Characterisation and Improved Performance of Molecularly Imprinted Polymers Prepared Using Room Temperature Ionic Liquids.

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Declaration

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other university or institution.

Katherine Booker
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Cheers!

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Abstract

Molecularly Imprinted Polymers (MIPs) are selective recognitions elements that have biological, chromatographic and sensory applications. While methods exist to reproducibly form MIPs with high affinity for a wide range of target molecules, improvements in production efficiency and selectivity are constantly being sought. Room Temperature Ionic Liquids (RTILs) represent a reasonably novel class of ‘green solvents’ which have been widely utilised for polymerisation reactions, resulting in enhanced polymerisation rates and polymer yields. In this study, the performance of imprinted polymers prepared in RTILs compared against equivalent formulations prepared using conventional organic solvents (Volatile Organic Compounds, or VOCs) is reported.

RTILs were found to greatly improve reaction efficiency compared to VOCs whilst maintaining reasonable levels of selectivity. The effect of variables such as RTIL structure, polymerisation temperature and solvent volume on the efficiency and structure of a cocaine-imprinted MIP were investigated and were found to affect polymer properties such as surface morphology, swelling, zeta potential, degree of crosslinking as well as selectivity for the template. Imprinting factors as high as 2.2 were observed for RTIL-prepared MIPs under initial rebinding conditions when using [bmim]BF4 as polymerisation solvent, compared with the highest imprinting factor of 1.6 for the polymers prepared in CHCl3. Optimisation of rebinding conditions resulted in ~26% improvement in selectivity for both RTIL and VOC-prepared polymers when using DCM as rebinding solvent.

Molecular modelling and NMR analysis of R, S-propranolol-imprinted polymers indicated some interaction between the RTIL anion and the template and monomer, although this was not found to prevent template/monomer associations in the RTIL [bmim]PF6. [bmim]PF6-prepared polymers showed good selectivity (imprinting factor of 2.3) and affinity for the template over other compounds, although binding capacity was lower than for the VOC-
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Abbreviations

$\Delta H_f$  Heat of formation

$[\text{bmim}]\text{BF}_4$  1-Butyl-3-methylimidazolium tetrafluoroborate

$[\text{bmim}]\text{CF}_3\text{SO}_4$  1-Butyl-3-methylimidazolium trifluoromethane sulfonate

$[\text{bmim}]\text{HSO}_4$  1-Butyl-3-methylimidazolium hydrogen sulfate

$[\text{bmim}]\text{MeSO}_4$  1-Butyl-3-methylimidazolium methylsulfate

$[\text{bmim}]\text{PF}_6$  1-Butyl-3-methylimidazolium hexafluorophosphate

$[\text{emim}]\text{PO}_4$  1-Ethyl-3-methylimidazolium diethylphosphate

$[\text{emim}]\text{SO}_4$  1-Ethyl-3-methylimidazolium ethylsulfate

AIBN  Azobisisobutyronitrile

ATRP  Atom Transfer Radical Polymerisation

BET  Brunauer Emmett Teller analysis

CAFF  Caffeine

CH$_3$CN  Acetonitrile

CHCl$_3$  Chloroform

DSC  Differential Scanning Calorimetry

EGDMA  Ethylene Glycol Dimethacrylate

EP  Ephedrine

FTIR  Fourier Transform Infrared Spectroscopy

SEM  Scanning Electron Microscope

MAA  Methacrylic Acid

MIP  Molecularly Imprinted Polymer

MMA  Methyl methacrylate

NIP  Non-Imprinted Polymer

NMP  Nitroxide Mediated Polymerisation

NMR  Nuclear Magnetic Resonance

PALS  Positron Annihilation Lifetime Spectroscopy

PNL  $R, S$-Propranolol

RTIL  Room Temperature Ionic Liquid

S.I  Selectivity Index

$T_g$  Glass transition temperature

TGA  Thermogravimetric analysis

$T_m$  Melting temperature

UV  Ultraviolet

VOC  Volatile Organic Compound
Chapter 1

Introduction

1.1 Molecularly Imprinted Polymers

Molecularly Imprinted Polymers (MIPs) have received significant attention in recent times as selective identifiers of compounds. The technique of molecular imprinting has become an increasingly popular option when looking for materials to detect a wide variety of target molecules and has found applications in many areas of chemistry and biology.

MIPs are polymers created to possess binding sites that have affinity for a particular template (or target) molecule. These sites are created through interactions between the template and selected monomer units possessing complementary functional groups that form a cavity within the gross polymer, allowing the MIP to selectively recognise and bind the template molecule (as well as, in some cases, structurally related template analogues) from a solution matrix. This high level of selectivity, combined with the fact that MIPs are inexpensive, robust and reusable, has led to widespread interest into possible applications. Particular areas of interest include separation chromatography, use as artificial antibodies and as sensor materials.\(^1\)

1.1.1 Applications
The scope of MIP applications is extraordinarily broad and constantly expanding. There appears to be almost limitless possibilities to the areas where MIPs may be successfully incorporated as recognition elements. The area that currently holds the most commercial promise is that of chromatography, as MIPs can be used as solid supports to recognise and remove certain molecules from a sample\(^2\). The fact that MIPs can be easily and cheaply produced, have long shelf lives and possess the capacity to be re-used makes them an attractive option for the production of specialist, commercial, chromatographic media.

Another area of significant interest is the use of MIPs as synthetic biological receptors. As the mechanism of MIP rebinding so closely resembles that of an antibody-antigen interaction, it has been a natural progression for MIPs to be considered as a synthetic receptor substitute for targets such as saccharides, DNAs, viruses, cells and proteins in sensory devices. In addition to the simplicity of their synthesis, MIPs possess the advantage over natural receptors of being able to perform in a wider range of environmental conditions (organic solvents, high/low pH etc.\(^3\)). MIPs produced for these sensory applications have been shown to exhibit affinity and specificity comparable to those of biological receptors and antibodies\(^4\).

MIPs have also been widely used as sensor materials. Due to the versatility of MIP-based systems and the fact that a wide range of template molecules can be detected, they have found many industrial, environmental and forensic applications\(^5, 6\). They can be used either as films or as column-packed particles and detection methods can include fluorescence\(^7\), optical reflectometry\(^8\), change in mass\(^9\) or surface plasmon resonance\(^10\). Many studies have also detailed the use of conducting polymers which will give rise to an electrical signal in the absence of a transducer element\(^11\) and, more recently, work has focused on the incorporation of MIPs with integrated gold nanoparticles for sensor applications\(^12\).
In order to utilise MIPs most effectively for any of these applications, it is important to fully understand the factors related to MIP synthesis which give rise to their specific rebinding ability. By understanding the mechanisms involved and working to optimise their performance, the scope of MIP application can be greatly enhanced.

1.1.2 MIP Synthesis

MIPs are generally prepared by the addition of one or more functional monomers to a solution containing the template molecule, which bind at functional sites. A crosslinker is added to the mixture to impart structural rigidity to the resultant polymer. Polymerisation then takes place in the presence of a solvent, or porogen and is usually initiated by a radical initiator, such as azobisisobutyronitrile (AIBN) by heating or UV irradiation. Post-polymerisation, the template molecule is extracted by exhaustive washing and filtration, leaving behind a binding site which is specific for that molecule. This site can then be used to selectively rebind to the template. A schematic overview of this process is shown in Figure 1.1.

![Figure 1.1](image_url)
Template/monomer interactions can generally be classed as covalent or non-covalent (although some semi-covalent\textsuperscript{13, 14} approaches have also been reported). The association in covalent bonding is of a formal nature, whereas non-covalent binding arises only from pre-association in solution, usually in the form of hydrogen-bonding, \(\pi\)-\(\pi\) interactions or electrostatic interactions. Whilst covalent binding can lead to the production of high-affinity sites in MIPs, template extraction is made difficult by the need to cleave bonds\textsuperscript{15}. Non-covalent binding is generally preferred in MIP production as it is considered to be the more versatile of the two techniques. A greater variety of functionality can be introduced to the MIP binding sites using non-covalent processes\textsuperscript{15} and it also facilitates template extraction as there is no need to cleave bonds. This enables the template to be removed from the polymer by simple washing. The degree of template/monomer associations in the pre-polymerisation mixture can be investigated through the combined approach of molecular modelling and NMR titration\textsuperscript{16, 17} in order to determine the optimal template:monomer ratio.

In addition to the specific binding behaviour exhibited by MIPs, there is usually a degree of non-specific binding taking place between the template and the polymer. Non-specific binding arises because non-covalent interactions are not strong and require the use of excess functional monomer to ensure adequate template/monomer complex formation. As a result, some of the functional monomer will be randomly distributed through the bulk polymer, forming non-specific binding sites\textsuperscript{18, 19}. In order to gauge the prevalence of these non-specific associations, a Non-Imprinted Polymer (NIP) is always prepared as a control measure. NIPs are synthesised employing an identical formulation and preparation procedure as for the MIP, but without the addition of the template. This allows for the levels of non-specific binding to be quantified and removed from consideration when evaluating MIP performance.

There are a variety of different methods based on the general principles outlined above that are currently utilised for MIP synthesis. In many instances, polymerisation is conducted using the free radical mechanism. It involves the use of an initiator, such as AIBN, which liberates
radicals upon heating or UV irradiation. The free radical then attacks the double bond in a monomer, thereby initiating the propagation phase of polymer growth. Propagation continues until functional monomer concentrations fall, allowing for chain termination events to proceed. An example of this process utilising methacrylic acid (MAA) as monomer is shown in Figure 1.2.

Figure 1.2. Free radical mechanism illustrated with azobisisobutyronitrile (AIBN) as initiator and methacrylic acid (MAA) as monomer.

MIPs can be synthesised to produce bulk polymers, precipitation polymers or thin films, according to the intended application. In both bulk and precipitation polymer processes, the polymers grow by the formation of highly cross-linked oligomer radicals which aggregate and cross-link together to form polymer spheres. Where there is a high monomer : solvent ratio, these spheres grow and coalesce together to form a macroporous or ‘bulk’ polymer structure. Traditionally, the synthesis of MIPs as bulk polymers has been the prevalent preparation
method as polymerisation times are relatively short and require only minimal amounts of solvent. However, this method can pose problems because the bulk polymers need to be ground to a suitable size in order to successfully extract the template and to increase the polymer surface area to increase rebinding efficiency. The grinding process is labour intensive and, more crucially, can damage or destroy high affinity binding sites\textsuperscript{20}. The process also results in polymer particles that are non-uniform, which can compromise the quality of results and affect batch-to-batch reproducibility\textsuperscript{21}.

The issues associated with polymer grinding can be overcome by producing polymers via the precipitation polymerisation method. This requires the use of excess solvent volume, which leads to the formation of discrete polymer spheres in place of a monolithic bulk structure. This method, instead of creating a solid macroporous network, produces polymer microspheres with some degree of control over polymer size and porosity\textsuperscript{22}. These microspheres do not then require grinding, which protects the integrity of polymer binding sites. However, polymerisation times may be lengthy using this procedure and the use of excess amounts of organic solvent is undesirable from an environmental perspective.

MIPs can also be produced by various pathways such as spin-casting and the phase-inversion technique\textsuperscript{23, 24}, to create polymers in a thin film format. Film preparation generally involves a different imprinting process to that employed in the formation of particles, as imprinting is often conducted post-polymerisation. Thin films offer a number of advantages in terms of applications, particularly in relation to sensory applications, as the film can be integrated with transducer devices to produce an electrical signal\textsuperscript{25}. However, film preparation can be time consuming and it can be difficult to create films with a uniform surface.

Ultimately, each MIP synthesis technique possesses advantages and disadvantages which need to be weighed up, and potential applications to be considered, before embarking on polymer production.
1.1.3 The Role of the Solvent

The solvent, or porogen, plays a key role in MIP synthesis. Its role is to impart porosity to the mixture through the phase separation of the solvent and the polymer during polymerisation. The porosity of the polymer plays an important role in allowing the template to access the polymer’s imprinted sites and it has been well documented that MIPs prepared in the absence of any solvent show no rebinding selectivity for the template\textsuperscript{15}.

Typical MIP morphologies involve the aggregation of MIP microspheres into clusters which then join to form larger clusters and produce a bead-like network. The porosity of the MIP is therefore made up from the void between microsphere clusters (macropores), within the cluster of microspheres (mesopores) and within the microspheres themselves (micropores). The diagram in Figure 1.3 describes this relationship.

![Figure 1.3. Pore structure in MIPs. Adapted from Spivak (2005)\textsuperscript{15}.](image)

There is evidence to show that monomer/solvent combinations exert a significant effect on the porosity of the resultant polymer. Haginaka et al (2008)\textsuperscript{26} conducted a study of the effect of monomer/solvent composition on the selectivity of a MIP towards \textit{d}-chlorpheniramine. MIPs were prepared with methacrylic acid or 2-(trifluoromethyl)acrylic acid as functional monomer using toluene, phenylacetonitrile, benzylacetonitrile or chloroform as solvent, resulting in a
range of porosities and surface areas. Despite this range, there appeared to be little impact on the ultimate selectivity of the polymer for the template.

Polymer surface area is highly dependent on the solubility of the polymer in the chosen solvent. Poorly solvating media typically promote early phase separation and produce polymers with larger pores and lower surface areas, while highly solvating media will lead to the formation of smaller pores and higher surface area. Polymer surface area affects MIP performance in a number of ways. It is generally accepted that an increase in surface area will increase the number of available binding sites, leading to higher polymer uptake and faster binding kinetics. However, as the number of non-specific sites also increases, the ultimate selectivity of the MIP will depend on the final distribution of binding sites. In some cases the ratio of imprinted to non-imprinted sites decreases with increasing surface area, leading to a decrease in selectivity; in others the distribution will remain constant and have no impact on the selectivity at all.

The other important consideration to bear in mind when choosing a solvent for MIP synthesis is the association between the strength of the non-covalent template/monomer interactions and solvent polarity. Conventional organic solvents used in molecular imprinting are generally known as volatile organic compounds, or VOCs. Typically, apolar organics such as halohydrocarbons, hexanes or acetonitrile are used for MIP synthesis and rebinding as they promote strong non-covalent intermolecular interactions (e.g. H-bonding). Stronger interactions promote complex formation and increase the number of binding sites created according to Le Chatelier’s principle. Care must be taken when selecting which solvent is the most appropriate for a given system as it is imperative that the template and functional monomers interact in preference to self-association or interaction with the solvent. If this is not the case, there will be a significant impact on the MIP performance as it is the nature and strength of the template/monomer pre-association that ultimately determines the selectivity and sensitivity of the MIP.
1.1.4 Other Factors Affecting MIP Morphology and Performance

In addition to the choice of solvent, there are a number of other synthesis parameters which have a significant influence on MIP characteristics, such as polymerisation temperature and solvent volume. The polymerisation temperature can affect the MIP in a number of ways. Reports have shown that synthesising MIPs at lower temperatures can lead to improved MIP binding performance due to reduced system internal energy which leads to stronger interactions between the template and monomer. Decreasing the polymerisation temperature may also lead to incomplete reaction (and hence incomplete crosslinking) of the polymer, reducing the structural rigidity. Whilst this may assist in mass transfer of the template and increase the binding capacity of the polymer, it may also cause collapse of some imprinted cavities within the MIP, decreasing selectivity.

Polymerisation temperature will also affect the viscosity, solvation and diffusion properties of the polymerisation solvent. These factors will have an effect on the polymer morphology and surface area, which will translate to effects on the ultimate polymer performance.

The volume of solvent used will not only differentiate between the type of polymer formed i.e. bulk or precipitation, but will also affect the pore volume of the MIPs. Increasing the volume of polymerisation solvent has been shown to increase polymer pore volume, leading to an increase in binding capacity.

The rebinding performance of MIPs has been shown to be highly dependant on the conditions used for rebinding and particularly on the choice of rebinding solvent, as the rebinding solvent can play an important role in MIP/template interactions. Traditionally, the imprinting solvent is also used for rebinding as it is thought that the solvation properties of the porogen may influence the shape and distance parameters in the binding cavities and it is therefore beneficial to recreate these conditions upon rebinding. However, this is not necessarily the best option in
all systems and studies have shown that enhanced rebinding performance can be achieved through alteration of the rebinding solvent polarity to best complement the predominant template/monomer interactions\textsuperscript{7}.

The choice of rebinding solvent may also impact on polymer swelling. The swelling behaviour of MIPs can impact on selectivity in two ways. On one hand, swelling of the polymer may increase the accessibility of the binding sites and would therefore be expected to increase the level of specific binding\textsuperscript{36}; however, there is also the possibility that the degree of swelling will affect the size and shape of the binding cavity and act to reduce these specific interactions\textsuperscript{37}. Optimisation of these various factors is an essential feature in the synthesis and application of well-performing MIPs.

1.2 Room Temperature Ionic Liquids

1.2.1 Definition and Properties

Room temperature ionic liquids (RTILs), once referred to as molten salts, are defined as organic salts which are liquid at room temperature. They have extreme liquid properties and occur as liquids between -100°C to 200°C, although by definition they can be classed as an RTIL as long as the melting point is lower than 100°C\textsuperscript{38}. Generally, they are colourless and odourless.

RTILs have been around for over 150 years, however it was some time before their useful properties became widely recognised. Early applications centred on their effectiveness as electrolytes in batteries, as they produced batteries that could be quickly activated and remained inert for long periods of time\textsuperscript{38}. Other early applications were limited by inherent moisture
sensitivity and it was not until the early 1990’s that RTILs which were air and water stable at room temperature were prepared and their benefits were more fully realised.

RTILs are composed wholly of ions; generally made from an organic cation and inorganic anion. The cation and anion generally differ greatly in size, with the cations being typically far larger and bulkier than the anion, which results in poor packing. This means that instead of crystallising to form a salt (as is the case with traditional monatomic and polyatomic salts such as NaCl) the RTIL remains as a liquid over a very large temperature range. The list of possible cation/anion combinations which can form these compounds is extensive, predicted to be in excess of one trillion. Most commonly, RTILs involve anions such as hexafluorophosphate, tetrafluoroborate, nitrate or triflate and cations such as imidazolium and pyridinium (Table 1.1).

