template - MAM interactions, but may affect template - MAA polymer binding.

Different approaches to reducing non-selective interactions and increasing the observable selectivity of the EPH MIPs were undertaken. Two of the indicator dyes screened in the in silico investigations were selected as indicator agents for the signalling of EPH adsorption by MAM MIPs. Unfortunately, due to the incomplete models of the polymer structures, no real conclusions could be reached about the effectiveness of the dye molecules from the computational scheme in this matter. Thus, the dye used by Shimizu and Greene, and its structural analogue, Dyes A and B were selected for application.

The effects of ionic strength on template recognition have been investigated by Turner et al.$^{[140, 141]}$, and Chianella et al.$^{[222]}$, who utilised SPE to optimise a MAA-co-EGDMA (1-2) binding for caffeine and microcystin L-R, and by Andersson et al.$^{[222]}$, who optimised the binding of a MAA-co-EGDMA polymer for the binding of propranolol. A similar approach was taken for the acidic MAA MIP. In this case, pH and ionic concentration were modulated to determine the effect on template rebinding. Ionic interactions are fast, strong and readily apparent in any charged system. They are also easily modulated, allowing relatively simple control over the pH and the ionic concentration of the recognition environment. The work presented in Chapter 6 is
indicative of the success of such an approach to tuning the recognition environment for EPH adsorption.

The presence of $[\text{PO}_4]^3-$ in solution is beneficial to template recognition at low concentrations (5mM). As $[\text{PO}_4]^3-$ concentration increased, the ability of the polymer to adsorb the template diminished. This suggests that in water, electrostatic interaction plays a critical role in the performance of the MAA MIPs. pH was also determined to play a significant role in the adsorption from aqueous solutions, with large amounts of EPH being adsorbed specifically and non-specifically at pH9. This is attributed to the protonation of the EPH amine functional group below its pKa of 9.56.$^{[213]}$

The divergent performance of the EPH MAA polymers synthesised in toluene and chloroform during optimisation confirms that the use of a more hydrophobic porogen produces better MIP performance in aqueous environments. Comparison of the performance of the acidic and neutral MIP systems in the optimised ionic environment showed that for EPH, there is a marginal difference in EPH adsorption. The difference in selectivity, shown in Table 6.1, is due only to the NIP adsorption. The interactions between EPH and the MAA polymers are likely to be electrostatic in character, while MAM polymers are more likely to have a significant contribution from hydrophobic interactions.

In the indicator (dye) displacement assay, the EPH imprinted, MAM MIP displayed a greater affinity for EPH relative to other compounds tested. The selectivity can be explained by the ionic interactions being individually stronger in the MAA MIP, and virtually absent in the MAM MIP. The MAM MIP contains a larger network of weaker interactions whose combination must be responsible for the better performance of this MIP system for the discrimination of the template from its analogues in SPE methods. Equilibrium based experiments showed that dye displacement occurs quickly, (<5 minutes), and selectivity for the MIPs involved in this study can be observed, with the best performance being $I=4.71$ for the EPH imprinted: MAM 1:5 polymer synthesised in toluene.

Overall, the computational design method, followed by experimental validation, has shown that it is possible to design biomimetic receptors using the same types of interaction in synthetic systems.
7.2 - **Further Work**

Further characterisation of endogenous receptors will improve the understanding of receptor/ligand function *in vivo* and thus will enable the development of more effective biomimetic synthetic receptors.

The modelling scheme may be improved by conducting the initial molecular modelling in a variety of implicitly and explicitly modelled solvent environments. Subsequent calculation of the interaction energies from these models, using higher levels of theory, could be used to determine the point at which accurate models are produced for the least computational effort.

An improved understanding of MIP structure, particularly the importance of the crosslinker in defining cavity morphology, could allow more accurate *in silico* models to be developed (computational limitations not withstanding).

Investigations could be carried out to synthesise MIP polymers for other ATS to determine the relative efficiency of different ATS on the selective adsorption of both ATS and non-related drugs. This may enable the creation of MIP arrays that could simultaneously detect and presumptively identify not only the general class of compound, but molecular identity.

Assessing the effect of different mobile phases and different mobile phase modifiers on the adsorption of both template analogues and dissimilar molecules could be performed. This would help to determine the best performing types of mobile phase and the best performing mobile phase modifier in each case, and could lead to further enhancement of the MIP performance.

To better provide for a field portable sensor, grafting the various MIPs for a range of compounds onto multi-channel micro capillary channels could be performed. Further to this, coupling of the indicator displacement assay on a micro capillary flow cell to increase the number of parallel presumptive tests that can be performed at one time could be a significant step forward in the commercial application of MIP systems for everyday use.

An investigation into the effect of pressure on the compressibility of MIP structures and the effect that this has on the selective adsorption capability of the MIP could provide further improvements in MIP performance. There was a significant pressure/compression effect upon the polymers produced in acetonitrile. It would be
interesting to determine how the effect of pressure in the polymerisation and recognition environments would affect the behaviour and properties of the produced polymers.

More accurate control of solvent flow rates, and pushing the solutions through the cartridges with a syringe pump, rather than pulling the solutions through the cartridges with vacuum pressure may allow for further optimisation of the method. It may be possible to calculate the real time kinetics of the displacement if a UV-Vis spectrometer light path could be passed through the outflow from these cartridges. This would allow for the optimisation of the flow-rate conditions for the process, and a more complete characterisation of the adsorption and desorption processes in the indicator displacement assays. This was not attempted in these experiments, as while using the vacuum manifold system, precise flow rate control proved impossible. It was difficult to generate a method by which the cartridges could be eluted through at the same flow rate in all cases. This would also remove a potential influx of operator bias in these experiments, through mechanical control of flow-rate.

Surface area analysis (BET - Brunauer-Emmett-Teller analysis / gas adsorption isotherms) could also provide significant information about the physical structure of the created MIPs based on their porogen. It is possible that dynamic measurement of the swelling kinetics could be obtained using laser diffraction particle analysis, and importantly as the particle size gets smaller, the aggregation behaviour of these particles to determine the effective particle size for micro and nanometre sized MIP particles.

For the field use of MIP loaded SPE cartridges, double blind trials with both pure and impure “street” samples of illicit drugs would need to be conducted to determine the reliability with which the MIP based displacement scheme performs when adulterants that are commonly found in seized samples are present.

As the DVB polymers were observed to exhibit a large adsorption of EPH from solution, incorporation of an aromatic functional monomer into the MAA and MAM functional MIP recipe may provide further mimicry of the natural receptors for ATS and thus improve selective recognition of the template.

As all polymers in this work have been created as monoliths by free radical polymerisation at 60 °C, investigations into precipitation and emulsion polymers, phase inversion films, spin cast films and surface grafted MIP elements on anchor particles may provide further improvement to the systems created. It would also be interesting to
investigate the potential for polymerisation reactions to be carried out within cryostatic environments to increase the number of high specificity sites in the imprinted polymer structures. This may produce less non-specific binding to functional monomer units incorporated into the networks when not associated with a template.

The potential for greater control in the architecture of the produced polymer could be investigated through the use of living radical polymerisation, or through nitroxide mediated polymerisation. Variation to the manner in which polymerisation is initiated such as pulsed laser, UV and microwave initiated decomposition of the free-radical may also provide improvements to the homogeneity of the created imprint sites and the physical properties of the resulting polymers.

Further investigations into the polymerisation porogen could allow enhancement for the selective characteristics of the generated MIPs. Application of a variety of polymerisation techniques in mixed organic solvent systems, room temperature ionic liquids and possibly even aqueous polymerisations may allow for better tailoring of the imprinting environment depending on the target compound. Utilisation of unusual cross linking monomers and custom synthesised functional monomers may also improve the performance of the produced MIPs.
Chapter 8 - Experimental Methods and Materials

8.1 - Molecular modelling, screening for functional monomer identity and stoichiometry

Materials

Dell PC 2GHz processor with 2GB RAM running Windows XP pro, SPARTAN04 (Wavefunction, Inc. 18401 Von Karman Avenue, Suite 370 Irvine, CA 92612 USA).

-METHODS-

Molecular models were constructed using SPARTAN 04 software package. Each molecule was constructed within Spartan '04 by building each component in an individual molecular area, and minimising the individual molecules using MMFF94. Subsequently, the final models were constructed by placing all components of the desired model (template, functional monomer, and number of functional monomers) within one molecular area, and in such a close proximity that such locations are physically impossible. A local minimum energy conformation for the cluster was then generated using the minimise command (utilising MMFF94 force field). Due to the degrees of freedom present in the molecular clusters, there is little likelihood of strain within a molecule. Thus the energy minima represent the geometries at which the interactions between template and monomer are greatest.