RTILs are non-volatile and have no vapour pressure, making them not only safer experimentally than VOCs, but also useful as solvents for carrying out reactions involving volatile materials. The relative thermal and chemical stability of RTILs compared to VOCs makes them an attractive solvent choice for reactions which are highly exothermic as they allow mild conditions to be maintained.
Table 1.1. Commonly used cation and anions in RTILs

<table>
<thead>
<tr>
<th>Cations</th>
<th>Anions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazolium</td>
<td>Tetrafluoroborate (BF₄⁻)</td>
</tr>
<tr>
<td>Tetraalkylphosphonium</td>
<td>Hexafluorophosphate (PF₆⁻)</td>
</tr>
<tr>
<td>Pyridinium</td>
<td>Trifluoromethanesulfonate (CF₃SO₄⁻)</td>
</tr>
<tr>
<td></td>
<td>Nitrate (NO₃⁻)</td>
</tr>
<tr>
<td></td>
<td>Aluminium tetrachloride (AlCl₄⁻)</td>
</tr>
<tr>
<td></td>
<td>Hydrogen sulfate (HSO₄⁻)</td>
</tr>
<tr>
<td></td>
<td>Methylsulfate (MeSO₄⁻)</td>
</tr>
</tbody>
</table>

1.2.2 RTILs as VOC Replacements

The unique properties of RTILs have lead to widespread interest into their use as VOC replacements in a number of areas. Finding alternative solvents to the widely used VOCs is a highly desirable prospect, particularly from an environmental standpoint, as VOCs currently represent the majority of hazardous substances released into the environment by the chemical industry. They are a popular choice of solvent as they can be used with a wide range of reactants and, importantly, the fact that they are volatile means that they can be easily separated from reaction products. However, these compounds are then allowed to enter the atmosphere, where they have been found to contribute to smog formation and ozone depletion⁴². VOCs are also potentially carcinogenic⁴³. These toxic effects give some reason for concern about their widespread use and more environmentally friendly alternatives need to be considered.
RTILs can effectively be viewed as a ‘green’ solvent option. Their lack of volatility means that as a general rule they will not create toxic vapours, the exception being some BF₄⁻ salts which liberate HF upon heating. This represents clear advantages in terms of a safe working environment, reducing the need for precautions such as fumehoods as well as allowing the use of vacuums without loss of solvent⁴⁰. Another significant advantage of RTILs is their ability to be easily recycled and reused, minimising wastage and pollution.

Aside from the environmental aspects, there are a number of desirable chemical outcomes resulting from the use of RTILs as opposed to VOCs. One of the most potentially significant benefits is the ability to alter the properties of the RTIL by changing the cation-anion combination and making a ‘designer solvent’, tailor-made with the desired physical and chemical properties for a given reaction. As mentioned previously, the predicted range of combinations is over one trillion, compared to approximately 600 available molecular solvents³⁹. Changes in functionality and reactivity of the RTIL can be easily controlled through choice of anion (which is largely responsible for air and water stability, as well as viscosity) or cation (which can affect melting point and organic solubility⁴³). There have been a number of studies detailing the property changes that can be brought about by cation/anion manipulation; for example, Rogers et al (2000)⁴⁴ altered the hydrophobicity, melting point and water content of 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF₆) by changing the alkyl chain from butyl to decyl. The alteration of solvent properties in this manner is extremely useful and can have a significant effect on the outcome of the reaction⁴⁵.

The high level of thermal stability observed in RTILs can also be of great use, allowing (as mentioned previously) highly exothermic reactions to be conducted. It also means that there can be greater kinetic control in some processes, and that reaction self-heating can be contained⁴⁶. As the thermal stability range of some RTILs is over 300°C, it can be possible to carry out some temperature-dependent separations where a product is extracted into the RTIL at high
temperature and then precipitated by cooling, with the RTIL maintaining its liquid state throughout\textsuperscript{40}.

The lack of volatility of RTILs would in many cases be viewed as a disadvantage as it means the solvent cannot be easily removed from the reaction products; however, it does enable any volatile low molecular weight products to be easily removed from the RTIL once the reaction is complete. This is particularly beneficial in the case of heterogeneous catalysis reactions as the product of the reaction can in general be removed, leaving the catalyst in the RTIL ready to be reused\textsuperscript{41}. This has been successfully demonstrated by Bernini et al (2003)\textsuperscript{47} with a MeReO\textsubscript{3}-catalysed oxidation carried out in [bmim]BF\textsubscript{4} and the product extracted in ether, leaving the catalyst to be reused without apparent loss in function.

In addition to the applications mentioned above, RTILs also have the potential to outperform VOCs as a reaction solvent. These include uses in electrochemical processes (where the ionic nature of the RTIL could play an important role), reactions involving enzymes (where RTILs can enhance enzyme stability\textsuperscript{48}) and in polymerisation processes.

RTILs are not the only candidate for a more effective, environmentally friendly VOC replacement. Others, such as water and supercritical carbon dioxide, are also being investigated as potential alternatives. However, there does not appear to be another class of solvent with the solubility range of RTILs in normal conditions; neither do any of the alternatives possess the capabilities of RTILs in terms of tuneable properties.

1.2.3 RTILs and Polymerisation

To date, there are many different types of polymerisation reactions that have been conducted in RTILs including: electrochemical, charge transfer and polyaddition polymerisation processes.
Of these, the most extensively investigated has been that of free radical polymerisation. Free radical polymerisation is widely used because it works with a wide range of monomers, is cost effective and does not require highly controlled conditions.43

One immediate advantage of using RTILs in polymerisation reactions is the fact that some RTILs can act as a reducing agent, meaning that some polymerisation reactions can be initiated at room temperature without the need for a thermal or UV light source.39 An example of this is the RTIL trihexyltetradecylphosphonium bis(2, 4, 4-trimethylpenyl) phosphinate, which can react with radical initiators at room temperature to initiate the polymerisation of methylmethacrylate (MAA).43

There have been numerous reports of RTILs accelerating polymerisation rates in some free radical reactions, as well as allowing control over the reaction temperature.39 In a study conducted by Benton et al. (2004)43, the rate of polymerisation was found to be four times higher in [bmim]PF₆ than in benzene, with an accompanying tenfold increase in polymer molecular weight. This was achieved without adversely affecting the resultant polymer, which displayed similar mechanical and thermal properties to those produced in conventional solvents. Other independent studies have noted similar improvements in thermal stability of RTIL formed polymers.49 The ability to increase the rate of reaction is highly desirable in polymerisations, particularly when producing polymers via the precipitation method, where reaction times can be lengthy when performed in VOCs.

Observed increases in polymerisation rates are attributed to both an increase in the propagation rate coefficient (kₚ) and a reduction in the activation energy of propagation that are caused by the monomers and radical chains undergoing H-bonding with the cation and anion of the RTILs. Additionally, the high viscosity of most RTILs is responsible for substantial decreases in termination rates (kₜ), as the likelihood of two radicals colliding is reduced.
The increase in molecular weight observed when using RTILs as polymerisation solvents is attributable to the fact that RTILs have low chain transfer constants that act to stabilise the radical during polymerisation\(^{49,51}\). The effect on the molecular weight was clearly described in work conducted by Qi et al (2008)\(^{52}\) where incremental increases in the RTIL fraction in the reaction solvent were directly correlated with an increase in molecular weight (shown by shorter Gel Permeation Chromatography (GPC) retention times). This increase in molecular weight will weaken the segmental motions in polymer chains which, in turn, will cause an increase in the glass transition temperature \(T_g\), where the polymer moves from a rigid glass state to a rubbery state. Again, this was observed upon incremental increases in RTIL concentration in the polymerisation solvent which resulted in a steadily increasing \(T_g\).

Many of the benefits associated with using RTILs in polymerisation reactions can be taken advantage of (at least to some degree) when using a mixture of RTILs and VOCs. Wu et al (2005)\(^{53}\) showed that the addition of the RTIL \([\text{Me}_3\text{NC}_2\text{H}_4\text{OH}]\text{[ZnCl}_3\text{]}\) to a polymerisation mixture of MMA in ethanol (EtOH) and \(N, N\)-dimethylformamide (DMF) resulted in significant increases in polymer molecular weight (from \(2 \times 10^4\) g/mol in EtOH and DMF to \(1.3 \times 10^6\) g/mol in DMF/RTIL solution with 40% vol of RTIL). However, using an EtOH/RTIL mixture did not give the same result, with the molecular weight increase being diminished. So while there may be some scope for the application of RTIL/VOC polymerisation mixtures, it is by no means guaranteed to produce desirable outcomes. It must also be taken into consideration that, from an environmental standpoint, a pure RTIL reaction is preferable to a mixture of RTIL and VOC, provided that comparable (or better) results can be produced when using the RTIL alone.

There is still very little known about the actual mechanisms involved in free radical reactions in RTILs. One report which attempts to explain this to some degree is a study conducted by Lui et al (2005)\(^{54}\) in which the FTIR result of a styrene/MAA copolymerisation in a RTIL/VOC mixture was analysed. It showed that the high polarity of RTILs can prolong radical life and improve reaction conversion. The mole fraction of styrene that was present in the reaction
mixture was found to be directly proportional to that produced in the copolymer, indicating that the reaction is a radical propagating process similar to that observed in traditional organic solvents.

A study by Thurecht et al (2008) proposed a novel mechanism by which RTILs may cause the observed increases in reaction rates and molecular weight of polymers. They proposed a ‘protected radical’ mechanism, in which monomer domains form within the RTIL, allowing the reaction to be carried out in bulk-like conditions. This would explain the increase in reaction rate that is observed with almost all polymerisation reactions involving RTILs. As there are reports of RTILs forming organic-rich and ionic-rich domains within themselves, it is hypothesised that the introduction of the monomer causes these domains to swell and become monomer-rich regions where polymerisation can take place. This domain effect is then coupled with the protection of the radical initiator, which is partitioned in the RTIL-monomer interface, limiting their availability for reaction initiation. This could explain the very high molecular weights of polymers resulting from RTIL-based reactions.

One specific type of free radical polymerisation which has been studied extensively in RTILs is atom transfer radical polymerisation (ATRP). ATRP is viewed as the most simple, versatile form of polymerisations known as controlled ‘living’ polymerisations. This terminology is based on the fact that the radicals involved are reversibly deactivated to form a dormant species. They can then be reactivated, stimulating polymer growth, and the process can be repeated until the polymer is of the desired length. This technique allows control over the polymerisation rate as well as producing a well-defined end group. Conducting these polymerisations in RTILs has proven to reduce unwanted side reactions; reactions which can lead to macromolecules being irreversibly terminated.

The success of incorporating RTILs into free radical polymerisations, while extensive, is not completely universal. Attempts to use RTILs in nitroxide mediated free radical polymerisations
(NMP) have met with mixed results. Some literature suggests that polymerisation rates and molecular weight can be increased when using RTILs but that high temperatures (over 140°C) were required for there to be reasonable control over the reaction\textsuperscript{48}. Also, in the NMP of styrene and MMA in [bmim]PF\textsubscript{6}, lower molecular weights were observed in the resultant polymer as well as broader polydispersities when compared to the same polymer prepared by ATRP\textsuperscript{57}. This is likely to be due to degradation of the initiator in the RTIL as well as limited polymer solubility.

RTILs have been used in various other polymerisation processes, such as electropolymerisations, phase transfer, ionic charge transfer and polyaddition polymerisations\textsuperscript{58,59,60}. Of all these, it is electropolymerisations where RTILs have attracted the most interest. It has been found that using RTILs in electropolymerisations not only increases polymerisation rates, but also produces polymer films with a smoother morphology, increased electroconductivity and electrochemical capacity\textsuperscript{61}.

Thus far, the studies mentioned have been in relation to linear polymer systems. However, RTILs have also been used in the polymerisation of highly crosslinked polymers\textsuperscript{62,63} (similar to those which are used in most molecular imprinting systems). The RTIL [omim]Tf\textsubscript{2}N was found to produce polymers displaying increased pore size and lower surface area compared to those prepared in toluene.
1.2.4 Nanodomains and the Alkyl Chain Length Effect

The proposed ‘protected radical’ mechanism and other effects observed in RTIL polymerisations can possibly be explained by the formation of nanostructured ‘domains’, or nanodomains, within the RTIL itself. There are a number of reports which put forward the notion of an ordered structure present in imidazolium-based RTILs, particularly those with a long alkyl chain \((n > 4)\) attached to the imidazolium ring\(^{56, 64, 65}\). While most materials exhibit an homogeneous structure in the liquid phase, there is evidence to suggest that this is not the case in many RTILs. This effect is directly related to the fact that there are both polar and non-polar regions present which will affect the structural organisation. Computer simulation studies performed by Canongia Lopez et al (2006)\(^{56}\) indicated that imidazolium-based RTILs showed structuring similar to that of microphase separation between polar and non-polar domains, and that the type of structure in the non-polar domain is directly related to the length of the alkyl side chain. The butyl side chain appeared to be the transition point, with shorter side chains producing a dispersed microphase and longer chains (such as hexyl, octyl or dodecyl) resulting in a continuous microphase.

Experimental evidence of the heterogeneities present in RTILs has been produced in the form of X-ray diffraction experiments where nanometre-scale heterogeneities were identified, the actual size of which was found to be dependant on the length of the alkyl chain\(^{65}\). It was hypothesised that these occur as the result of alkyl chain segregation, where the neutral alkyl tails aggregate and are surrounded by charges which produce electrostatic interactions leading to an even charge distribution around them. The temperature-dependence of this observed phenomenon was also investigated, showing a strong effect of diffusion processes on the morphology of the nanodomains. This is displayed by the increase in spatial correlation with decreasing temperature, despite the increasing density, until such a stage where diffusion stops. At this point the domain size was observed to correlate with the density data\(^{65}\) (see Figure 1.4).
These structural properties were further investigated to examine the effect of the anion on the size of the nanodomains. It was discovered that an increase in the size of the anion resulted in a decrease in the size of the heterogeneities, possibly due to the decreasing ability (with increasing size) of the anion to form three dimensional hydrogen bonding lattices, which leads to larger interlayer spacing.

This study also compared the structure of RTILs with other nanostructured systems such as linear alcohols, where the alkyl chains tend to interdigitate. The measured growth in size of the structural heterogeneities in RTILs would indicate that this is not the case in these systems and it is proposed that the bulky methyl-imidazolium groups produce such strong steric hindrance that the RTILs are forced into a configuration where no interdigitation occurs at all, forming a ‘micellar’ structure (Figure 1.5).
When conducting polymerisation reactions in RTILs the size and structure of these nanodomains may have an effect on the process and, consequently, on the properties of the resultant polymers.

### 1.2.5 Disadvantages

Much of the focus on RTILs in the literature is on the advantages of their use over conventional solvents. However, there are a number of potential drawbacks that must be considered. Some polymers have limited solubility in RTILs which, although allowing for easier separation, may result in early stage termination of the polymerisation reaction. This occurs even with some of the most commonly used RTILs and polymers, such as polystyrene in [bmim]PF₆.
Another issue often encountered relates to difficulties associated with the complete removal of the RTIL from the polymer once the reaction is complete. The high molecular weight of the polymer and the inherent thermal stability of the RTIL limit the ability to separate them effectively as neither is sufficiently volatile to enable separation by conventional methods. This may mean that an organic solvent is necessary to remove the polymer from the RTIL, negating some of the environmental benefits of using RTILs.

Although RTILs are generally regarded as a ‘green’ solvent, there are instances in which they can degrade to form corrosive or volatile products. Some chloroaluminate RTILs can degrade to form HCl which may decompose polymer films. As a result of this, many chloroaluminate RTILs have been replaced by imidazolium-based species with a stable counter anion. However, even some imidazolium-based RTILs can decompose to form undesirable products, such as HF.

One major difference between RTILs and VOCs which becomes immediately apparent from their laboratory use is the much higher level of viscosity present in most RTILs. This can make them more difficult to work with and, more importantly, can affect reaction rates and solubilities, especially at low temperatures. It should be remembered, however, that any issues encountered regarding this feature can often be circumvented by using the tuneable properties of the RTILs.

The lack of knowledge about different RTILs and their properties can also be an issue. Although there is certainly an advantage in having such a vast array of cation-anion combinations available, it makes a comprehensive data catalogue or a predictive property chart difficult to formulate. If RTILs are to be used as a substitute for VOCs, it is important that data exists on a variety of different cation-anion combinations, encompassing information such as solubility, thermophysical properties, density, surface tension and other physicochemical properties. To date, few reports describing this information about RTILs have been published.
Such disadvantages, however, appear to be substantially outweighed by the many benefits associated with RTIL use. Their unique properties make them an attractive candidate for VOC replacement as reaction solvents in general; and particularly for reactions involving polymerisation.

1.3 RTILs as Solvents for Molecular Imprinting

Due to the large amount of published material detailing the potential of RTILs as polymerisation solvents, there is good reason to expect that RTILs may have an application as solvents for molecular imprinting. To date, there is very little literature associated with the combination of RTILs and MIPs into a single system. Our group was the first to report on the use of RTILs as MIP solvents\textsuperscript{70,71}, with some very promising initial findings.

In our studies, the RTIL solvent system was found to improve both reaction efficiency and polymer yield while maintaining good selectivity for the templates \textit{trans}-aconitic acid and cocaine. In the case of \textit{trans}-aconitic acid, a twofold improvement in selectivity was obtained compared to the corresponding polymer prepared in a VOC. Additionally, many other benefits were observed, such as reduced rebinding times, the ability to conduct polymerisation at low temperatures, the elimination of the need to grind the polymers and the ability to recycle the RTIL after the polymer had been removed.

Scanning Electron Micrograph (SEM) images of the polymers showed a considerable difference in morphology between the polymers prepared in RTILs and VOCs. While polymers prepared using VOCs show a monolithic structure when using low solvent volumes and typically form disperse microspheres in high solvent volumes, the RTIL morphologies appear relatively
unaffected. Figure 1.6 shows the polymer morphology obtained by bulk polymerisation in RTILs compared to typical polymers prepared by both bulk and precipitation methods in VOCs\textsuperscript{72}. The elimination of monolithic structure meant that the RTIL polymers did not require grinding and sieving in order to be usable, which is highly desirable in terms of the efficiency of MIP synthesis and also avoids the issue of damage to the binding sites through the grinding process.