Subsequently, the clusters were submitted for AM1 semi-empirical, Equilibrium geometry optimisation and calculation of the heats of formation, in addition to high level electron density potential surfaces. Isolated functional monomer clusters were constructed in the same fashion as the template containing clusters to enable the calculation of the estimated energy of interaction between functional monomer units. Each individual model was constructed in triplicate, following the same protocol to ensure that sampling bias through the model construction process was not incorporated into the results.
The following equations were utilised to calculate the magnitude of the association between the template and monomer clusters.

\[
\text{Estimated } \Delta E_{\text{interaction}} = \Delta_{\text{HF system}} - (\sum (\Delta_{\text{HF monomer cluster}} + \Delta_{\text{HF template}}))^{[167]} \text{ Equation 2.1}
\]

A description of the total intermolecular interaction within the whole system, including any functional monomer self association/repulsion and template - functional monomer (T:FM) interaction is described by:

\[
\text{Estimated } \Delta E_{\text{interaction}} = \Delta_{\text{HF system}} - (n.\Delta_{\text{HF monomer unit}} + \Delta_{\text{HF template}}) \quad \text{Equation 2.2}
\]

Where ‘n’ is the number of individual functional monomer units present in the cluster.

By performing these two slightly different calculations, the total interactions between all molecules in each model can be identified by equation 2.2, while the specific interaction between template and monomers can be isolated by equation 2.1

The molecules modelled were:

- templates; EPH, MDMA, N-MPEA, HER.

A density potential surface was added to each system before semi-empirical modelling to display the surface potential of each molecule, allowing sites of electrostatic interaction to be identified. Models were terminated upon reaching a gradient = 0.001 and were considered to be at the global minimum state upon the termination of the modelling script.

It must be emphasised that the models were not placed in such an orientation as to artificially select possible interactions. To do so would be to circumvent the entire purpose of the in silico screening process. The positions of all molecules in the calculation of the heats of formation were generated through the iterative calculation process. Each model consisted of ONE template molecule and the appropriate number of monomer units for the ratio for that model. Each of these models was constructed 3 times, and the results averaged.
8.2 - Molecular Modelling, *in silico* screening for association

Cluster geometry against template analogues and potential displacement indicators.

In Spartan, the template molecule was selected and removed from the template and monomer clusters described in chapter 2 that suggested that there was a favourable interaction between the template and the monomer cluster. Movement / torsion constraints were placed upon the functional monomer cluster atoms to prevent movement during subsequent minimisation sequences using MMFF94, and the calculation of the interaction between the pseudo receptor cavity and screened library.

Each member of the library was individually placed in the general area of the frozen pseudo receptor site with no attempt to force the fit on the molecules into the pseudo receptor site. The energy was initially minimised, followed by submission for Equilibrium Geometry optimisation protocols using AM1 method to identify the energy of interaction between the frozen monomer cluster and screened analytes. Calculated energies of interaction were then compared as an estimate of potential binding affinity with the cavity using the same formula as in the initial screening process as described by equation 2.1.

Effects generated by the cross linker (CL) on associations between monomer and template species was investigated through the addition of 20 CL molecules, and calculation of the interaction magnitude between frozen cluster and template.

Subsequently, the frozen monomer clusters were copied to the AccelrysDS DS package, and the same procedure was carried out in the gas phase using the CHARMm force field to give a baseline comparison between the performances of the two gas phase screening calculation methods. The final calculation was not carried out using the AM1 semi empirical method this time, but was simply a molecular mechanics calculation using the CHARMm FF.

To determine the effect of solvent character on template – functional monomer binding a generalised Born-Oppenheim with simple switching method was used to incorporate the dielectric constant term into the calculation of interaction energies. The frozen monomer clusters with, and without the incorporated library molecules, were
subjected to the same calculation scheme with the addition of the dielectric constant term to incorporate the implicit presence of a variety of solvent molecules on the association clusters. The solvents that were implicitly modelled were toluene ($\varepsilon=2.41$), chloroform ($\varepsilon=4.81$), acetonitrile ($\varepsilon=37.5$) and water ($\varepsilon=80.1$) and had their dielectric constants rounded to the nearest whole value to allow entry into the AccelrysDS DS program. Each cluster and ligand was screened 3 times and the results averaged.

8.3 - Confirmation of association, polymer synthesis and initial assessment of performance.