**Figure 1.6** SEM images of (a) Precipitation-prepared polymer in VOC\textsuperscript{72}, (b) Bulk-prepared polymer in VOC\textsuperscript{72} and (c) Bulk-prepared polymer in the RTIL [bmim]PF\textsubscript{6}\textsuperscript{71}.

The morphology of RTIL-prepared polymers, however, was affected by both the RTIL used and the temperature of polymerisation, with morphologies ranging between aggregated clusters of microspheres, space-filling macrogels and gels (as described in studies by Stover’s group\textsuperscript{73, 74}) across the various preparations. The effects on morphology were even more pronounced at lower temperatures, indicating a possible relationship between morphology and viscosity\textsuperscript{75}. 

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In addition to our own studies, there are also a few reports involving RTILs in sol-gel synthesis of silica-based MIPs\textsuperscript{76-78}. Silica-based monoliths are seen as an attractive alternative to organic polymers as the organic polymer can shrink or swell in mobile phases which can affect the positioning of the functional groups and hence the selectivity of the polymer\textsuperscript{77}. Using silica increases the mechanical strength of the material while maintaining high permeability; however the traditional hydrolytic sol-gel preparation can cause cracking and shrinking of the polymer during drying. RTILs were seen as a solvent additive that would enable non-hydrolytic sol-gel synthesis to be performed, eliminating the aging and drying steps at high temperatures, thus preserving polymer integrity.

The sol-gel studies, conducted using \textit{R}, \textit{S}-naproxen\textsuperscript{76} and zolmitriptan\textsuperscript{77} as templates, both showed good chiral recognition using polymers with RTILs incorporated into the solvent. In the case of the zolmitriptan this was especially significant as the polymer prepared \textit{without} the addition of a RTIL could not adequately separate the \textit{R}, \textit{S} enantiomers. The observed increase in selectivity of the polymers is attributed to the increase in surface area and porosity obtained by the addition of RTILs to the solvent mixture. This follows earlier data obtained by Klingshirn et al\textsuperscript{79}, which detailed the effect of using RTILs in sol-gel polymer synthesis (albeit not of \textit{imprinted} polymers). This study indicated that as more RTIL was added to the solvent mixture, pore sizes decreased but total pore volume increased, resulting in an overall increase in available surface area. The effect on pore area was consistent regardless of whether the pores in question were micro- or meso- and macropores. Wang et al\textsuperscript{77} noted this same effect when synthesising their MIPs in RTILs, with smaller pores sizes and hence more uniform topography of polymers observed as the amount of RTIL increased, but overall porosity changed from $\varepsilon = 0.48$ with polymers prepared in pure acetonitrile, to $\varepsilon = 0.70$ with the RTIL added.

To date, only one other study detailing the use of a RTIL as a MIP preparation solvent has been reported. A covalent imprinting approach using a sacrificial spacer imprinting technique was
applied. This resulted in a MIP with good homogeneity wherein mesopores displaying high specific recognition properties for testosterone relative to structural analogues\textsuperscript{78}.

It is important to note, however, that in nearly all the studies mentioned above (with the exception of those conducted by our own research group), the RTIL is used in conjunction with other organic solvents such as acetonitrile or chloroform. Hence, while some properties of the RTILs are being taken advantage of in terms of producing polymers with increased porosity, the improvements in relation to environmental impact are minimised. It is highly desirable that the benefits of RTILs as imprinting solvents are realised without the incorporation of VOCs, and our preliminary work in this area indicates that this is indeed possible.

### 1.4 Project Aims

Molecularly Imprinted Polymers (MIPs) have proven to be excellent recognition devices, with many desirable attributes such as robustness, reusability and high levels of selectivity. However, factors such as protracted polymerisation times, the need for grinding and sieving of polymers and frequent problems with batch-to-batch reproducibility place limitations on their use.

RTILs have been established throughout the literature as solvents possessing great potential as reaction solvents compared to traditional organic solvents, particularly in the field of polymerisation, making them a justifiable choice as polymerisation solvent for a study in imprinted polymers. In studies conducted to date, cocaine and \textit{trans-}aconitic acid were studied by our group as template molecules for MIP production. The data obtained across these studies, although tending towards improved rebinding selectivity in RTIL prepared polymers, remains somewhat inconclusive as to the precise role of the RTIL in polymer formation. There was
substantial variation in the imprinting factors observed over the various temperatures, volumes and between the two RTILs under investigation. The reasons for these variations are unclear as there were no obvious patterns or definitive observations that could be made to explain why rebinding would be improved in one case over another. There also appeared to be problems with batch-to-batch reproducibility, a recurrent issue throughout MIP literature. So while the results obtained from these studies are extremely promising, there remains much to be explained about the interactions taking place within the pre-polymerisation mixture, during polymerisation and in the course of the rebinding process.

Further physical characterisation of the MIPs is an important part of understanding polymer behaviour. Measurement of characteristics such as particle size, zeta potential and surface area has not yet been conducted on any RTIL-prepared MIPs and can yield valuable information. Thermal analysis (Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)) is another area of some interest. Measurement of the decomposition behaviour by thermogravimetric methods can give data regarding the thermal stability and structure of the polymer, and show if there are differences from MIP to NIP and between the different solvent preparations. The glass transition temperature ($T_g$) of the polymer can be determined via DSC and will impact on the rebinding behaviour of the polymer, as at temperatures above the $T_g$, selectivity of the MIP will decrease due to the collapse of imprinted cavities\textsuperscript{14}. The $T_g$ of these type of crosslinked polymers will be influenced by a variety of factors, including the crosslink density\textsuperscript{80}. A sound understanding of characteristics such as these is critical in increasing our knowledge of the physical nature of the polymers.

The first section of this project involves the evaluation of cocaine-imprinted polymers and the many factors related to polymer synthesis and rebinding analysis which influence polymer performance. Cocaine was selected as initial studies showed that comparable template selectivity could be achieved using both RTILs and VOCs as polymerisation solvent. In addition to this, our group has particular interest in developing MIPs for cocaine as it represents
the possibility of more immediate, practical applications, due to our affiliation with the Australian Federal Police (AFP).

As only the RTILs [bmim]BF$_4$ and [bmim]PF$_6$ have thus far been investigated as MIP polymerisation solvents, initial studies will involve MIP preparation in of a variety of RTILs, to include a wide range of viscosities as well as anions and cations of various sizes. Other factors related to polymer synthesis i.e. polymerisation temperature, solvent volume and the choice of crosslinking agent will then be included in the analysis and all systems compared against the corresponding VOC preparation.

Characterisation of these MIPs will be discussed to give information which has not previously been determined in any RTIL-MIP system. Techniques such as zeta potential, TGA, DSC and the measurement of polymer swelling will be utilised to provide information which can then be used to explain the polymers’ rebinding behaviour.

This section will also assess the influence of the rebinding solvent on rebinding performance. This will allow for optimisation of the polymer systems as well as give some indication as to the predominant interactions occurring during the rebinding process.

The final aspect of the cocaine-imprinted polymer system to be examined will be that of a mixed RTIL/VOC polymerisation solvent. This will be compared to both a pure VOC and pure RTIL-prepared polymer to determine the effects on the physical characteristics of the polymer and selectivity for the template.

The second section of this study concerned the evaluation of MIPs using $R,S$-propranolol as the template molecule. This selection was made based on the need to validate the RTIL-MIP system against a standard, or ‘model’ MIP template. $R, S$-propranolol has been extensively studied as a MIP template molecule, providing a large library of data available for comparison purposes. By
substituting RTILs into a MIP system which has been well developed with a commonly used target molecule, we are able to best evaluate the influence of the RTIL on the polymer.

Examination of the interactions that take place within the pre-polymerisation mixture were investigated to determine if the RTIL itself was involved in interactions with the polymerisation components, followed by polymer synthesis and rebinding analysis.

Physical characterisation of these polymers was taken further than with the cocaine-imprinted polymer system to include studies of surface area and porosity. This provided additional information to explain the polymers’ rebinding behaviour. The \( R, S \)-propranolol-imprinted system was also used to examine the effect of the length of the imidazolium alkyl side chain in the RTILs. A pattern in the behaviour of polymers corresponding to the imidazolium alkyl side chain length gave some insight into the structure of the RTIL and how this affected polymer formation.

To date, the role of RTILs in MIP systems is poorly understood. While RTILs show promise for use as MIP polymerisation solvents, this is limited by the fact that very little information is currently available regarding the physical characteristics of MIPs prepared using RTILs. This study addresses these issues by thorough investigations using two different templates. An understanding of the factors driving selectivity for the template in these polymers is necessary in order for RTILs be used to their full advantage in MIP-based systems.
1.5 References


Chapter 2

Experimental

2.1 Materials

All solvents used were of bulk grade and redistilled. Cocaine base (1) was provided by the Australian Federal Police services and used as received. Methacrylic acid (2) (MAA, Sigma-Aldrich), ethylene glycol dimethacrylate (3) (EGDMA, Sigma-Aldrich) and divinylbenzene (4) (DVB, Sigma-Aldrich) were distilled under reduced pressure prior to use. Caffeine (5), ephedrine (6) and R, S-propranolol (7) were purchased from Sigma-Aldrich and used as received. Azobisobutyronitrile (AIBN) was recrystallised from acetone and dried under vacuum prior to use. Chlorobutane, bromohexane, bromoethane and bromooctane (Sigma-Aldrich) were purified by washing with conc. H₂SO₄ until washings were colourless, neutralised with NaHCO₃ and dried with MgSO₄.

RTILs 1-butyl-3-methylimidazolium trifluoromethane sulfonate ([bmim]CF₃SO₄), 1-butyl-3-methylimidazolium methylsulfate ([bmim]MeSO₄), 1-ethyl-methylimidazolium diethylphosphate ([emim]PO₄), and 1-ethyl-methylimidazolium ethylsulfate ([emim]SO₄) were purchased from Solvent Innovations and used as received.

R, S-Propranolol hydrochloride (Sigma-Aldrich, 99%) was converted from the hydrochloride salt by NaOH solution, filtered and dried under vacuum.
2.2 Synthesis of RTILs

2.2.1 1-Butyl-3-methylimidazolium chloride ([bmim]Cl)

1-Methylimidazole (76mL, 0.9mol) was refluxed for 72 hours under nitrogen with a mixture of chlorobutane (110mL, 1mol) and ethyl acetate (50mL, 0.5mol) following the procedure of Whitehead et al (2004). The product was then washed with ethyl acetate and dried by rotary evaporation followed by $^{13}$C NMR analysis to confirm the identity of the product ($^{13}$C NMR (DMSO-d$_6$) 138.09, 123.75, 122.06, 49.88, 36.65, 32.26, 19.55, 13.51 ppm).

1-Hexyl-3-methylimidazolium bromide ([hmim]Br, $^{13}$C NMR (DMSO-d$_6$) 137.23, 123.94, 122.35, 51.87, 34.24, 32.27, 29.56, 27.12, 23.01, 14.09 ppm) and 1-octyl-3-methylimidazolium bromide ([omim]Br, $^{13}$C NMR (DMSO-d$_6$) 137.00, 123.79, 122.05, 49.95, 36.58, 31.47, 30.15, 28.81, 28.75, 26.06, 22.36, 13.84 ppm) and were prepared using an identical method but with bromohexane and bromooctane as starting materials, respectively.

![Figure 2.1](image_url)  Synthesis of 1-butyl-3-methylimidazolium chloride.

2.2.2 1-Butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF$_4$)

[Bmim]Cl was prepared according to the procedure used by Whitehead et al (2004). [Bmim]Cl (60g, 0.28mol) was dissolved in water (60mL) and cooled in an ice bath. To this chilled solution HBF$_4$ (60mL, 0.9mol) was added over 15 minutes. After complete addition the solution was stirred at RT overnight. The aqueous layer was extracted using dichloromethane (DCM, 3x 50mL), the extracts combined and dried over MgSO$_4$ and the solvent removed by rotary
evaporation. Product identity was confirmed by $^{13}$C NMR ($^{13}$C NMR (DMSO-d$_6$) 135.09, 122.80, 121.77, 51.53, 34.21, 32.02, 21.59, 13.81 ppm).

![Figure 2.2](image.png)

**Figure 2.2** Synthesis of 1-butyl-3-methylimidazolium tetrafluoroborate.

### 2.2.3 1-Butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF$_6$)

[Bmim]PF$_6$ was prepared according to the procedure used by Whitehead et al (2004)$^1$. [Bmim]Cl (120g, 0.56mol) was dissolved in distilled water (100mL) and HPF$_6$ (68mL, 60% aqueous solution, 0.56mol) slowly added over 20 minutes. The aqueous upper phase was decanted and water added (50mL). The mixture was mixed vigorously, allowed to settle and the upper phase decanted. This procedure was repeated until the pH of the upper phase was ~7. The water was removed by rotary evaporation. Product identity was confirmed by $^{13}$C NMR ($^{13}$C NMR (DMSO-d$_6$) 135.00, 122.21, 120.87, 48.14, 34.31, 30.23, 17.75, 11.45 ppm).

![Figure 2.3](image.png)

**Figure 2.3** Synthesis of 1-butyl-3-methylimidazolium hexafluorophosphate.
2.2.4 Imidazolium–based RTILs of Various Alkyl Chain Lengths

1-Hexyl-3-methylimidazolium hexafluorophosphate [hmim]PF₆ (¹³C NMR (DMSO-d₆) 136.65, 123.62, 122.27, 49.29, 35.69, 30.86, 29.61, 25.41, 22.13, 13.74 ppm) and 1-octyl-3-methylimidazolium hexafluorophosphate [omim]PF₆ (¹³C NMR (DMSO-d₆) 135.62, 123.65, 122.22, 49.97, 35.95, 31.57, 29.79, 28.86, 26.01, 22.46, 13.91 ppm) were prepared using the same method as [bmim]PF₆ but with [hmim]Br and [omim]Br as starting materials, respectively.

2.2.5 1-Butyl-3-methylimidazolium hydrogen sulfate ([bmim]HSO₄)

![Synthesis of 1-butyl-3-methylimidazolium hexafluorophosphate.](image)

[bmim]HSO₄ was prepared according to the procedure used by Whitehead et al (2004)¹. [bmim]Cl (30mg, 0.14mol) was dissolved in water (30mL) and conc H₂SO₄ (96mL) added. The solution was refluxed for 4 hours at 100°C then heated at 120°C until the vapour given off was no longer acidic. The remaining water was removed by rotary evaporation.
2.3 Preparation and Rebinding of MIPs

2.3.1 Cocaine-Imprinted Polymers

MIPs were prepared following the procedure of Holdsworth\(^2\) with cocaine base (0.14mmol, 42.6mg), MAA (0.28mmol, 24.1mg) and EGDMA (1.4mmol, 280mg), in desired amount of solvent. The reaction mixture was degassed with N\(_2\) before AIBN (10mg) was added and the solution was heated at 60°C in a Syncore Polyvap Plateform (Buchi). Photoinitiated polymerisation was done at 5°C for 3 hours in an ice bath using a UV probe (Acticure). The solvents used were CHCl\(_3\) (control), \([\text{bmim}]\text{BF}_4\), \([\text{bmim}]\text{PF}_6\), \([\text{bmim}]\text{HSO}_4\), \([\text{bmim}]\text{CF}_3\text{SO}_4\), \([\text{bmim}]\text{MeSO}_4\), \([\text{emim}]\text{PO}_4\), and \([\text{emim}]\text{SO}_4\) as well as mixtures of \([\text{bmim}]\text{BF}_4\) and CHCl\(_3\). NIPs were prepared using the same method without the addition of the cocaine base.

Extraction of the template was performed by washing in methanol (5 x 30mL) until no cocaine peak was registered by GCMS analysis. The polymer was then filtered and dried under vacuum.

Rebinding was carried out by suspension of 10mg polymer in 2mL of 25\(\mu\)M cocaine solution in various solvents for 1 hour. The resulting solution was filtered using a 2\(\mu\)m PTFE membrane filter and analysed using GCMS.

The amount of cocaine bound by the polymer was calculated from the difference in solution concentration before and after rebinding. The total selective binding of the polymer (\(\Delta B\)) was calculated as the difference between the MIP binding and the NIP binding (\(B_{\text{MIP}}-B_{\text{NIP}}\)). Imprinting factors (I) were calculated as \(B_{\text{MIP}}/B_{\text{NIP}}\).
2.3.2 \textit{R, S-Propranolol-Imprinted Polymers}

\textit{R, S-Propranolol} MIPs were prepared following the procedure described by Castell et al. (2006). \textit{R, S-Propranolol base} (389mg, 1.5mmol), MAA (509µl, 6mmol) and DVB (4.103 mL, 28.8mmol) were dissolved in 30mL of selected solvent and degassed with N\textsubscript{2} before AIBN (312mg, 1.9mmol) was added and the mixture was heated at 60°C for 24 hours. NIPs were prepared using the same method without addition of \textit{R, S-propranolol base}.

Template extraction was achieved by washing with CH\textsubscript{3}CN, toluene, methanol/acetic acid 70/30 v/v, methanol, CH\textsubscript{3}CN, toluene, CH\textsubscript{3}CN and methanol before being dried under vacuum.

Rebinding was conducted by suspending 20mg of polymer in 5mL of 25µM \textit{R, S-propranolol} solution in CHCl\textsubscript{3} and placed on a shaker table at 100rpm for desired rebinding duration. The solution was filtered using a 2µm PTFE membrane filter and the concentration measured using fluorescence. The amount of rebound \textit{R, S-propranolol} was calculated in the same manner as above.

Competitive rebinding studies using ephedrine and caffeine were conducted under the same conditions followed by filtration of the rebinding solution. The filtrate was evaporated and the template redissolved in water prior to analysis by HPLC.

Scatchard analysis was conducted using 20mg polymer in 5mL solvent over a 6 hour time period in the concentration range of 2.5-50µM prior to filtration and analysis via fluorescence.
2.4 Characterisation

2.4.1 Particle Sizing

Particle size is a measure of the diameter of the sphere that diffuses at the same speed as the particle being measured. It can be determined by measuring the speed of Brownian motion (random movement of particles in a liquid due to bombardment by the molecules that surround them) of the particles through the use of Dynamic Light Scattering and establishing a size via theoretical calculations.