Azobisisobutyronitrile (AIBN, DuPont, 99.95%) initiator was recrystallised from acetone prior to use. Functional monomers and crosslinking agents (methacrylic acid, divinyl benzene, EGDMA) were purchased from Sigma-Aldrich Australia ($\geq 98\%$ purity) and distilled under reduced pressure prior to use. Methacrylamide was purchased from Sigma Aldrich Australia (98% purity) and recrystallised from a solution of toluene and methanol prior to use dried under vacuum for 4 hours after crystallisation then stored under nitrogen at 4°C. ($1R,2S$)-(-)-Ephedrine hemihydrate (Sigma Aldrich, 98%), was used as received.

Bulk grade solvents (methanol and acetonitrile) and were twice distilled prior to use. HPLC grade solvents (Merck, 99.8%): toluene acetonitrile, methanol, chloroform were used as received. All water used was prepared by reverse osmosis using a MilliQ system.

All other reagents: glacial acetic acid (AcOH, Sigma Aldrich), orthophosphoric acid ($H_3PO_4$, BASF, 85%), hydrochloric acid (HCl, Sigma Aldrich, 37%), triethylamine (TEA, Sigma Aldrich, 99.5%), potassium dihydrogen phosphate ($KH_2PO_4$, Fluka, 99.5%), potassium hydrogen phosphate ($K_2HPO_4$, Fluka, 99.5%), potassium chloride (KCl, Sigma Aldrich, $>99\%$), chloroform-d (+0.05% v/v TMS, Cambridge Isotope, 99.8%) were used as received.
8.4 - **NMR Experiments**

$^1$H and $^{13}$C Nuclear Magnetic Resonance (NMR) spectra were recorded at 300.13 and 75.47 MHz respectively using a Bruker Advance 300MHz Spectrometer in conjunction with Bruker Topspin v1.3 software. Deuterated chloroform was used as the solvent for all investigations.

$^1$H titration experiments were conducted by sequentially adding molar equivalents of the functional monomer to 1mL of a $6.0525 \times 10^{-3}$ M. solution of ephedrine hemihydrate (CDCl$_3$). All titrations were performed at 50°C. The chemical shift of each nuclei was plotted as a function of the amount of titrant added.

Continuous variation experiments were performed using solutions of ephedrine, methacrylic acid and methacrylamide at a concentration of $6.0525 \times 10^{-3}$ M. The molar fraction gradient was constructed via addition of changing volumes of each component solution, at a constant volume (1mL). $^1$H spectra and $^{13}$C were recorded for each individual solution. Plots were constructed as the mole fraction ephedrine vs. the chemical shift (ppm) x mole fraction.

8.5 - **Polymer synthesis**

Both the imprinted and non-imprinted polymers (*Table 8.1*) were synthesised using thermally induced (60 °C) radical polymerisation using AIBN (azo-iso-butyronitrile) for 24 hours. Non-imprinted polymers were identical in all respects save for the absence of the template from the formulation. Following polymerisation the polymers were ground using a mortar and pestle, then wet sieved in methanol to collect particles between 38-45µm for use in binding studies. The ground particles were then subjected to Soxhlet extraction for 24 hours using 10% v/v acetic acid in methanol to remove the template and unreacted monomers. Template removal (exhaustive extraction) was monitored using GCMS. Initial binding experiments were performed using particles below 25 µm in size, with ultrafine particles removed by suspension in acetone. This caused sample handling issues and significant filtration issues, so a slightly larger mesh size was used.
Polymer compositions were selected from the functional monomer and stoichiometry screening previously conducted in the previous chapters, in tandem with their non-imprinted counterparts. These selections were methacrylic acid at a ratio of 1:2, and methacrylamide at a ratio of 1:5. Polymers were also using the arbitrary selection of stoichiometry, 1-4, employed by the groups of Piletsky et al.\textsuperscript{[222]}, Cormack et al.\textsuperscript{[223]}, and Liu et al.\textsuperscript{[224]}.

Table 8.1: Polymer synthesis recipes for the MIP involved in this project. NIP partners follow the same recipe; however they do not include template molecules in the synthesis solution. 15mL of porogen was used in each case.

<table>
<thead>
<tr>
<th>Template</th>
<th>Mass/µmol/ratio</th>
<th>FM</th>
<th>Mass/µmol/ratio</th>
<th>XL</th>
<th>Mass/µmol/ratio</th>
<th>Porogen</th>
</tr>
</thead>
<tbody>
<tr>
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<td>MAA</td>
<td>0.0417g/48.4/2</td>
<td>EGDMA</td>
<td>0.95832g/484/20</td>
<td>TOL</td>
</tr>
<tr>
<td>EPH</td>
<td>0.040g/24.2/1</td>
<td>MAA</td>
<td>0.0417g/48.4/2</td>
<td>DVB</td>
<td>0.63012g/484/20</td>
<td>TOL</td>
</tr>
<tr>
<td>EPH</td>
<td>0.040g/24.2/1</td>
<td>MAA</td>
<td>0.0417g/48.4/2</td>
<td>EGDMA</td>
<td>0.95832g/484/20</td>
<td>CHCl\textsubscript{3}</td>
</tr>
<tr>
<td>EPH</td>
<td>0.040g/24.2/1</td>
<td>MAA</td>
<td>0.0417g/48.4/2</td>
<td>DVB</td>
<td>0.63012g/484/20</td>
<td>CHCl\textsubscript{3}</td>
</tr>
<tr>
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<td>MAA</td>
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<td>EGDMA</td>
<td>0.95832g/484/20</td>
<td>MeCN</td>
</tr>
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<td>MAA</td>
<td>0.0417g/48.4/2</td>
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<tr>
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<td>MAA</td>
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</tr>
<tr>
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<td>MAA</td>
<td>0.0383g/96.8/4</td>
<td>DVB</td>
<td>0.63012g/484/20</td>
<td>TOL</td>
</tr>
<tr>
<td>EPH</td>
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<td>MAA</td>
<td>0.0383g/96.8/4</td>
<td>EGDMA</td>
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<td>CHCl\textsubscript{3}</td>
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<tr>
<td>EPH</td>
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<td>MAA</td>
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<td>CHCl\textsubscript{3}</td>
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<td>MAM</td>
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<td>DVB</td>
<td>0.63012g/484/20</td>
<td>TOL</td>
</tr>
<tr>
<td>EPH</td>
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<td>MAM</td>
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<td>CHCl\textsubscript{3}</td>
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<td>MAM</td>
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<td>MeCN</td>
</tr>
<tr>
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<td>MAM</td>
<td>0.0824g/96.8/4</td>
<td>DVB</td>
<td>0.63012g/484/20</td>
<td>MeCN</td>
</tr>
</tbody>
</table>

8.6 – Additional Modelling Studies

Additional modelling studies were carried out to assess the reliability of the absolute output values from the original AM1 experiments. These simulations were carried out using SPARTAN\textsuperscript{04} at the PM3 and H-F 6-31G levels of theory.
8.7 - Polymer Swelling Studies

50mg of dried polymer was added to SPE tubes, sequestered between porous frits then placed on a tared analytical balance. Solvent (Acetonitrile, toluene, chloroform and MQ water) was added and allowed to incorporate with the polymer for 24 hours. Excess solvent was then removed using vacuum filtration. The tubes were then re-weighed and solvent uptake calculated by mass difference (Figure 8.1). Swelling observations were conducted in triplicate and the results averaged.

To allow a comparison between solvents of differing densities and the volumetric expansion of the polymer in different solvents, the specific gravity of the solvent must be accounted for. SI data values were utilised for the specific gravity of each solvent. The amount of swelling was assessed using the following equation;

\[
\text{Polymer swelling} \% = \frac{\Delta M}{S_g \times 0.05g} \times 100
\]

Where \( \Delta M = (M_{\text{swollen}} - M_{\text{unswollen}}) \)

8.8 - Rebinding Studies

SPE was used as the method for control of the polymer particles following the published work of Zou et al.,\cite{234} Turner et al.\cite{140,141} and Chianella et al.\cite{222,235}. Dry polymer samples (in triplicate), with masses ranging from 5mg to 60mg were placed in 1mL SPE tubes between polyethylene frits. These polymers were conditioned with
their porogen for 24 hours, and then excess solvent drained under vacuum using a Preppy SPE vacuum manifold. 1 mL of a 4 mM ephedrine solution was then passed through the polymer and collected in 1.8 mL chromatography vials before the solvent was evaporated to dryness using \( \text{N}_2(g) \). Subsequently, 1 mL of milliQ water was added to each sample vial and the sample vials were sonicated for 10 minutes to dissolve the sample. The solutions were then quantified using a Shimadzu High Performance Liquid Chromatograph (HPLC) (LC-20AD) fitted with an Econosphere\textsuperscript{TM} C18, 5\( \mu \text{m} \) column (Grace\textsuperscript{TM}) using a photodiode array detector.