Large particles will move more slowly than small particles, so by measuring the speed of movement and knowing the relationship between diffusion speed and size, the size can be determined.

2.4.2 Zeta Potential

Zeta potential, or electrokinetic potential, relates to the surface charge of the particles in question and is a commonly used technique in colloidal systems to determine the electrostatic interaction between particles. It is defined as the electric potential in the interfacial double layer of a particle i.e. between the dispersion medium and the stationary fluid layer closest to the particle surface. Calculations are generally based on the Smoluchowski theory which is valid for particles of all shapes and concentrations, although it does not account for surface conductivity.
The Smoluchowski equation is given as:

\[ \zeta = \frac{\mu \eta}{\varepsilon} \]

where \( \zeta \) = zeta potential, \( \mu \) = particle mobility, \( \eta \) = viscosity and \( \varepsilon \) = dielectric constant.

A zeta potential of zero will indicate an uncharged surface. Typically, if a material gives a zeta potential of greater than \( \pm 25 \text{mV} \), it is regarded as being “highly charged”\(^6\). This charge is directly related to the colloidal stability of the material as a highly charged particle will repel adjacent particles in the dispersion medium and prevent aggregation. Conversely, a low zeta potential indicates there is little surface charge on the particles: attraction will therefore exceed repulsion and the particles will tend to aggregate. This is particularly relevant when examining RTIL-prepared polymers as initial SEM analysis indicates that the polymers do, in fact, generally consist of an aggregation of smaller particles and the degree of aggregation may well have an impact on the rebinding performance of the polymer. In addition to giving an indication of the relative stability of the polymer in solution, the surface charge of the polymer may be related to number of monomer groups (in this case methacrylic acid groups) on the polymer surface.

2.4.3 Thermal Analysis

2.4.3.1 Thermogravimetric Analysis (TGA)

TGA analysis involves heating the polymer from room temperature and monitoring the loss in mass until such a point that it has completely decomposed. The onset and rate of decomposition of the material will differ according to the polymer structure and will be highly influenced by the degree of crosslinking of the polymer\(^7\).
2.4.3.2 Differential Scanning Calorimetry (DSC)

Additional information about the thermal behaviour of the polymers can be garnered through DSC analysis, which monitors the heat flow in and out of a sample as it is heated, cooled or held under isothermal conditions. These fluctuations can be attributed to phase changes such as glass transition, melting and crystallisation of the material. Processes such as melting and crystallisation are considered to be 1st order transitions as they involve both a change in latent heat as well as a change in heat capacity. Glass transition (Tg) on the other hand is a 2nd order transition as it only involves a change in heat capacity and is the temperature at which the polymer goes from a rubbery to a rigid glass state. These types of measurements can provide useful data in terms of the degree of crystallinity of the polymers, as well as the physical state of the polymers during rebinding processes.

2.4.4 Positron Annihilation Lifetime Spectroscopy (PALS)

PALS is a novel technique for the quantification of pore size down to pores the size of positronium (Ps, Bohr radius 0.53Å). Ps formed in the solid phase of the material diffuses into the pores and its lifetime is related to the size of the pore. This is a useful alternative to the adsorption method which involves the diffusion of gases into spaces which must be in the range of 3.3-3.6Å.
2.4.5 Polymer Swelling

Swelling analyses were conducted by packing 30mg polymer in a graduated syringe and measuring the dry volume. Solvent was added (CHCl$_3$ unless otherwise stated) and the polymer left to stand for 1 hour (cocaine-imprinted polymers) or 6 hours (PNL-imprinted polymers). Excess solvent was forced through the syringe and the volume of the swollen polymer measured. Percentage swelling was calculated using the equation: (swollen volume/dry volume) x 100.

2.5 Instrumentation

2.5.1 Gas Chromatography-Mass Spectrometry (GCMS)

All GCMS analyses were carried out on a Shimadzu 2010 gas chromatograph coupled to a Shimadzu QP2010 mass spectrometer and used a Shimadzu AOC-20s auto sampler at 71kPa with a column flow rate of 1mL/min and a total flow rate of 9mL/min. The carrier gas was high purity helium. The column was a ZB-5MS capillary column which was 30 m x 0.25mm, coated with 0.25µm of stationary phase. 1µl samples were injected and run on a specified method, using a split ratio of 15.

Cocaine (retention time 8.37 min) was quantified using an external calibration method with a 7 point linear curve where $R^2 = 0.992$ at the concentration range of 5-30µM. The GCMS program commenced with an initial hold at 100°C for 1 min, followed by an increase of 10°C min$^{-1}$ to 300°C.
2.5.2 High Performance Liquid Chromatography (HPLC)

HPLC studies were conducted using a Shimadzu High Performance Liquid Chromatograph (LC-20AD) fitted with an econosphere™ C₁₈, 5µm column (Grace®). For ephedrine analysis, the mobile phase consisted of 75% A (50mM phosphate buffer adjusted to pH3.5) and 25% B (3:7 water: acetonitrile, with 10mM TEA) (gradient elution). A 10µL injection volume was used with a run time of 10 minutes, flow rate of 0.8mL/min and detection wavelength of 190nm. Quantification was conducted using an external calibration method with a 7 point linear curve where $R^2 = 0.995$ at the concentration range of 10-1000µM. Results were analysed using Shimadzu LC Solution software. EPD concentration was monitored using the UV/VIS Photodiode Array Detector at a wavelength of 190nm. The mobile phase for caffeine consisted of a 70%/30% mixture of water/methanol. A 20µl injection volume was used with a run time of 5 minutes, flow rate 0.7mL/min and detection wavelength of 210nm. Quantification was conducted using an external calibration method with a linear curve of $R^2 = 0.993$ in the concentration range of 10-100µM.

2.5.3 Particle Sizing and Zeta Potential

Particle sizing and zeta potential measurements were conducted on a Malvern Zetasizer Nano using a plastic disposable zeta cell. All readings were done over a 10 run sequence (run time 10s) at 25°C.

Polymer particles were analysed using the recommended concentration of particles (0.001-0.010%) and measurements were taken after 15 minutes sonication in methanol.
2.5.4 Scanning Electron Microscopy (SEM)

SEM images were recorded on a Philips XL30 scanning electron microscope and Oxford ISIS EDS (1997) software.

2.5.5 Molecular Modelling

Computer modelling was conducted using Spartan 04®. The equilibrium geometries of the mixtures were calculated using semi-empirical optimisation at the AM1 level. Interaction energies (ΔE_{int}) were calculated using heats of formation (H_f) by the equation:

\[ \Delta E_{\text{int}} = \Delta H_{\text{products}} - (\Sigma \Delta H_{\text{reactants}}) \]

2.5.6 Fluorescence

Fluorescence measurements were conducted on a Cary Eclipse fluorescence spectrophotometer (excitation λ 290nm, emission λ 338nm for R, S-propranolol,) at medium voltage. Calibration curves were produced using a 7 point linear curve in the range of 0-50μM, with minimum R^2 values of 0.99.
2.5.7 Nuclear Magnetic Resonance (NMR)

$^1$H and $^{13}$C NMR experiments were conducted using a Bruker Avance 300 MHz spectrometer. NMR titrations were conducted by placing a capillary tube filled with deuterated solvent inside the NMR tube. This was used as an external lock to ensure that the solvent is not in contact with the RTIL, monomers, crosslinker or template and cannot affect their interactions. Following titration additions, the NMR tube was inverted and to ensure complete mixing of the components.

2.5.8 Thermogravimetric Analysis (TGA)

TGA was conducted on Perkin-Elmer Diamond TG/DTA using Pyris software version 8.0.0.0172. Analysis was conducted using 5mg of polymer in platinum pans. Samples were heated from 30ºC to 600ºC at a ramp rate of 10ºC/min.

2.5.9 Differential Scanning Calorimetry (DSC)

DSC measurements were carried out on a Shimadzu DSC 60A with a TA-60WS thermal analyser using TA60 software version 2.11. All samples were weighed and sealed in aluminium pans. Samples (approximately 5mg) were heated from -100ºC to 250ºC at a ramp rate of 10ºC min$^{-1}$ then cooled. All data was taken from the second run. A three point calibration using cyclohexane (m.p. 7ºC) indium (m.p. 157ºC) and zinc (m.p. 419.59ºC) as temperature standards.
2.5.10 Positron Annihilation Lifetime Spectroscopy (PALS)

PALS analysis was conducted by Dr Cara Doherty at CSIRO Materials Science and Engineering, Melbourne, Australia.

2.5.11 Brunauer-Emmet-Teller (BET) Analysis

BET surface area measurements were conducted on a Micromimetics ASAP 3030 surface area and porosity analyser using a 5-point surface area analysis. Approximately 100mg of polymer samples were analysed using $N_2$ as the adsorption gas.

2.5.12 Transmission Electron Microscopy (TEM)

TEM analysis was conducted by dispersing polymers in acetonitrile and examined using a JEOL JEM-1200EXII instrument with digital imaging software at various magnifications.

2.5.13 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analyses were conducted on a Shimadzu FTIR 8400S spectrophotometer using IR Solution software and a PIKE technologies 30SPEC specular reflectance accessory.
2.6 References


Chapter 3

RTILs as MIP preparation solvents

3.1 Introduction

The aim of this chapter is to give a general overview of the effect that RTILs have on the preparation, physical characteristics and rebinding capabilities of MIPs. Although the information regarding RTILs as polymerisation solvents is extensive, there has been very little research published using RTILs as solvents for the synthesis of crosslinked polymers in general\(^1,2\) and MIPs in particular\(^3-6\). This chapter compares the properties and performance of imprinted polymers prepared in VOCs with those prepared in RTILs and investigates the experimental parameters (such as temperature of polymerisation, solvent volume, rebinding conditions and template-RTIL combination,) which may have a role to play in these systems. This study aimed to further develop previous work in this area and evaluate these RTIL-based MIPs not only in terms of rebinding selectivity but also look at various physical characteristics of the polymers such as morphology, zeta potential, swelling and thermal stability.

The process of molecular imprinting depends primarily on the selection of an appropriate template/functional monomer pair for the chosen porogenic solvent. The target molecule must possess a number of desirable characteristics, the most important of these being the presence of functional groups for complementary pre-association with the chosen functional
monomer/s. Pre-association between the functional groups (in the case of non-covalent imprinting) must yield a template-functional monomer cluster of sufficient stability such that it is maintained during the polymerisation process. The template must also be soluble in the intended solvents, which in this case involves both the traditional VOCs as well as RTILs.

The template selected for investigation in this chapter was the illicit drug cocaine (I- see Chapter 2, Section 2.1). It was chosen based on the fact that, in addition to meeting the criteria outlined above, it is a template of relevance to our group due to an ongoing collaboration with the Australian Federal Police Forensic Services (AFPFS). Our group has been working with the AFPFS for the past 6 years in developing MIPs as illicit drug and explosive detection elements, with cocaine being one substance of particular interest.

Prior studies conducted by our group using cocaine as a template molecule have produced data showing that polymers selective for cocaine could be generated in both a VOC (CHCl₃) and RTILs ([bmim]BF₄ and [bmim]PF₆). These results provide a good foundation for the current study.

Previous research conducted by our group indicated that a change in the anion of the RTIL used as polymerisation solvent (from BF₄⁻ to PF₆⁻) influences polymer selectivity for the template for both trans-aconitic acid and cocaine-imprinted polymers when prepared under otherwise identical conditions. Therefore, one of the factors to be investigated in this chapter is the effect of various cation/anion combinations on the polymers’ physical characteristics and rebinding behaviour.

Details of all the RTILs used are outlined in Table 3.1. A range of RTILs were selected from those commercially available to include anions and cations of various sizes as well a range of viscosities.
Both [bmim] and [emim] cations have been considered (with butyl and ethyl imidazolium side chains, respectively), as the length of the alkyl side chain can result in differences in solubility\(^9\) which may affect polymer morphology. Viscosities of the RTILs range from 83.6 to 900 mPa s; substantially more viscous than the VOC chloroform (CHCl\(_3\), 0.51 mPa s\(^{10}\)) that has been used.

Many of the RTILs considered have previously been used as polymerisation solvents ([bmim]PF\(_6^{11}\), [bmim]BF\(_4^{11}\), [bmim]CF\(_3\)SO\(_4^{12}\) and [emim]SO\(_4^{13}\)), while others such as [bmim]HSO\(_4\), [bmim]MeSO\(_4\) and [emim]PO\(_4\), to the best of our knowledge, have not. Of these, only [bmim]PF\(_6\) and [bmim]BF\(_4\) have been used in the production of MIPs\(^{3\text{-}6}\). The studies discussed in this chapter aim to identify RTILs that reproducibly create well-performing MIPs, and assess factors contributing to variations in polymer performance.
Table 3.1  Structure and viscosity for all VOCs and RTILs used as porogens.

<table>
<thead>
<tr>
<th>Solvent*</th>
<th>Structure</th>
<th>Viscosity at 25°C (mPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td><img src="image1" alt="Cl-Cl" /></td>
<td>0.54</td>
</tr>
<tr>
<td>[bmim]BF₄</td>
<td><img src="image2" alt="N+" />BF₄⁻</td>
<td>104.2</td>
</tr>
<tr>
<td>[bmim]PF₆</td>
<td><img src="image3" alt="N+" />PF₆⁻</td>
<td>195.9</td>
</tr>
<tr>
<td>[bmim]HSO₄</td>
<td><img src="image4" alt="N+" />HSO₄⁻</td>
<td>900</td>
</tr>
<tr>
<td>[bmim]MeSO₄</td>
<td><img src="image5" alt="N+" />MeSO₄⁻</td>
<td>173</td>
</tr>
<tr>
<td>[bmim]CF₃SO₄</td>
<td><img src="image6" alt="N+" />CF₃SO₄⁻</td>
<td>83.6</td>
</tr>
<tr>
<td>[emim]SO₄</td>
<td><img src="image7" alt="N+" />SO₄⁻</td>
<td>120.4</td>
</tr>
<tr>
<td>[emim]PO₄</td>
<td><img src="image8" alt="N+" />PO₄⁻</td>
<td>553.7</td>
</tr>
</tbody>
</table>

*For full RTIL names refer to Chapter 2, Section 2.1
In addition to the choice of polymerisation solvent, polymerisation temperature is an important consideration for the preparation of well-performing MIPs. It is well documented that conducting polymerisations at low temperatures (photoinitiated by UV) with imprinted polymers can allow for a better ‘freezing’ of the interaction between the template and monomer and can therefore produce a better imprinted framework and produce improved levels of MIP selectivity. This highlights one of the major advantages of using RTILs for imprinting investigations, as studies have shown faster reaction rates in RTILs compared to VOCs under photoinitiated conditions, as well as the fact that some polymerisations do not proceed at all in VOCs at low temperatures (0-5°C).

Conducting polymerisations at low temperature, however, does have drawbacks in terms of the extent of the conversion of double bonds of the crosslinker. Lower polymerisation temperatures have been associated with decreased double bond conversions in VOCs, which may have an adverse effect on the binding site integrity within the polymer, although this effect is yet to be investigated in RTIL-produced polymers.

Another significant consideration in terms of MIP synthesis is volume of solvent used in the reaction, as this typically differentiates between two of the main imprinting formats i.e. bulk or precipitation. Bulk (or monolith) polymers are prepared using minimal solvent volumes, where the polymer particles coalesce during polymerisation to create a monolithic polymer structure. By contrast, precipitation polymers are prepared in a large solvent volume (usually >95% of the polymerisation mixture by volume), allowing the polymer to form in solution as discrete particles.

The effect of solvent volume on MIP morphology and selectivity has been documented in both VOCs and RTILs. In the case of VOCs, minimal amounts of solvent (5mL) produce polymers with a monolithic structure, whereas high solvent volume (25mL) promotes the formation of nanoparticles. In terms of pore size, high solvent volumes have been shown to
increase the pore volume of MIPs which, in turn, increase the rebinding capacity of the polymers\textsuperscript{17}. The effect of RTIL solvent volume on MIP morphology, however, is less distinct. Even at 5mL solvent volume, clusters of irregular shaped nanoparticles have been found to form. Increasing the volume to 25mL resulted in only minor visible changes, although differences in selectivity were observed over the various template/solvent/polymerisation temperature combinations studied\textsuperscript{8}. Only a limited number of characterisation studies have been conducted on the polymers and much remains to be understood about the precise nature of the polymers and how this leads to the observed variations in template selectivity.

Both polymerisation temperature and solvent volume will be considered here for a range of different RTILs to assess both qualitatively and quantitatively the impact of these factors on both MIP physical characteristics and selectivity for the template.

### 3.2 MIP Synthesis and Analysis

The cocaine-imprinted MIPs to be examined here are analogous to those prepared previously within our research group\textsuperscript{18}. Template (cocaine (1)), functional monomer (MAA (2)) and crosslinker (EGDMA (3)) were used in a ratio of 1:2:10 as this was shown to produce MIPs with good selectivity. \(\text{CHCl}_3\) was used as VOC porogen for comparison to the RTIL system, based on the high solubility of cocaine in \(\text{CHCl}_3\) and the fact that it had been successfully applied previously.
3.2.1 Polymer Synthesis

In order to investigate the effects of RTILs on polymerisation kinetics, yields and MIP selectivity, polymers were synthesised in a range of RTILs under different reaction temperatures (0°C and 60°C) and solvent volumes (5mL and 25mL). A summary of the polymers produced is given in Table 3.2.

Overall, the use of RTILs showed marked advantages over CHCl₃ in terms of production efficiency. Yields were increased by up to 50% for some RTIL-solvated polymerisations, for example with BF₄-60-5 where a 94% yield was obtained, compared to 46% in CHCl₃-60-5. Reaction times were reduced markedly in RTILs, with particularly fast reaction times observed in the low temperature photoinitiated polymerisations. At 60°C in 5mL solvent, reaction time was reduced from 6 hours in CHCl₃ to 2 hours in the RTILs and in 25mL, from 18 hours in CHCl₃ to 8 hours in RTILs. At 0°C, using UV-initiated polymerisation, a high polymer yield was obtained in RTIL polymerisations within 30-45 minutes, while there was no polymer formed in CHCl₃ (after a reaction time of 6 hours).