Following these experiments, 50mg of imprinted and non-imprinted polymers, (synthesised in acetonitrile, chloroform and toluene respectively) were rebound in each of the above solvents with milliQ water also used as a conditioning and rebinding solvent. After conditioning in the solvent for 24 hours, the conditioning solvent was drained under vacuum and 1mL of 4mM (approx) ephedrine containing solution was eluted through the polymer cartridges, collected in 1.8mL vials and the solvent was evaporated to dryness using a stream of nitrogen. 1mL of water was added to the vials, and they were sonicated for 10 minutes before being assessed on the Shimadzu High Performance Liquid Chromatograph (HPLC) (LC-20AD) fitted with an Econosphere\textsuperscript{TM} C18, 5\( \mu \text{m} \) column (Grace\textsuperscript{TM}) photodiode array detector.

**8.9 - Optimisation of aqueous recognition**

\( N,N\)-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediame was prepared according to method of Shimizu and Greene\textsuperscript{151}. 500mg (2.5 mmol) of 4-Chloro-7-nitro-2,1,3-benzoxadiazole was dissolved in 20 mL MeCN and added to a dripping funnel under nitrogen. \( N,N\)-dimethylethyl-1-2-diamine (220mg, 2.5mmol) was dissolved in 20 mL acetonitrile in a two-necked round bottom flask containing 0.206mg NaHCO\textsubscript{3} and stirred until fully dissolved. The sample was placed in an ultrasonic bath to ensure complete dissolution. Over 1 hour, the diazole was added drop wise, under nitrogen and constant stirring. The solution was then heated to 60°C for 15 minutes then cooled and filtered under vacuum to remove particulate matter. The mixture was purified using silica gel chromatography, and then dried under vacuum to yield a brown/orange solid.
Figure 8.2: calibration of \( N,N' \)-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine absorbance vs concentration.

\[
\begin{align*}
    y &= 7860.6x + 0.0151 \\
    R^2 &= 0.9995 \\
    (459\text{ nm})
\end{align*}
\]

\[
\begin{align*}
    y &= 5535.4x + 0.0824 \\
    R^2 &= 0.9992 \\
    (214\text{ nm})
\end{align*}
\]

\[
\begin{align*}
    y &= 2751.3x + 0.0156 \\
    R^2 &= 0.9994 \\
    (350\text{ nm})
\end{align*}
\]

Figure 8.3: calibration of \( N,N' \)-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine absorbance vs concentration.

\[
\begin{align*}
    y &= 9812x + 0.043 \\
    R^2 &= 0.9987 \\
    (204\text{ nm})
\end{align*}
\]

\[
\begin{align*}
    y &= 1465.8x + 0.0145 \\
    R^2 &= 0.9972 \\
    (302\text{ nm})
\end{align*}
\]

\[
\begin{align*}
    y &= 1010.2x + 0.0006 \\
    R^2 &= 0.9951 \\
    (468\text{ nm})
\end{align*}
\]

\( N,N' \)-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine was prepared from 4-Chloro-7-nitro-2,1,3-benzoxadiazole (500 mg, 2.5 mmol) by dissolving
in 20mL MeCN and placing in a dripping funnel under nitrogen. \(N,N'\)-dimethylene-1-2-diamine (220mg, 2.5mmol) was dissolved in 20mL acetonitrile in a two-necked round bottom flask containing 0.206mg NaHCO₃, and stirred until fully dissolved. The sample was placed in an ultrasonic bath to ensure complete solvation. Over 1 hour, the diazole was added drop wise, under nitrogen and constant stirring. The solution was heated to 60°C for 15 minutes, after which the reaction flask was cooled, and filtered under vacuum to remove particulate matter. The mixture was purified using silica gel chromatography, and dried under vacuum to yield a brown solid.

4-Chloro-7-nitro-2,1,3-benzoxadiazole (≥99.0% (HPLC)), \(N,N\)~dimethylethyl-1-2-diamine (≥98.0% (GC) (Aldrich)) were purchased and used with further purification. MDMA, cocaine hydrochloride and heroin were obtained from the Australian Federal Police under license. Heroin and cocaine were used as received while MDMA was purified from tablets. The heroin was a mixture of diacetyl and 6’ mono acetyl morphine, and was not further purified before use.