The effect of increased reaction rate in RTIL polymerisations is well documented in the literature and is attributed to a combination of an increased rate of propagation coupled with decreased rates of termination (due to the RTILs’ high viscosity)\(^{13}\).
Table 3.2  Cocaine-imprinted polymer synthesis conditions, reaction time and yield. All polymers were prepared using MAA as functional monomer and EGDMA as crosslinker.

<table>
<thead>
<tr>
<th>Polymer code</th>
<th>Polymerisation solvent</th>
<th>Reaction temperature (°C)</th>
<th>Solvent volume (mL)</th>
<th>Reaction time (hours)*</th>
<th>Polymer yield (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃-60-5</td>
<td>CHCl₃</td>
<td>60</td>
<td>5</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>CHCl₃-60-25</td>
<td>CHCl₃</td>
<td>60</td>
<td>25</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>BF₄-60-5</td>
<td>[bmim]BF₄</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>94</td>
</tr>
<tr>
<td>BF₄-60-25</td>
<td>[bmim]BF₄</td>
<td>60</td>
<td>25</td>
<td>8</td>
<td>86</td>
</tr>
<tr>
<td>BF₄-0-5</td>
<td>[bmim]BF₄</td>
<td>0</td>
<td>5</td>
<td>0.75</td>
<td>78</td>
</tr>
<tr>
<td>BF₄-0-25</td>
<td>[bmim]BF₄</td>
<td>0</td>
<td>25</td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td>PF₆-60-5</td>
<td>[bmim]PF₆</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>PF₆-60-25</td>
<td>[bmim]PF₆</td>
<td>60</td>
<td>25</td>
<td>8</td>
<td>84</td>
</tr>
<tr>
<td>PF₆-0-5</td>
<td>[bmim]PF₆</td>
<td>0</td>
<td>5</td>
<td>0.5</td>
<td>70</td>
</tr>
<tr>
<td>PF₆-0-25</td>
<td>[bmim]PF₆</td>
<td>0</td>
<td>25</td>
<td>2</td>
<td>73</td>
</tr>
<tr>
<td>HSO₄-60-5</td>
<td>[bmim]HSO₄</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>69</td>
</tr>
<tr>
<td>HSO₄-60-25</td>
<td>[bmim]HSO₄</td>
<td>60</td>
<td>25</td>
<td>8</td>
<td>52</td>
</tr>
<tr>
<td>HSO₄-0-5</td>
<td>[bmim]HSO₄</td>
<td>0</td>
<td>5</td>
<td>0.75</td>
<td>55</td>
</tr>
<tr>
<td>HSO₄-0-25</td>
<td>[bmim]HSO₄</td>
<td>0</td>
<td>25</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>MeSO₄-60-5</td>
<td>[bmim]MeSO₄</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>57</td>
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<tr>
<td>MeSO₄-0-5</td>
<td>[bmim]MeSO₄</td>
<td>0</td>
<td>5</td>
<td>0.75</td>
<td>61</td>
</tr>
<tr>
<td>CF₃SO₄-60-5</td>
<td>[bmim]CF₃SO₄</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>CF₃SO₄-0-5</td>
<td>[bmim]CF₃SO₄</td>
<td>0</td>
<td>5</td>
<td>0.75</td>
<td>60</td>
</tr>
<tr>
<td>eSO₄-60-5</td>
<td>[emim]SO₄</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>99</td>
</tr>
<tr>
<td>eSO₄-0-5</td>
<td>[emim]SO₄</td>
<td>0</td>
<td>5</td>
<td>0.5</td>
<td>86</td>
</tr>
<tr>
<td>ePO₄-60-5</td>
<td>[emim]PO₄</td>
<td>60</td>
<td>5</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>ePO₄-0-5</td>
<td>[emim]PO₄</td>
<td>0</td>
<td>5</td>
<td>0.5</td>
<td>21</td>
</tr>
</tbody>
</table>

* Average of MIP and NIP values. Yield was determined gravimetrically.
3.2.2 Polymer Morphology

SEM images of cocaine-imprinted polymers prepared in both CHCl₃ and RTILs are displayed in Figures 3.1-3.3 and show the variations in polymer morphology over the different solvents, polymerisation temperatures and solvent volumes.

The polymers prepared at 60°C in 5mL solvent (Figure 3.1) showed marked differences between the various solvent preparations. The CHCl₃-prepared polymer (image a, Figure 3.1) showed the monolithic structure and amorphous polymer morphology typical of polymers prepared under similar conditions in VOCs¹⁹.

However, the RTIL polymers prepared under these same conditions differ structurally, possessing a more defined structure composed of clusters of nanoparticles with diameters of <300 nm, a feature that results in a much higher visible surface area. This morphology is attributed to increased solubility of the polymer in the RTIL, promoting later phase separation, resulting in a higher surface area²⁰. The high viscosity of the RTIL also limits polymer diffusion, thereby preventing the separation of the particles into discrete units, leading to the formation of the observed aggregated structure.

The exceptions to the trend are the CF₃SO₄-60-5 and HSO₄-60-5 (image f and image d, Figure 3.1) which formed a space-filling macrogel and a gel, respectively, according to Stover’s system of naming²¹. The differences in morphology may be attributed to difference in viscosity, as these solvents appear at either end of the viscosity range studied here ([bmim]CF₃SO₄ = 83.6 mPa s and [bmim]HSO₄ = 900 mPa s).
Figure 3.1  SEM images of cocaine-imprinted polymers prepared in 5mL solvent at 60°C. (a) CHCl₃-60-5, (b) BF₄-60-5, (c) PF₆-60-5, (d) HSO₄-60-5, (e) MeSO₄-60-5, (f) CF₃SO₄-60-5, (g) ePO₄-60-5 and (h) eSO₄-60-5.
Figure 3.2  SEM images of cocaine-impanted polymers prepared in 5mL solvent at 0°C. (a) BF₄₀₋₀·₅, (b) PF₆₀₋₀·₅, (c) HSO₄₀₋₀·₅, (d) MeSO₄₀₋₀·₅, (e) CF₃SO₄₀₋₀·₅, (f) ePO₄₀₋₀·₅ and (g) eSO₃₀₋₀·₅.
Figure 3.3  SEM images of MIPs prepared in 25mL solvent at low and high temperature. (a) CHCl₃-60-25, (b) PF₆-60-25, (c) PF₆-0-25, (d) BF₄-60-25, (e) BF₄-0-25, (f) HSO₄-60-25 and (g) HSO₄-0-25.
Figure 3.2 shows the change in morphology observed when the polymers are prepared at 0°C. A decrease in visible surface area was apparent in nearly all polymer preparations. This can presumably be linked to the very high viscosity of the RTILs at such low temperatures, which will decrease the solvation of the polymer in solution during polymerisation as well as limiting diffusion, causing a bulk-like rather than particulate structure.

Polymer sample eSO_4-0-5, however, maintained a clear particulate morphology even under these low temperature polymerisation conditions. This cannot be directly related to the viscosity as [bmim]BF_4 and [bmim]CF_3SO_4 both possess lower viscosity than the [emim]SO_4 and both of these polymer preparations show a smoother surface than when prepared at 60°C. This may possibly be accounted for by differences in polymer solvation.

The effect of polymerisation temperature on polymer morphology is by no means unexpected as it has been well documented that the temperature of polymerisation has a direct influence on polymer particle size and porosity in traditional solvent systems. Increased temperatures of polymerisation have been associated with both higher and lower polymer surface areas and pore volumes in polymers prepared in CHCl_3 depending on the template/monomer/crosslinker used.

The effect of increasing the solvent volume was then examined at both high and low temperatures. However, due to the limited availability of some of the RTILs in this study, precipitation studies were limited to [bmim]BF_4, [bmim]PF_6, [bmim]HSO_4 and CHCl_3 (in the high temperature polymerisation only). SEM images of all polymers are given in Figure 3.3.

CHCl_3-60-25 (image a, Figure 3.3) showed a distinctly different morphology to CHCl_3-60-5. Nano-sized particles (~300nm) became evident with increased solvent volume, whereas in the 5mL preparation a monolithic structure was observed. In the RTIL-prepared polymers, there was little observable difference between the bulk and precipitation polymer preparations.
Both BF₄-60-25 and PF₆-60-25 (images b and d, Figure 3.3) maintained an agglomerated particulate structure analogous to that of the 5mL preparation (consistent with previous findings⁴), while HSO₄-60-25 (image f, Figure 3.3) still lacked any obvious visible surface features. This suggests limited movement of the polymer in the RTIL due to the high levels of viscosity.

At high solvent volumes, reducing the polymerisation temperature to 0°C again resulted in the polymers undergoing a marked reduction in visible surface area (images c, e and g, Figure 3.3). The polymers displayed more amorphous surface morphologies which lacked the particulate structure of the 60°C preparation. In the case of the [bmim]HSO₄-polymer preparation, relatively smooth polymer morphologies were displayed across the board. This is attributed to the extremely high viscosity of this RTIL and low polymer solvation, as discussed previously.

3.2.2 Thermogravimetric Analysis

The thermal stability of the polymers was analysed using TGA and the results plotted in Figure 3.4. As the NIP data closely resembled the MIP data, it has not been presented here. The 60°C 5mL prepared polymers (image a, Figure 3.4) showed some differences between the solvent preparations. All the polymers showed good thermal stability up to 260°C and generally contained multiple stages of decomposition as the polymer broke down. Polymer eSO₄-60-5 in particular showed distinct stages of polymer breakdown which were not complete until the polymer reached nearly 580°C. Other systems, such MeSO₄-60-5 and ePO₄-60-5, underwent much faster decomposition processes with less distinct phases. As the MIPs and NIPs gave very similar TGA profiles, the major cause of these variations in
polymer behaviour is ascribed to the influence of the preparation solvent. The exception to this is HSO₄-60-5, where the NIP showed
Chapter 4

R, S-Propranolol: A Model Template

4.1 Introduction

The selection of $R, S$-propranolol (PNL) as a template molecule for imprinting studies was based on the need to validate and benchmark the investigations against well established literature standards. PNL (7, see Chapter 2, Section 2.1) is an anti-hypertensive, beta blocking agent possessing a diverse range of functional groups (including an arene pi donor and a heteroatom containing alcohol and amine groups), making it an ideal imprinting template. The considerable amount of literature available on VOC-prepared MIPs imprinted with PNL\textsuperscript{1, 2}, and the fact that a high level of selectivity has been achieved in these previous studies, made it an ideal choice for this type of investigation. PNL-imprinted MIPs have previously been synthesised using both bulk and precipitation polymerisation methods\textsuperscript{3, 4} with imprinting factors as high as $I = 11$ observed. Analysis of the PNL-imprinted polymer system in RTILs was taken from first principles and the performance of RTIL-prepared MIPs was compared to those prepared in VOCs.

In traditional molecular imprinting studies, solvents are selected that will promote template/monomer interactions (typically apolar organics\textsuperscript{5}). However, RTILs consist of charged species (an anion and cation) and exhibit behaviour different to that typically observed in VOCs, such as the formation of structural domains within the liquid itself\textsuperscript{6}. These
characteristics may have an influence on the template/monomer associations in the pre-polymerisation mixture and on the physical characteristics of the final polymer (such as surface area and porosity).

As well as selecting a well-studied template for this study, it was necessary to choose RTILs which have been widely used as polymerisation solvents. [bmim]BF$_4$ and [bmim]PF$_6$ have received much attention in the literature as solvents for many types of polymerisation studies and therefore both of these RTILs were studied here. Methyl methacrylate (MAA) and divinylbenzene (DVB) were chosen as monomer and crosslinker, respectively. These choices were based on literature data showing that well-performing PNL-imprinted MIPs can be generated using this system.

4.2 Molecular Modelling and NMR Interaction Studies

Molecular modelling and NMR studies were conducted to determine if there were any interactions occurring in the pre-polymerisation mixture between the RTIL (as the porogen) and the monomer (MAA (2)), template (PNL (9)) or crosslinker (DVB (4)) which would potentially interfere with the creation of selective imprinted cavities in the polymer. A recent study involving the theoretical interaction between a BF$_4^-$ anion and glycine indicates that F-H interactions are likely between the BF$_4^-$ and alcohol and/or amine moieties of neighbouring compounds. For these modelling studies, a semi-empirical (AM1) modelling method was used. The semi-empirical method relies on experimental data sets for performing calculations and, as such, it is less accurate than ab initio modelling techniques that rely solely on quantum physics. However, ab initio methods are time consuming and computationally
expensive. Semi-empirical methods represent a significant advantage when there are many calculations to be performed, as is the case in this study.

All bond distances are given in Angstroms (Å) and can be generally classed as very strong (1.2-1.5 Å), strong (1.5-2.2 Å) and weak (2.0-3.0 Å). While it is acknowledged that the modelling studies cannot be thought of as being representative of the actual experimental conditions within RTIL solution, they do provide some insight into the relative strength of potential interactions between species within the solution, which can then be validated through the use of NMR analysis.

4.2.1 Molecular Modelling

Molecular modelling of the monomer, MAA, and RTILs showed hydrogen bonding between the OH group of the MAA and the fluorine molecules of both the [bmim]BF₄ (bond distance 2.3 Å, Figure 4.1) and [bmim]PF₆ (bond distance 1.6 Å, Figure 4.2). The shorter hydrogen bond distances with PF₆⁻ compared to BF₄⁻ (a strong vs. weak association) indicated a more favourable interaction between the MAA and PF₆⁻. The [bmim]⁺ cation showed no direct interaction with MAA in either system.

Figure 4.1  Modelling image of [bmim]BF₄ and MAA.
The geometry minimised cluster of the template (PNL) and [bmim]BF₄ is given in Figure 4.3 and indicated that there is strong hydrogen bonding occurring between the amine group of PNL and the BF₄⁻ anion, with an NH-F bond distance of 2.2Å.
The [bmim]PF$_6$ template modelling study revealed a similar hydrogen bonding interaction with the H-F bond distance being even stronger at 1.8Å (Figure 4.4).

![Modelling image of PNL and [bmim]PF$_6$.](image)

**Figure 4.4.** Modelling image of PNL and [bmim]PF$_6$.

In both template modelling studies it is of interest to note that the principal interaction with the anion occurred through the PNL amine rather than the more polarised hydroxyl group. The proximity of the latter to the bulky naphtyl ring and associated steric hindrances would appear to be the reason behind this observation.

The PNL-imprinted polymer system involved the use of divinylbenzene (DVB) as crosslinking monomer. Molecular modelling studies showed no observable interaction between the DVB and the BF$_4^-$ or PF$_6^-$ molecules (Figure 4.5 and 4.6). This was to be expected as the DVB does not possess potential hydrogen bonding sites.
The template/monomer mixture was first modelled in the absence of RTIL molecules. As template:monomer (T:M) ratios of 1:3 and 1:4 are the most commonly used in MIP literature in the synthesis of PNL-imprinted polymers, ratios of 1:1, 1:2, 1:3 and 1:4 were considered (Figure 4.7). At a 1:1 T:M ratio (image a, Figure 4.7), hydrogen bonding was evident between MAA hydroxyl group and the NH group of PNL (bond distance 2.3Å). Upon addition of another monomer unit (image b, Figure 4.7), additional hydrogen bonding occurred at the hydroxyl group of PNL (bond distance 2.3Å). At a 1:3 T:M ratio (image c, Figure 4.7), strong hydrogen bonding was evident at the PNL hydroxyl (bond distances 1.6Å and 1.9Å), amine
(bond distance 2.3Å) and ether (bond distance 1.9Å) groups, with the MAA hydroxyl group acting as both hydrogen bond donor and acceptor. A weaker monomer self-association interaction (bond distance 2.5Å) was also indicated. At a T:M ratio of 1:4 (image d, Figure 4.7), similar interactions to the 1:3 ratio were observed, as the fourth MAA unit does not appear to associate with the PNL, instead forming a relatively strong monomer/monomer association (bond distance 1.7Å) with another of the MAA molecules. This suggests that the optimal stoichiometry for cluster formation occurs with three MAA units surrounding the PNL template.
Figure 4.7. Modelling images of MAA and PNL. Template:monomer ratios of (a) 1:1, (b) 1:2, (c) 1:3 and (d) 1:4.

Modelling of the PNL/MAA mixture in the presence of [bmim]BF$_4$ and [bmim]PF$_6$ (Figure 4.8 and 4.9, respectively) was then conducted to ascertain changes in the template/monomer interactions. In each case, inclusion of the RTIL cation and anion showed a reorientation of some of the MAA monomer units away from PNL, to align with the fluorine atoms of the respective polyanions.

With [bmim]BF$_4$, H-F bond distances ranging from 1.6 to 2.2Å were observed between the BF$_4^-$ and both MAA and PNL at T:M ratios of 1:1 and 1:2. (images a and b, Figure 4.8). Interaction between the MAA hydroxyl and PNL ether oxygen lone electrons (which were not observed at these ratios in the absence of RTIL) were promoted due to preferential bonding between the BF$_4^-$ and the hydroxyl and amine groups of PNL. Hydrogen bonds were generally weaker ($\geq$2.4Å) at higher T:M ratios (1:3 and 1:4, images c and d, Figure 4.8) as steric hindrances increased.
Figure 4.8  Modelling images of MAA, PNL and [bmim]BF$_4$. Template:monomer ratios of (a) 1:1, (b) 1:2, (c) 1:3 and (d) 1:4.

With [bmim]PF$_6$, (Figure 4.9) the H-F bonds were stronger than those observed in the [bmim]BF$_4$ ($\leq$1.7Å). However, while the PF$_6^-$ and PNL interacted at a T:M ratio of 1:1
(image a, Figure 4.9, bond distance 1.7Å), there was no PF$_6$-PNL association evident at higher T:M ratios (1:3 and 1:4, images b-d, Figure 4.9) as MAA/PNL and MAA/PF$_6$- interactions predominate.

**Figure 4.9.** Modelling images of MAA, PNL and [bmim]PF$_6$. Template:monomer ratios of (a) 1:1, (b) 1:2, (c) 1:3 and (d) 1:4.
These results indicate that whilst the PF$_6^-$/MAA associations were stronger than the BF$_4^-$ /MAA associations, anion/PNL interactions were reduced in the [bmim]PF$_6$ system. Strong hydrogen bonds between MAA and PNL were observed despite the PF$_6^-$/MAA associations (2.0-2.5Å compared to 2.6-2.8Å in the presence of BF$_4^-$), suggesting that good template/monomer associations can be achieved.