The tablets containing MDMA were crushed in a mortar and pestle before being added to a flask containing 20mL of methanol. After 20 minutes, the solids were filtered using a Hirsch funnel under vacuum and both the filtrate and filter cake saved. MDMA was confirmed via GCMS of the filtrate solution. The solvent was then removed and 20mL of ethyl acetate and 2M NaOH (aq) added the crude MDMA freebase then transferred to a separatory funnel. The dye was extracted into the aqueous phase with shaking. MDMA purity was confirmed by thin layer chromatography (silica gel on Al - 50% hexane/ethyl acetate mobile phase). Recrystallisation from ethyl acetate produced slightly off white crystals. Further recrystallisation from anhydrous acetone resulted in brilliant white crystals with a melting point of 148-152 °C.

\((1R,2S)-(-)-\)Ephedrine hemihydrate (Sigma Aldrich, 98%), was used as received, and the waters of crystallisation taken into account during mass measurements for synthesis and rebinding.
-Methods

Separate aqueous solutions of 50mM KH₂PO₄ and K₂HPO₄ were made and allowed to dissolve overnight with continuous stirring. In a separate container, a pH probe (Horiba D-51) was placed, and while the solution was magnetically stirred, parts of either KH₂PO₄ or K₂HPO₄ were added to construct aqueous solutions of 50mM PO₄³⁻ at pH values of 5, 6, 7, 8, and 9. The solution was determined to be at the desired pH if the pH value remained constant for 2 minutes after the last addition. Subsequent dilution of the master stock solutions followed by the pH monitoring of the mixture allowed solutions of the required pH range to be constructed at 25mM, 10mM and 5mM.

These solutions were used as the conditioning and rebinding solvent for the assessment of the effect of pH and ionic concentration on the binding performance of the imprinted and non-imprinted polymers synthesised using methacrylic acid – co – EGDMA.

A triplicate series of dry polymer samples weighing 50mg were placed into 1mL SPE tubes on between two polyethylene frits. These polymers were conditioned with their porogen for 24 hours, and the excess solvent was drained under vacuum using a Preppy SPE vacuum manifold. 1 mL of a 4mM ephedrine solution was passed through these polymer samples and collected in 1.8mL chromatography vials. These samples were subsequently delivered for HPLC quantification on a Shimadzu High Performance Liquid Chromatograph (HPLC) (LC-20AD) fitted with an Econosphere™ C18, 5μm column (Grace®) photodiode array detector.

HPLC mobile phase contains 75% aqueous buffer solution (25mM KH₂PO₄, modulated to pH 3.5 with H₃PO₄ (aq) ) and 25% 3:7 water: acetonitrile (with 10mM TEA(l)). A 10μL injection volume was used with a run time of 20 minutes, flow rate of 0.8mL/min and detection wavelength of 200nm. A calibration curve was generated using solutions in the range of 0.1 to 10mM. Results were analysed using Shimadzu LC Solution software.

Cocaine, Heroin and MDMA samples were constructed at 5mM in 5mM phosphate salt solution at pH6. Calibration samples were constructed by serial dilution of this stock solution with the aqueous phosphate salt solution to construct 5 solutions
of 5, 4, 3, 2, and 1 mM in 5mM aqueous phosphate solution. Adsorption solutions were constructed from the master solutions at 4mM using serial dilution.

UV visible spectra recorded on a Hitachi u-2000 ultraviolet spectrophotometer in specific wavelength mode for concentration determination.

The drug samples were eluted through the SPE cartridges after conditioning, and the depleted solution kept in sealed 2mL vials to prevent evaporation before the UV visible spectra absorbance of each solution was recorded on a Hitachi u-2000 ultraviolet spectrophotometer in specific wavelength mode for concentration determination.

8.10 - Displacement scheme

50mg dried polymer samples were weighed into SPE cartridges between polyethylene frits. These samples were conditioned in 5mM K$_2$HPO$_4$(aq) in milliQ water at pH6 for 24 hours. Following this conditioning time, 1mL of aqueous phosphate solution containing 63mg.L$^{-1}$ of Dye A was added to the tubes, and the volume eluted slowly under vacuum over the course of an hour. To desorb as much non-specifically bound indicator from the surface as possible, 20mL of aqueous phosphate solution was passed through the loaded cartridges, after which time the eluent solution visually contained no hint of the vivid yellow colour of the dye.