### 4.2.2 NMR Interaction Studies

The modelling behaviour serves only as an indicator of interactions and is not entirely representative of the compound's behaviour in solution. To confirm the nature of the interactions in the pre-polymerisation mixture, NMR spectroscopy was utilised. While it would be preferable to utilise $^1$H NMR spectroscopy for the study, an external proton lock could not be obtained, leaving $^{13}$C NMR spectroscopy as the only viable alternative. The $^{13}$C NMR spectrum of pure MAA is given in Figure 4.10.

![MAA structure, $^{13}$C NMR spectrum of MAA in CHCl$_3$, with chemical shifts given in ppm.](image)
One RTIL system was selected for NMR studies i.e. [bmim]PF$_6$. The rationale for this choice was based on the molecular modelling data, which indicated that whilst the PF$_6^-$ interacted more strongly with the MAA and PNL than BF$_4^-$, hydrogen bonds of $<2.6\text{Å}$ between MAA and PNL were still observed. $^{13}$C NMR was then be used to substantiate these findings and determine if template/monomer associations can be observed even in the presence of a strongly interacting RTIL.

The interaction between MAA and [bmim]PF$_6$ was analysed by successive additions of MAA to 0.5mL [bmim]PF$_6$. The carbonyl carbon (C1) of MAA was selected for analysis as the modelling data indicated that this carbon was the most likely to experience a change in chemical shift as a result of changes in electron density of the attached hydrogen bond donor atoms. Partial $^{13}$C NMR spectra for the MAA-RTIL analysis showing the C1 resonances are given in Figure 4.11.

![Figure 4.11](image)

**Figure 4.11**  $^{13}$C NMR titration of MAA in 0.5mL [bmim]PF$_6$. MAA additions of (a) 6µl, (c) 8µl, (d) 10µl, (e) 12µl, (f) 14µl and (g) 26µl. Shifts are in ppm.
In the $^{13}$C NMR spectrum of pure MAA (Figure 4.10), C1 displayed a chemical shift ($\delta$) of 173ppm. When added to [bmim]PF$_6$ (image (a), Figure 4.11) the initial change in chemical shift for C1 shift was substantial (over 5ppm upfield) indicating a change in the electronic environment of the carbon in the presence of the RTIL compared with CHCl$_3$. The movement to a higher field chemical shift is consistent with a strong shielding effect resulting from hydrogen bonding interactions with the PF$_6^-$ fluorine atoms, as predicted by the modelling studies. Such interactions would result in an increase in electron density around the C1 atom. Further additions of MAA, however, resulted in the C1 shift moving progressively downfield, indicating increased deshielding of the C1 nucleus as a result of monomer/monomer self-associations (hydrogen bonding), resulting in electron density being withdrawn. The finding is consistent with previous modelling-NMR studies of MAA in conventional organic solvents where increasing MAA concentrations resulted in increased monomer/monomer self-association and a downfield shift in the NMR due to deshielding at C1$^{11}$. The predominance of monomer self-association over interaction with the RTIL suggests that favourable associations between monomer and template may still be likely in RTILs.

Interaction studies between [bmim]PF$_6$ and PNL were also conducted (see Figure 4.12 for PNL structure and $^{13}$C NMR assignations). NMR analysis of template/RTIL interactions were observed after adding PNL (13mg, 50µmoles) to [bmim]PF$_6$ (0.5mL), the same proportions that were used in the subsequent experimental polymerisation mixture. Only a single addition was performed in this case, as the aim was simply to determine if the template was interacting with the RTIL prior to the addition of MAA.
There were some interactions between the template and RTIL evident through $^{13}$C NMR shifts in the PNL resonance relative to the signal in CHCl$_3$ (Figure 4.12). These shifts were most pronounced for carbons located near potential hydrogen bond donor sites on the PNL (i.e. C3, C4 and C5). The $^{13}$C NMR shift of C3, which moved 0.46 ppm upfield, is given as an example in Figure 4.13. This is consistent with an increase in electron density caused by hydrogen bonding of the PNL hydroxide and amine moieties with PF$_6^-$, as indicated by the molecular modelling studies.
The chemical shift was less pronounced in carbons not directly attached to a hydrogen bond donor/acceptor group. The example of the $^{13}$C NMR spectrum of C10 on the napthyl ring of the PNL showed upfield movement of less than 0.1 ppm upon addition to the [bmim]PF$_6$ (Figure 4.14).
Figure 4.14  Partial $^{13}$C NMR spectra of (a) 13mg PNL in 0.5mL [bmim]PF$_6$ and (b) 13mg PNL in CHCl$_3$ showing the observed C10 shifts in ppm.

Subsequently, PNL/MAA associations were evaluated. $^{13}$C NMR studies were performed by dissolving 13mg of PNL in [bmim]PF$_6$ and adding successive amounts of MAA to the solution up to 6.5 mole equivalents. One molar equivalent of MAA to PNL is equal to a 4µl MAA addition.

The C1 carbon of the MAA underwent an initial upfield shift (4.53 ppm, Figure 4.15) upon addition to the solution and continued to track upfield as more MAA was added. The shielding of C1 may be due to hydrogen bonding between the MAA hydroxyl group with the ether of the PNL, or the F of the PF$_6$$. These interactions would donate electron density to C1 of MAA, causing the upfield movement observed in these NMR studies. The downfield shift upon addition of excess MAA (26µl, image g, Figure 4.15) is attributed to monomer self association.

Figure 4.15.  Partial $^{13}$C NMR spectra showing C1 resonances of MAA in a titration of MAA with 13mg PNL in 0.5mL [bmim]PF$_6$  (a) MAA,  (b-g) 13mg PNL in [bmim]PF$_6$ with MAA additions of (b) 6µl, (c) 8µl, (d) 10µl, (e) 12µl, (f) 14µl and (g) 26µl. Shifts are in ppm.
To confirm interaction between the MAA and the PNL ether group, the chemical shift of an adjacent carbon (C7) on PNL was also examined. Figure 4.16 shows upfield movement upon addition of MAA thus confirming the proposed interactions. Similar movements were observed at C6; C4 and C5 shifts however were obscured by the [bmim]PF$_6$ $^{13}$C NMR peaks.

![Partial $^{13}$C NMR spectra showing C7 resonances of PNL. Solution contains 13mg PNL in [bmim]PF$_6$ with MAA additions of (a) 0µl MAA, (b) 6µl MAA, (c) 8µl MAA, (d) 10µl MAA, (e) 12µl MAA (f) 14µl MAA and (g) 26µlMAA. Shifts are in ppm.](image)

A summary of the change in chemical shift for C1 in both the RTIL/monomer and the RTIL/monomer/template systems is displayed in Figure 4.17. Initial shifts are those observed in CHCl$_3$ and monomer additions are given as molar equivalents relative to the PNL mass. Without the template present, the monomer was observed to interact with the RTIL, but as more monomer was added, monomer/monomer interactions increased resulting in the gradual downfield movement of the MAA C1 carbon. By contrast, in the presence of both RTIL and template, template/monomer interactions resulted in continued upfield movement of the C1
carbon due to an increased shielding effect. Shielding continued until the potential hydrogen bonding donor/acceptor sites on the template had been exhausted (template:monomer ratios of ≥1:3) and monomer/monomer interactions could again predominate.

Figure 4.17 $^{13}C$ NMR shift of MAA C1 upon incremental MAA additions to [bmim]PF$_6$ with and without the presence of PNL.

Monomer/template titrations were repeated in CHCl$_3$ in order to compare the shifts observed in the RTIL with a VOC system. The movement of C1 (MAA) in CHCl$_3$ and [bmim]PF$_6$ in the presence of PNL is given in Figure 4.18.
The change in the C1 movement (from upfield to downfield) in the presence of the template occurred at the same monomer concentration in both the [bmim]PF₆ and CHCl₃ system, giving similar results (with the exception of the large initial shift in the [bmim]PF₆ system associated with MAA: RTIL interactions).

Finally the interaction of PNL with MAA in CHCl₃ and [bmim]PF₆ were studied by monitoring the movement of selected PNL carbon atoms (Figure 4.19). Carbons not directly attached to potential bonding sites with MAA (i.e. C8) showed little movement in either solvent. In [bmim]PF₆, C7 shifted 0.18ppm upon initial MAA addition, with no further movement observed as more MAA was added. In CHCl₃, C7 showed the same behaviour but with a larger initial shift of 0.4ppm. C5 underwent an initial 0.2ppm shift in [bmim]PF₆, and 1.5ppm in CHCl₃, indicating a much stronger association between MAA and the OH group of PNL when in CHCl₃. In [bmim]PF₆, C6 showed the most significant interaction with MAA with a gradual increasing shift up to 0.8ppm at 6 molar equivalents of MAA added, while in CHCl₃, C6 underwent an initial shift of 0.9ppm and the titration curve then levelled out as more MAA was added.
Figure 4.19 \[^{13}\]C shifts of selected PNL carbon atoms in (a) [bmim]PF\(_6\) and (b) CHCl\(_3\) upon the incremental additions of MAA. Refer to Figure 4.12 for carbon assignments.
These modelling and $^{13}$C NMR results show that hydrogen bonding between MAA and PNL occurs at low template:monomer ratios in CHCl$_3$ at carbons adjacent to the OH and ether groups of the PNL. In [bmim]PF$_6$, these interactions are suppressed somewhat by interaction between the PF$_6^-$ and MAA, although there is still some template/monomer association evident. This indicates that the formation of specific bonding sites in the [bmim]PF$_6$ system can still occur, although the site abundance and specificity may not be as high as in the CHCl$_3$ system.
4.3 Polymer Synthesis and Rebinding Analysis

Polymers were synthesised in both VOCs and RTILs for rebinding and characterisation studies. The behaviour of the RTIL-prepared systems was compared in detail to the VOC system using binding isotherm data and various physical characterisation techniques. Additionally, aspects such as solvent volume and choice of rebinding solvent were examined in order to see if template selectivity can be generated in the RTIL-prepared MIPs which is comparable to, or better than, those prepared in VOCs.

4.3.1 Polymer Synthesis

The selected solvent systems for preparation of the PNL-imprinted system were a 82.5:17.5 v:v mixture of acetonitrile:toluene (VOC), [bmim]BF₄ and [bmim]PF₆. The procedure followed was that reported by Castell et al³ for the creation of PNL-imprinted polymers via precipitation polymerisation. This procedure was followed for the preparation of polymers in both the VOC and RTILs. Unless stated otherwise, all polymers were prepared at 60°C in 30mL of solvent with a template:functional monomer:crosslinker ratio of 1:4:20.

Polymerisation in the RTILs was found to proceed at a much faster rate than in the VOC. Polymer yield was increased from a MIP/NIP average of 21% in the VOC after 18 hours to 65% in the RTIL after 6 hours, once again demonstrating the efficiency of an RTIL-based polymerisation system.

Following polymer synthesis, a series of studies were conducted to determine the optimum rebinding parameters for both the VOC and RTIL-prepared polymer systems. All measurements were conducted in triplicate; the error bars represent the standard deviation.
4.3.2 Polymer Mass Saturation Studies

Rebinding of PNL was conducted as a function of polymer mass to determine saturation binding conditions for a given solution concentration and exposure time period. CHCl₃ was used as rebinding solvent in all systems. The selection of CHCl₃ was based on reported data showing CHCl₃ to be the optimum rebinding solvent for similar PNL-imprinted systems out of a range of solvents studied. Figure 4.20 shows the rebinding behaviour of the VOC-prepared MIP (MIP<sub>VOC</sub>) and the VOC-prepared NIP (NIP<sub>VOC</sub>) towards 5mL of a 25µM solution of PNL after a rebinding time of 24 hours. The results show high levels of differentiation between MIP and NIP polymers due to the presence of selective sites for the template. MIP<sub>VOC</sub> reached saturation binding of 46% of the PNL present in rebinding solution with 20mg polymer (compared to 15% with NIP<sub>VOC</sub>), with only minimal increase in template uptake when polymer mass was increased to 30mg, indicating that the selective binding sites have been filled.
Figure 4.20  Rebinding of PNL in CHCl₃ as function of polymer mass for PNL-imprinted polymers prepared in VOC measured after 24 hours rebinding time.

Figure 4.21  Rebinding of PNL in CHCl₃ as function of polymer mass for PNL-imprinted polymers prepared in [bmim]BF₄, measured after 24 hours rebinding time.

Figure 4.22  Rebinding of PNL in CHCl₃ as function of polymer mass for PNL-imprinted polymers prepared in [bmim]PF₆, measured after 24 hours rebinding time.
The [bmim]BF₄ preparation, however, did not show the same differentiation between MIP and NIP (Figure 4.21), with no selectivity for PNL observed (within error) over the polymer mass range studied. The [bmim]BF₄-prepared MIP (MIP[bmim]BF₄) saturation point (37%) was reached when using 20mg polymer, with high levels of non-specific binding observed (32%).

When [bmim]PF₆ was used as polymerisation solvent (Figure 4.22), selectivity for the template was clearly evident. At the [bmim]PF₆-prepared MIP (MIP[bmim]PF₆) saturation (20mg polymer) binding values of 26% were recorded, representing a decrease in binding capacity compared to MIP[bmim]BF₄ (37%) and MIP_VOC (47%). This may be attributed to a decreased number of template/monomer interactions, due to some MAA/PF₆⁻ associations, thereby reducing the number of imprinted sites. This was confirmed by the fact that [bmim]PF₆-prepared NIP (NIP[bmim]PF₆) binding levels were similar to those observed for NIP_VOC (13% compared to 15%).

The different behaviour between the [bmim]BF₄ and [bmim]PF₆ polymer preparations suggests that the RTIL anion has had a significant impact on the nature of the polymer produced and the creation of selective sites for the template. This correlates to the modelling behaviour and indicates that the BF₄⁻ /PNL interactions are preventing MAA/PNL associations, and therefore preventing the creation of selectively imprinted cavities within the polymer.

Once the polymer concentration required for saturation (20mg) had been identified for the 25µM rebinding solution used, these parameters were used to look at the variation of binding behaviour over time.
4.3.3 Time Dependant Rebinding Studies

Each polymer preparation was then subject to a time-based rebinding assessment to compare rebinding behaviour. Studies were conducted at saturation conditions (20mg polymer, 25µM solution of PNL in CHCl₃ over a 24 hour time period). As Figure 4.23 shows, both MIP and NIP for the VOC and RTIL systems reached a binding equilibrium after 6 hours rebinding time.

MIP_{VOC} and NIP_{VOC} (image a, Figure 4.23) showed increased template uptake up until the 6 hour mark, with little to no change in rebinding after this time. Clear differentiation between MIP and NIP was observed, giving an imprinting factor at 6 hours of \( I = 4.8 \).

The [bmim]BF₄-prepared polymer (image b, Figure 4.23) did not show any selectivity for the template. The high levels of NIP_{[bmim]BF₄} binding observed in Section 4.3.2 did not change over the 24 hour time period studied, again indicating that the [bmim]BF₄ preparation has not led to the generation of template-specific cavities, consistent with the molecular modelling studies.

MIP_{[bmim]PF₆} and NIP_{[bmim]PF₆} (image c, Figure 4.23) showed rapid uptake of the template in the first 2 hours. This rapid absorption (possibly due to a high surface area polymer) may be the reason behind the disjointed nature of the curve and leads to a high degree of error in measurements. MIP binding (B_{MIP}) is higher than NIP binding (B_{NIP}), indicating the presence of template selective sites in the polymer. The imprinting factor in this case, however, was not as high as in the VOC preparation, giving \( I = 2.3 \) at 6 hours.
Figure 4.23  Rebinding from 5mL of a 25µM PNL solution in CHCl₃ over 24hr time period with polymers prepared in (a) VOC, (b) [bmim]BF₄ and (c) [bmim]PF₆₅
4.3.4 Binding Isotherms and Scatchard Analysis

Based on polymer mass saturation and time rebinding studies, binding isotherms were conducted in triplicate on both MIP\textsubscript{VOC} and MIP\textsubscript{[bmim]PF\textsubscript{6}} using 20mg polymer in 5mL of PNL solutions of various concentrations over a 6 hour time period; the error bars represent the standard deviation of the measurements. The saturation rebinding curves are shown in Figure 4.24 and show a higher binding capacity in MIP\textsubscript{VOC}, with a bound concentration of PNL ([B]) of 7µM compared to 5µM in MIP\textsubscript{[bmim]PF\textsubscript{6}}, consistent with the mass saturation and time rebinding studies. High levels of reproducibility were observed over the three trials for both polymer systems.

As MIP binding sites are generally heterogeneous in nature, they cannot be evaluated by standard binding isotherms. Scatchard plots provide a means of evaluating these different classes of binding site through manipulation of the binding isotherm data using the Scatchard equation:

\[
[B]/[C] = -(1/K_d[B]) + (N/K_d)
\]

where \( B \) = amount of template bound, \( C \) = free concentration of template, \( N \) = total recognition cavity concentration and \( K_d \) is the dissociation constant for the template.

Scatchard analysis has been used widely in MIP-based systems and can be a useful tool in the determination of the presence of different classes of binding sites as well as their relative dissociation constants\textsuperscript{12}. The influence of RTILs as porogens on these MIP characteristics can be evaluated through comparison of the Scatchard plots for a VOC and RTIL-prepared system. Due to the fact that the \([\text{bmim}]\text{BF}_4\)-prepared system had shown no template
selectivity up to this point, only the [bmim]PF$_6$-prepared system was used to compare VOC and RTIL.

**Figure 4.24** Saturation binding data for PNL-imprinted MIP prepared in (a) VOC and (b) [bmim]PF$_6$ using 20mg polymer in PNL solutions of various concentrations over 6 hours. The selected region indicates data used for Scatchard calculations.
The binding isotherm data was transformed and replotted as a Scatchard plot by dividing [B] by the free template concentration [C] and plotting against [B]. The binding constant (K_a) was then determined from the slope. The Scatchard plots of MIP_{VOC} and MIP_{[bmim]PF_6} are shown in Figure 4.25 and the calculated properties of the polymer in Table 4.1. The linear nature of both plots in these concentration ranges indicates a homogeneous distribution of binding sites, consistent with other reports involving polymers prepared via precipitation methods at similar concentrations\textsuperscript{13, 14}.