The drug containing solutions used above (4mM in 5mM pH6 K$_x$H$_{3-x}$PO$_4$) were eluted under vacuum and collected in 2mL vials to be assessed on a UV visible spectra absorbance of each solution was recorded on a Hitachi u-2000 ultraviolet spectrophotometer in specific wavelength mode for dye concentration determination.

UV visible spectra recorded on a Hitachi u-2000 ultraviolet spectrophotometer in wavelength scanning mode to identify the absorbance maxima, and then in specific wavelength mode for the concentration determination.

Multiple calibration solutions of $N,N$-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine and $N,N'$-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine were constructed between $5.06261 \times 10^{-1}$M and $8.4377 \times 10^{-3}$ M in milliQ water, and the absorbance of the solutions calculated at each of the wavelength maxima observed for each dye. A wavelength that would not be impinged upon by the drug molecules was selected for the determinations.
100mg of dried, selected polymer (ephedrine imprinted, methacrylamide-co-EGDMA stoichiometry 1-5 and 0-5, toluene porogen) was added to a dialysis sack containing 1mL saturated dye (0.5mM) solution. This sack was tied off, and at t=0 was added to 24mL of milliQ water, under constant stirring. An aliquot was sampled for its absorbance ($\lambda$=350 nm for $N,N'$-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediame, and $\lambda$=302 nm $N,N'$-dimethyl-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediame at 10min intervals, before being returned to the test container until the system attained equilibrium (t = 120 min). A large excess of solid analyte was then added (1mg.mL$^{-1}$) and absorbance readings then taken at 5 minute intervals until t=240min, as outlined in Figure 8.2.

Figure 8.4: schematic representation of the process used to establish template mediated specific displacement of indicator from the imprinted polymer.


[5] Boyle, R: Experiments and considerations touching colours. First occasionally written, among some other essays, to a friend; and now suffer’d to come abroad as the beginning of an experimental history of colours London, Printed for Henry Herringman at the Anchor in the Lower walk of the New Exchange. MDCLXIV (1664)


[14] Martinelli, E; Design and test of an electronic nose for monitoring the air quality in the international space station Microgravity Science and Technology (2007), 19(5-6), 60-64


[18] Orzechowska, G; Use of solid-phase microextraction (SPME) with ion mobility spectrometry Anal. Lett., (30)1437


[23] Chattock, A.P; On the velocity and mass of the ions in the electric wind in air Phil. Mag., 48 (1899) 401

[25] Fallman, A; Rittfeldt, L; Detection of Chemical Warfare Agents in water by high temperature solid phase micro extraction-ion mobility spectrometry Int. J. Ion Mob Spect, 4(2001)85
[27] Borsdorf, H. and Rammler, A; Continuous on-line determination of methyl tert-butyl ether in water samples using ion mobility spectrometry J. Chrom. A, 1072(2005)45
[31] Kanu, A; A small subsurface ion mobility spectrometer sensor for detecting environmental soil-gas contaminants J Env Mon (2007), 9(1), 51-60
[34] Eiceman, G.A; Preston D., Tiano G., Rodriguez J.and Parmeter J. E., Quantitative calibration of vapour levels of TNT, RDX, and PETN using a diffusion generator with gravimetry and ion mobility spectrometry Talanta, 45(1997)57
[35] Yelverton, B.J; Analysis of RDX vapours in pre- and post-detonations using the ion mobility spectrometer under field conditions. J of Ener Mat, 6(1988)7380
[37] Daum, K.A; Atkinson, DA, Ewing RG; Formation of halide reactant ions and effects of excess reagent chemical on the ionization of TNT in ion mobility spectrometry Talanta, 55(2001)491
[41] Yinon, J; Field detection and monitoring of explosives TrAC, 21(2002)292


280


[113] Peterson BR, Mordasini-Denti T, Diederich F; Cavity depth and width effects on cyclophane-stereoid recognition: Molecular complexation of cholesterol and progesterone in aqueous solution. Chemistry & Biology 3(1995)139-46


[166] Okutucu B; Telefunca A; Optimization of serotonin imprinted polymers and recognition study from platelet rich plasma. Talanta 76(2008)1153-1158


[238] Uwe D Neue. WATERS HPLC Troubleshooting Guide
[260] Beer PD, Smith DK Anion Binding and recognition by inorganic based receptors *Prog Inorg Chem 46*(1997)1