![Figure 4.25](image_url)

**Figure 4.25**  Scatchard analysis of VOC and [bmim]PF_6-prepared MIP where [B]= PNL bound and [C]= free PNL concentration.

<table>
<thead>
<tr>
<th>Preparation solvent</th>
<th>K_a (M)</th>
<th>K_d (M)</th>
<th>N (mole)</th>
<th>ΔG_{bind} a (kJ mol\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOC</td>
<td>0.0299</td>
<td>3.34x 10\textsuperscript{-5}</td>
<td>1.37 x 10\textsuperscript{-5}</td>
<td>-25.54</td>
</tr>
<tr>
<td>[bmim]PF_6</td>
<td>0.0624</td>
<td>1.60 x 10\textsuperscript{-5}</td>
<td>9.77 x 10\textsuperscript{-6}</td>
<td>-27.36</td>
</tr>
</tbody>
</table>

\[ ΔG_{bind} = -RT \ln K_d, \quad T = 298 K, \quad R = 8.314 \text{ JK}^{-1} \text{ mol}^{-1}. \]

The K_d value of MIP_{VOC} is more than twice that of MIP_{[bmim]PF_6} (Table 4.1), indicating that the binding cavities are less well-defined in the VOC polymer preparation. However the total
The number of binding cavities (N) is greater in MIP\textsubscript{VOC} (1.37 x 10\textsuperscript{-5} vs. 9.77 x 10\textsuperscript{-6} in MIP\textsubscript{[bmim]PF\textsubscript{6}}), which explains the higher binding capacity of MIP\textsubscript{VOC}. The decrease in MIP\textsubscript{[bmim]PF\textsubscript{6}} binding capacity may be due to the fast kinetics of polymerisation, which limits the thermally driven template/functional monomer equilibrium processes necessary for the formation of MIP binding cavities\textsuperscript{15}.

### 4.4 Polymer Characterisation

#### 4.4.1 Polymer Morphology

Polymers prepared using excess solvent showed differences in morphology depending on the solvent used (Figure 4.26). The polymers prepared in the VOC (image a and b, Figure 4.26) produced discrete MIP particles in the size range 1-3µm. In the case of [bmim]PF\textsubscript{6} (image c and d, Figure 4.26) discrete particles were not obtained, however the relative size of the polymer particles was greatly decreased, with particles in the range of 300-500nm. This may explain the rapid uptake of PNL by these polymers in the previous section.

While the [bmim]PF\textsubscript{6}-prepared polymer particles maintained a similar structure and particle size between the MIP and NIP, this was not the case in the VOC preparation. The majority of MIP\textsubscript{VOC} particles (image a, Figure 4.26) had a fairly regular size and shape (1.5-2µm), while the NIP\textsubscript{VOC} particles (image b, Figure 4.26) showed some particles of larger size range (1-3µm). This is consistent with reported data in similar PNL imprinted systems\textsuperscript{3}. It has been observed that NIP particles of the same size distribution can be synthesised through adjustment of the acetonitrile:toluene ratio, however this would introduce another variable into the NIP system, compromising the legitimacy of MIP/NIP comparisons.
Transmission Electron Microscopy (TEM) analysis was conducted as an alternative visualisation method to SEM in the hope of gaining a clearer idea of the particle sizes present in the polymers (particularly in the case of the [bmim]PF<sub>6</sub>-prepared polymers) as the TEM technique allows for the dispersion of the polymer in solution prior to passing light through the sample. Acetonitrile (CH<sub>3</sub>CN) was used as dispersion solvent. NIP<sub>[bmim]PF<sub>6</sub></sub> was not examined due to its similarity to MIP<sub>[bmim]PF<sub>6</sub></sub>. 

**Figure 4.26** SEM images of PNL-imprinted polymers. (a) MIP<sub>VOC</sub>, (b) NIP<sub>VOC</sub>, (c) MIP<sub>[bmim]PF<sub>6</sub></sub> and (d) NIP<sub>[bmim]PF<sub>6</sub></sub>. 
The TEM analysis of the VOC-prepared polymers gave similar information to the SEM in terms of the size and shape of the polymer particles (Figure 4.27). Again, the NIPVOC particles (image b, Figure 4.27) consisted of a much larger range of sizes than the MIPVOC (image a, Figure 4.27).

As for MIP[bmim]PF6, TEM (image c, Figure 4.27) shows that the particles have remained in aggregated clusters in solution. Although some different dispersion solvents were investigated (i.e. acetone and methanol), similar TEM images were obtained, showing a very fine particulate structure.
4.4.2 Thermal Analysis

The thermal properties of the polymer systems were investigated by TGA and DSC (Figure 4.28). TGA showed similar decomposition processes in MIP\textsubscript{VOC} and MIP\textsubscript{[bmin]PF\textsubscript{6}} (NIP profiles were almost identical to those of the MIP so have been omitted). Both MIPs displayed an onset of decomposition from 300°C and appeared to undergo two clear decomposition phases, with MIP\textsubscript{VOC} showing increased thermal stability in the later decomposition phases.

DSC analysis was then conducted. As Figure 4.29 shows, there was no evidence of a $T_s$ or melting point in any of the polymers studied (this was true for both MIPs and NIPs). This shows that the PNL-imprinted polymers will not go through any transitional states before the onset of polymer decomposition, indicative of the highly crosslinked nature of the polymer\textsuperscript{16}. This is attributed largely to the use of DVB as crosslinking agent, as discussed in Chapter 3, Section 3.2.8.
Figure 4.28  TGA traces of PN L-imprinted MIPs.

Figure 4.29  DSC scans of PN L-imprinted MIPs.
4.4.3 Polymer Swelling

Swelling studies were conducted, as polymer swelling may influence both the binding capacity and binding site integrity of the polymer (as discussed in Chapter 3, Section 3.2.4). The [bmim]PF₆-prepared polymer showed significantly less swelling than the VOC-prepared polymers in CHCl₃ after 6 hours (Figure 4.30). These results indicate that the higher binding capacity observed in the VOC polymer preparation may be related to greater swelling of the polymer, facilitating template access to the polymers’ internal rebinding cavities. However, lower swelling in the [bmim]PF₆-prepared polymer suggests that it can maintain a higher level of binding site integrity (due to less cavity deformation) than the VOC-prepared polymer. This correlates with the Scatchard analysis which showed the MIP[bmim]PF₆ to have higher affinity sites. The higher levels of MIP swelling than NIP swelling observed in both polymer preparations indicates the presence of imprinted cavities within the polymer matrix, as these cavities will be occupied by the solvent in the absence of any template molecules.
4.4.4 Zeta Potential

Zeta potential measurements of all four polymer preparations were performed in methanol after a 15 minute sonication in order to disperse the polymer particles in solution. The data (Figure 4.31) clearly shows the difference in surface charge between the MIP$_{[\text{bmim}]PF_6}$ (-21mV) and MIP$_{\text{VOC}}$ (-37mV). This value for the VOC polymer preparation exceeds the -25mV necessary for the polymer to be colloidally stable and well dispersed in solution$^{17}$.

For polymers with a uniform distribution of MAA, zeta potential is expected to correlate with the polymer surface area$^{18}$ and may, therefore, be related to the degree of surface binding. The higher charge of these VOC-prepared polymers also indicates that there is a higher concentration of functional monomer groups present on the polymer surface than in the [bmim]PF$_6$-prepared polymer. This is not consistent with rebinding analysis where MIP$_{\text{VOC}}$ binding was higher than MIP$_{[\text{bmim}]PF_6}$, while NIP rebinding levels are comparable. However,
the high surface charge on the VOC-prepared polymers is responsible for the stabilisation of
the polymer dispersion, which may be the more favourable energy driven process in this case.

4.4.5 Pore Size and Surface Area

The pore size and distribution of PNL-imprinted polymers prepared in both VOC and
[bmim]PF₆ were analysed by Positron Annihilation Lifetime Spectroscopy (PALS). The
PALS technique involves bombarding the polymer samples with positrons and measuring its
lifetime and the intensity of the measurement. Longer lifetime of the positron is associated
with increased pore size, whilst the intensity correlates to the number of pores present¹⁹.
PALS was undertaken in place of the more commonly used gas adsorption methods (such as
Brunauer-Emmet-Teller analysis, or BET) for pore size analysis as literature has shown the
PALS method to be more accurate for the sensing of micropores¹⁹. This is due to
discrepancies observed in BET between the adsorption and desorption isotherms when
examining pores of < 2 nm diameter. PALS data is given in Table 4.2 as an average of two
samples measured.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Micropore diameter (nm)</th>
<th>Micropore intensity</th>
<th>Mesopore diameter (nm)</th>
<th>Mesopore intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOCMIP</td>
<td>1.10</td>
<td>10.33</td>
<td>2.18</td>
<td>8.45</td>
</tr>
<tr>
<td>VOCPNIP</td>
<td>1.14</td>
<td>10.62</td>
<td>2.23</td>
<td>11.69</td>
</tr>
<tr>
<td>[bmim]PF₆MIP</td>
<td>0.97</td>
<td>7.46</td>
<td>7.06</td>
<td>2.95</td>
</tr>
<tr>
<td>[bmim]PF₆NIP</td>
<td>0.92</td>
<td>5.15</td>
<td>11.15</td>
<td>2.94</td>
</tr>
</tbody>
</table>

Table 4.2 shows that in both the VOC and [bmim]PF₆ polymer preparations, there were two
distinct pore types present in both MIP and NIP i.e. micro- and mesopores. Micropores are
classified as those with pore diameters of < 2 nm, while mesopores typically have pore
diameters of > 2 nm\textsuperscript{19, 20}. The maximum pore diameter of the micropores across all polymer preparations was 1.1 nm (NIP\textsubscript{VOC}). The ability of the template to access these smaller size pores would be substantially restricted by the size of the PNL molecule (1.3 x 0.8 nm as measured using molecular modelling). It is therefore unlikely that the micropores will have a significant bearing on template binding.

A summary of the mesopore data is given in Figure 4.32 and shows the significant differences in both pore size and number between the polymer preparations. Whilst the pores measured in MIP\textsubscript{[bmim]PF\textsubscript{6}} (7.06 nm) and NIP\textsubscript{[bmim]PF\textsubscript{6}} (11.15 nm) were substantially larger than those in MIP\textsubscript{VOC} (2.19 nm) and NIP\textsubscript{VOC} (2.23 nm), the total number of pores was much lower in the [bmim]PF\textsubscript{6} preparations. This is consistent with the Scatchard data which showed MIP\textsubscript{VOC} to have a greater number of pores than MIP\textsubscript{[bmim]PF\textsubscript{6}}. There was also an increase in number of pores from MIP\textsubscript{VOC} to NIP\textsubscript{VOC} (intensities of 8.4 and 12.7, respectively). The broader distribution of particle sizes in NIP\textsubscript{VOC} (observed via SEM and TEM) may account for this difference.

The pore size discrepancy between MIP\textsubscript{[bmim]PF\textsubscript{6}} and NIP\textsubscript{[bmim]PF\textsubscript{6}} is consistent with other reports that have observed larger pore sizes in NIPs due to a compactness of the imprinted cavity in the MIP caused by the presence of the template upon cooling of the polymer from polymerisation temperature (60°C) to room temperature\textsuperscript{21}.
BET analysis (while not the preferred method for detailed pore analysis) can be used to measure the specific surface area of a polymer via gas absorption onto the solid surface. It is based on the adsorption-desorption isotherms generated at critical pressure and liquefaction temperature. N$_2$ gas absorption on the four different polymers (VOC and [bmim]PF$_6$, MIP and NIP) was conducted to see if the specific surface area measurements correlated with the physical morphology and porosity measurements of the samples. BET data is given in Table 4.3.

**Table 4.3** BET data for [bmim]PF$_6$ and VOC-prepared MIP and NIP

<table>
<thead>
<tr>
<th>Preparation solvent</th>
<th>Specific surface area (m$^2$/g)</th>
<th>Pore volume (cc/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIP</td>
<td>NIP</td>
</tr>
<tr>
<td>VOC</td>
<td>306</td>
<td>509</td>
</tr>
<tr>
<td>[bmim]PF$_6$</td>
<td>185</td>
<td>180</td>
</tr>
</tbody>
</table>
NIP\textsubscript{VOC} showed much larger pore size and surface area than MIP\textsubscript{VOC}. This supports the PALS data which showed a greater number of mesopores in the NIP. In the [bmim]\textsubscript{PF}_6-prepared polymer, there was only slight variation from MIP to NIP. In NIP\textsubscript{[bmim]PF}_6, the slightly lower surface area (180m\textsuperscript{2}/g compared to 185m\textsuperscript{2}/g in MIP\textsubscript{[bmim]PF}_6), along with the slightly larger pore volume (0.302cc/g compared to 0.295cc/g in MIP\textsubscript{[bmim]PF}_6), is consistent with the PALS findings of larger pore diameters in NIP\textsubscript{[bmim]PF}_6.

The substantial increase in specific surface area observed in the VOC-prepared polymers compared to the [bmim]\textsubscript{PF}_6-prepared polymers is attributed to the decreased number of mesopores present in the latter which result in a much lower specific surface area, whilst maintaining a high pore volume due to large pore size. This may explain the rebinding behaviour observed in the polymers, as the higher surface area of the VOC-prepared polymers will increase overall binding capacity compared to the [bmim]\textsubscript{PF}_6-prepared polymers. The larger mesopores present in NIP\textsubscript{[bmim]PF}_6 compared to MIP\textsubscript{[bmim]PF}_6 may be responsible for the higher proportion of non-specific binding.

These results correlate well to another study involving the preparation of crosslinked polymers in VOCs compared to RTILs, which have also shown larger pore sizes and decreased surface areas in RTIL-prepared polymer when using toluene and 1-octyl-3methylimidazolium bis((trifluoromethyl)sulfonyl)imide as VOC and RTIL, respectively\textsuperscript{22}. It is hypothesised that the large pore volumes observed in polymers prepared with RTILs as porogens arises from the more structured arrangement of RTIL molecules compared to VOCs\textsuperscript{6} which may result in the RTIL forming a ‘micellar’ structure (as proposed by Triolo et al\textsuperscript{23}). The relatively larger size of these ‘micelles’ compared to VOC molecules permeating the polymer structure would explain the discrepancy in pore size.
4.5 Cross-Reactive Rebinding Studies

In order to assess the selectivity of the imprinted polymers for PNL over other compounds, a cross-reactive binding assay was conducted using both a structurally analogous and a non-analogous compound (ephedrine and caffeine, respectively). Chemical structures of these compounds are shown in Figure 4.33.

As the ephedrine molecule possesses proximally oriented functional groups common to PNL (pi donor arene and hydrogen bond donor/acceptor amine and hydroxyl), it was hypothesised that the MIPs would exhibit the highest competitive binding affinity for ephedrine. Rebinding studies were conducted using 20mg polymer in 5mL of a 25μM solution of analyte for 6 hours in CHCl₃. Rebinding data for the various analytes are given in Figure 4.34.

![Chemical structures](image)

**Figure 4.33** Chemical structures of (a) caffeine (CAFF), (b) ephedrine (EPD) and (c) propranolol (PNL).
As expected, there was a large decrease in the levels of binding of CAFF compared to the PNL binding for both polymers. CAFF binding was higher in the [bmim]PF₆-prepared polymers than in the CHCl₃-prepared polymer for both MIP and NIP, but both exhibited no selectivity for the CAFF molecule. The low binding of CAFF was attributed to the difference in size and shape of the CAFF molecule as well as the absence of hydrogen bond donor groups. These differences are highlighted in Figure 4.35 which shows both the differences in size and electron potential of the three molecules. Areas of high and low electron density are indicated by red and blue, respectively, and show the presence of strong hydrogen bond donating groups in EPD and PNL (images b and c, Figure 4.35) which are not present in CAFF (image a, Figure 4.35).
In the presence of EPD, both polymer preparations showed predictably higher affinity than for CAFF. This was to be expected given the higher degree of structural similarity between EPD and PNL, both of which possess hydroxide and amine groups capable of acting as hydrogen bond donors for binding with MAA. MIP\textsubscript{VOC} displayed higher binding for PNL than EPD (48% compared to 37%) with NIP\textsubscript{VOC} binding remaining constant (10%), resulting in a reduction in $I$ value from $I = 4.8$ for PNL and $I = 3.6$ for EPD. A reduction in imprinting factor for the
[bmim]PF$_6$-prepared polymers was also evident (from $I = 2.3$ for PNL to $I = 1.5$ for EPD. However, the MIP and NIP binding levels in the [bmim]PF$_6$-prepared polymer for both analytes were within the range of experimental error. The fact that MIP$_{[bmim]PF6}$ did not appear to discriminate between PNL and EPD in the same way as MIP$_{VOC}$, does not correlate to the $K_d$ values generated via Scatchard analysis. The $K_d$ values indicated a more well-defined binding cavity in MIP$_{[bmim]PF6}$ than in MIP$_{VOC}$. However, the amounts of EPD bound for both MIPs was comparable and suggests that the relatively fewer number of binding sites in MIP$_{[bmim]PF6}$ and the corresponding lower rebinding capacity may be responsible for the lower levels of selectivity in MIP$_{[bmim]PF6}$. This would effectively mask any differences in selectivity for PNL over EPD in MIP$_{[bmim]PF6}$. Other studies have also reported high levels of binding for structurally analogous compounds with PNL-imprinted polymers, where binding levels for analytes such as pindolol and acebutolol are actually higher than for PNL itself. Discrimination of the MIP for PNL may also be limited slightly by the use of a mixture of enantiomers as template, which would increase the heterogeneity of binding sites and reduce the ability of the polymer to distinguish between structurally analogous compounds.

### 4.6 Factors Affecting Rebinding Performance

#### 4.6.1 Effect of Polymerisation Solvent Volume

As previous studies involving the use of cocaine as template molecule indicated that the volume of polymerisation solvent has an influence on the polymer’s rebinding performance of the resulting MIP (see Chapter 3), this effect was again investigated here in the PNL-imprinted system. Only the RTIL (not VOC) system was prepared at the lower solvent volumes, as the VOC reference standard for this section is that of the precipitation VOC polymer preparation.
Polymers were prepared under identical conditions to those prepared in [bmim]PF₆ in Section 4.3.1, but using 5mL of [bmim]PF₆ instead of 30mL. This did not appear to have a large impact on the polymer morphology, as the SEM images in Figure 4.36 shows.

![SEM images of PNL MIPs synthesised in (a) 30mL and (b) 5mL [bmim]PF₆.](image)

Subsequent rebinding analysis was conducted in triplicate using 20mg polymer in 5mL of a 25µM PNL solution in CHCl₃, conducted over a 6 hour rebinding period; the error bars represent the standard deviation of the measurements. An effect of polymerisation solvent volume on total polymer uptake was observed, with the [bmim]PF₆ 5mL polymer preparation binding approximately half that of the [bmim]PF₆ 30mL preparation (Figure 4.37), a further reduction from the binding capacity observed in the VOC system. In terms of the imprinting factor, there was an increase in selectivity from $I = 2.3$ in the [bmim]PF₆ 30mL-prepared polymer to $I = 2.6$ in the [bmim]PF₆ 5mL-prepared polymer (although these were not outside experimental error of each other), values which are still lower than $I = 4.8$ observed in the VOC system.
Figure 4.37 Rebinding results after 6 hours rebinding of a 25µM solution of PNL for PNL-imprinted polymers prepared in various solvents at different volumes. Imprinting factors are given in bold.

The lower uptake of template in the [bmim]PF₆ 5mL polymer preparation is unsurprising given the literature indicating that, firstly, a reduction in solvent (VOC) volume will lead to a reduction in polymer surface area²⁴ and, secondly, that a reduction in surface area will result in lower total amounts of template bound by the polymer²⁵. A higher surface area in the polymer prepared in 30mL [bmim]PF₆ can be explained by either increased permeation of the solvent into the polymer system, or higher levels of solubility of the polymer (which would lead to a later phase separation and higher surface area³). The latter explanation is the more likely, particularly given the results with cocaine-imprinted polymers (Chapter 3, Section 3.2.5), which indicated that permeation of [bmim]PF₆ into the polymer may be restricted by viscosity at high solvent volumes. As one of the limitations of using RTILs as solvents is the high initial cost of the solvents compared to VOCs, the fact that polymers with high selectivity for the template can be prepared using substantially lower solvent volumes than those used in traditional precipitation polymerisations (and not requiring grinding down as with monolithic polymers formed in low volumes of VOCs) is a significant advantage.
4.6.2 Effect of Rebinding Solvent

As it is generally accepted that an imprinted polymer will rebinding most effectively in the same solvent used for synthesis, it is a logical step to investigate the rebinding performance of RTIL-prepared polymers in an RTIL. As mentioned previously, this is not a very practical option due to the highly viscous nature of most RTILs. In this particular case, with polymers prepared in [bmim]PF$_6$, the viscosity would prove to be a limiting factor in the execution of rebinding studies. For this reason, rebinding was instead conducted in a VOC solution spiked with 20% [bmim]PF$_6$. Although the VOC of choice would be CHCl$_3$, as this is the solvent in which all previous rebinding studies have been conducted, CHCl$_3$ and [bmim]PF$_6$ are not miscible, so methanol (MeOH) was used as an alternative. MeOH is not generally utilised as a rebinding solvent for MIPs, as its polar nature can disrupt hydrogen bonding interactions between template and MIP and reduce selectivity. However, literature has shown that MeOH, although not the optimal choice, has been used to good effect when rebinding PNL-imprinted polymers.

The rebinding data of the PNL-imprinted polymers in a pure MeOH solution and the solution containing 20% [bmim]PF$_6$ is given in Figure 4.38. Rebinding was conducted over 6 hours, using a 20 mg polymer in 5mL of a 25µM solution of PNL. All measurements were made in triplicate; the error bars represent the standard deviation.
Rebinding results in MeOH and in MeOH containing 20% [bmim]PF$_6$ after 6 hours rebinding of a 25µM solution of PNL for PNL-imprinted polymers. Imprinting factors and rebinding solvent are given in bold.

Both VOC and [bmim]PF$_6$-prepared polymers showed a high level of selectivity for the template in MeOH, with imprinting factors of $I = 2.3$ and $I = 3.8$ in the VOC and [bmim]PF$_6$-prepared polymers, respectively.

Compared to the rebinding observed in CHCl$_3$, MIP$_{VOC}$ binding levels have remained the same while NIP$_{VOC}$ binding has increased from 10% in CHCl$_3$ to 20%, in MeOH, resulting in the decreased imprinting factor from $I = 4.8$ to $I = 2.8$. This was not the case with the [bmim]PF$_6$ polymer preparation, where NIP$_{[bmim]PF_6}$ rebinding levels have stayed the same but there is a substantial increase in MIP$_{[bmim]PF_6}$ rebinding from 31% to 48%, resulting in the imprinting factor increasing from $I = 2.3$ to $I = 3.8$.

The difference between the results obtained in MeOH to those in CHCl$_3$ in the previous section indicates that the increased polarity of the rebinding solvent is driving non-polar interactions. This is consistent with other reports into the rebinding of PNL-imprinted polymers which
suggest that, in polar environments, there may be hydrophobic processes occurring, such as π-π stacking interactions of the naphthalene rings of the template with the aromatic ring on the crosslinker DVB\textsuperscript{1}, that result in increased binding of the template to the polymer while the polar, specific hydrogen bonding interactions are reduced. This explains the rebinding behaviour of VOC\textsubscript{NIP} (where binding increased) and VOC\textsubscript{MIP} (where binding remained the same due to the net effect of reduced specific and increased non-specific interactions), but the effects on the [bmim]PF\textsubscript{6}-prepared polymer are not as clear. It is hypothesised that, due to the lower surface area of the [bmim]PF\textsubscript{6}-prepared polymers, they are less affected by the increased non-polar interactions observed in the VOC-prepared polymers and that the increase in MIP\textsubscript{[bmim]PF\textsubscript{6}} binding is, in fact, due to the better polymer dispersion observed in MeOH, resulting in increased access to MIP binding sites.

The rebinding solvent was then altered by introducing 20% [bmim]PF\textsubscript{6} to the rebinding solution. The difference in rebinding between the two polymer systems in this case was quite remarkable. There was a drastic decrease in the specific binding of the VOC-prepared polymer upon the introduction of some [bmim]PF\textsubscript{6} to the rebinding mixture. The amount of polymer rebound by the VOC-prepared MIP was reduced from 47% down to only 6%. The non-specific rebinding was less affected, although still decreasing significantly from 20% to 10%, resulting in an imprinting factor of \( I = 0.6 \).

In the case of the RTIL-prepared polymer, it was anticipated that the inclusion of some of the RTIL used for polymer synthesis in the rebinding solvent mixture would lead to an overall increase in the amount of template rebound, based on conventional MIP literature. This was not the case, with both MIP\textsubscript{[bmim]PF\textsubscript{6}} and NIP\textsubscript{[bmim]PF\textsubscript{6}} undergoing an average decrease in the total amount of template bound, giving an imprinting factor of \( I = 1.1 \).

The drop in rebinding levels for both polymer systems has two possible causes. Molecular modelling data indicated that the [bmim]PF\textsubscript{6} would interact with PNL and MAA to some extent.
Interaction between [bmim]PF$_6$ and PNL may hinder the ability of the template to bind at imprinted sites within the polymer. However, if this was the cause, a corresponding drop in NIP binding would be expected. This is not the case as it is clearly the MIP binding which was most affected. As a proportion of the binding observed in MeOH, MIP$_{\text{VOC}}$ was reduced by 87% and MIP$_{[\text{bmim}]\text{PF}_6}$ by 69%, while NIP$_{\text{VOC}}$ and NIP$_{[\text{bmim}]\text{PF}_6}$ were reduced by 50% and 15%, respectively. This suggests that the rebinding behaviour may in fact be caused by [bmim]PF$_6$ molecules binding in the MIP cavities, effectively blocking the selective binding sites and reducing PNL-MIP interactions. This result shows the importance of the choice of rebinding solvent in these systems and that care must be taken when using RTILs to ensure that maximum recognition between the template and imprinted polymer sites can occur.

The effect of rebinding solvent polarity was further investigated by using solvents of higher and lower polarity than those previously analysed. Figure 4.39 shows the rebinding performance of the VOC and RTIL-prepared polymers when rebound in H$_2$O and DCM, with polarities of 9.0 and 3.4, respectively. Selectivity in H$_2$O is a particularly desirable outcome from a field testing point of view, as this would avoid any health and safety issues associated with VOCs. Rebinding was conducted over 6 hours, using a 20 mg polymer in 5mL of a 25µM solution of PNL. All measurements were made in triplicate; the error bars represent the standard deviation.

In the H$_2$O rebinding system, the VOC-prepared MIP and NIP underwent an increase in binding capacity compared to the MeOH and CHCl$_3$-systems examined previously. This is analogous to similar studies performed on PNL-imprinted polymers prepared in VOCs where the increase in template uptake is caused by non-specific binding to the crosslinker\(^3\). The high levels of NIP binding resulted in a reduced imprinting factor of $I = 1.4$ compared to the $I$ values recorded in CHCl$_3$ ($I = 4.8$) and MeOH ($I = 2.3$).

When DCM was used as the rebinding solvent, MIP$_{\text{VOC}}$ rebinding was comparable to rebinding in both CHCl$_3$ and MeOH but NIP$_{\text{VOC}}$ rebinding was increased, again resulting in a lower $I$
value than the CHCl₃ and MeOH rebinding systems \((I = 1.2)\). This is attributed to increased hydrogen-bonding on the polymer surface due to the non-polar nature of the rebinding solvent.

![Figure 4.39](image)

**Figure 4.39** Rebinding results in H₂O and in DCM after 6 hours rebinding of a 25µM solution of PNL for PNL-imprinted polymers. Imprinting factors and rebinding solvent are given in bold.

In the [bmim]PF₆-prepared polymers, the effect of the highly polar H₂O as rebinding solvent only led to minor increases in template rebinding (compared to rebinding in CHCl₃ and MeOH). The fact that the high binding levels observed in VOC-prepared polymer were not replicated in the [bmim]PF₆-prepared system is attributed to the increased hydrophobicity observed in the [bmim]PF₆-prepared polymers, which reduced contact between the polymer and the template in solution, causing the lower rebinding values recorded here. This resulted in a lower \(I\) value of 2.2, compared to \(I = 3.8\) in the MeOH rebinding system. Essentially, the effects of increased hydrophobic interactions are cancelled out by reduced PNL-polymer contact.

In DCM, NIP[bmim]PF₆ rebinding levels were increased in a similar fashion to NIP\(_{\text{VOC}}\). Again, this is attributed to an increase in non-specific hydrogen bonding interactions. This results in an
imprinting factor of \( I = 1.2 \) for the DCM system, the lowest observed for the [bmim]PF\(_6\)-prepared polymer across all rebinding solvents.

### 4.6.3 Sonication Rebinding Study

The zeta potential data in Section 4.4.4 showed the VOC-prepared polymers have a higher surface charge than the [bmim]PF\(_6\)-prepared polymers and will remain dispersed in solution while the [bmim]PF\(_6\)-prepared polymers with zeta potential lower than the threshold surface charge form less stable colloids and tend to aggregate. In order to study the effect of this behaviour on particle size and rebinding selectivity, the technique of sonication was utilised to assist in breaking up the polymer aggregates.

Firstly, both VOC and [bmim]PF\(_6\)-prepared MIP polymers were subject to sonication and particle size measurements were taken at various time intervals as the polymer was sonicated then allowed to settle. Figure 4.40 shows the range of particle sizes measured in the VOC-prepared systems after 10 measurements.
Figure 4.40  Effect of sonication on the particle size of VOC-prepared MIPs. ‘Sit’ indicates the settling time after sonication. Measurements were made using 3mg polymer in 5mL MeOH.

No variation in polymer particle size was observed as sonication proceeded with sizes of ~1800nm recorded in each instance and all measurements within standard deviation. This was unsurprising given that SEM and TEM images showed the polymers to exist as discrete particles, combined with the high zeta potential of the polymers which indicated that the particles will remain well dispersed in solution.

By contrast, the [bmim]PF₆-prepared polymers underwent a marked change in the particle size and standard deviation measurements over the course of the experiment. Again, values were taken as an average of 10 measurements (Figure 4.41).
Figure 4.41  Effect of sonication on the particle size of [bmim]PF₆-prepared MIPs. ‘Sit’ indicates the settling time after sonication. Measurements were made using 3mg polymer in 5mL MeOH.

There is a direct correlation between the length of sonication and the particle size. The sizes decreased significantly as the sonication time increased (up to the 60 minute time period), indicating that the sonication process leads to break up of the polymer clusters initially present in solution. A minimum particle size of 1600nm was recorded after 60 minutes of sonication and represents an increase in the available surface area (compared to after a 15 minute sonication period) due to a 46% decrease in average particle size. The standard deviation of the measurements also appeared to be influenced by the sonication process, with decreases in standard deviation observed as the length of sonication time increased, indicating a more regular distribution of particle sizes.

As sonication demonstrated this effect on particle size and size distribution, rebinding studies were conducted on the sonicated polymer systems for comparison against the original binding results. Rebinding was conducted in triplicate using 20mg polymer in 5mL of a 25µM solution of template for 1 hour with and without sonication of the polymer solution. The error bars represent the standard deviation. The shortened rebinding time was used to minimise the heating effect of the sonication process.
Rebinding data for PNL in VOC (MeOH) and [bmim]PF₆-prepared polymers rebound with and without sonication from a 25µM PNL solution after a rebinding time of 1 hour. Imprinting factors are given in bold.

Figure 4.42 shows that there was no improvement in the total rebinding of the polymer when the rebinding was conducted under sonication. In MIP VOC rebinding was reduced from 41% to 19% and in NIP VOC from 12% to 3%. The more pronounced effect on NIP VOC is consistent with a decrease in the weaker, non-specific associations of the polymer with PNL caused by the effects of sonication and results in an improved imprinting factor from $I = 3.5$ (unsonicated) to $I = 5.5$ (sonicated).

In MIP [bmim]PF₆ rebinding was reduced from 23% to 16% and in NIP [bmim]PF₆ from 8% to 7% (although the NIP values were within error of each other). This could possibly be attributed to the fact that, although the sonication may aid in the separation of the polymer particles, as indicated by the particle sizing data, it may also prove too vigorous a process for PNL to form any additional associations with the polymer, resulting in the reduction in binding. Overall selectivity was reduced from $I = 2.8$ (unsonicated) to $I = 2.1$ (sonicated). The reduction in
binding capacity was less pronounced than with the VOC-prepared polymers and suggests that the predominant effect of sonication in the [bmim]PF₆ system is the de-aggregation of the particles and not the dissociation of PNL from the polymer as with the VOC system. The result further indicates that whilst there was less binding taking place on the [bmim]PF₆-prepared polymers, the associations were stronger in nature than those present on the VOC-prepared polymers. This is consistent with the Scatchard analysis, which showed the [bmim]PF₆-prepared polymer to have a lower Kₐ value than the VOC-prepared polymer (1.60 x 10⁻⁵ vs. 3.34x 10⁻⁵) meaning that the polymer-template associations in the [bmim]PF₆-prepared polymers will be stronger in nature.

4.7 Mixed Solvent Systems

Although the imprinting efficiency in the cocaine-imprinted system was not enhanced by using mixtures of VOC and RTIL as preparation solvents, this was related to solubility issues in the [bmim]BF₄ and may not be the case in all systems. Therefore, a study involving mixed solvents was also conducted for the PNL-imprinted polymers.

In contrast to the previous study which investigated the effect of incremental changes in RTIL proportion of the polymerisation solvent, the PNL study focussed on a single 50:50 mixture of the RTIL and VOC (acetonitrile/toluene). This would allow for the determination of whether the RTIL or VOC is having the greatest impact.

In terms of reaction rate and yield, there was again a marked acceleration effect due to the presence of the RTIL as a solvent component. The reaction time for pure RTIL and pure VOC systems had been previously recorded as 6 and 24 hours, respectively, with a threefold increase
in polymer yield. Although the 50:50 solvent mixture decreased the reaction efficiency slightly, there was still a remarkable improvement over the pure VOC system with polymerisation complete in 8 hours. This finding is in line with other studies relating reaction rates to the percentage RTIL composition of the polymerisation solvent\textsuperscript{27}. Polymer yield data is outlined in Table 4.4.

<table>
<thead>
<tr>
<th>Polymerisation solvent</th>
<th>50:50 [bmim]PF\textsubscript{6}:VOC</th>
<th>VOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. polymer yield (mg)</td>
<td>65%</td>
<td>51%</td>
</tr>
</tbody>
</table>

It was immediately clear from the SEM images (Figure 4.43) that the influence of the [bmim]PF\textsubscript{6} substantially outweighed that of the VOC in terms of the impact on polymer morphology. The polymer prepared in 50% proportions (image b, Figure 4.43) bore a greater resemblance to the pure [bmim]PF\textsubscript{6}-prepared polymer (image a, Figure 4.43), with the larger, spherical, discrete particles that were seen in the VOC polymer (image c, Figure 4.43) no longer present.
Figure 4.43  SEM images of PNL-imprinted MIPs prepared in (a) [bmim]PF₆, (b) 50:50 RTIL: [bmim]PF₆ and (c) VOC.

TGA analysis for the mixed solvent systems showed a very similar curve profile to the pure VOC and pure [bmim]PF₆ preparations with two clear stages of decomposition evident (Figure 4.44). The onset of decomposition was approximately 300°C for all polymers.
The swelling data again indicated the predominance of the effects of \([\text{bmim}]\text{PF}_6\) in the polymerisation mixture (Figure 4.45), with substantial decreases in MIP and NIP swelling in the 50:50-prepared polymer compared to pure VOC. MIP\([\text{bmim}]\text{PF}_6\) however, maintained a higher degree of swelling than MIP\(_{50:50}\), indicating a more porous structure.

![TGA spectra graph](image)

**Figure 4.44** Overlay TGA spectra of MIPs prepared in VOC, \([\text{bmim}]\text{PF}_6\) and a 50:50 mixture.

**Figure 4.45** Swelling after 6 hours in CHCl\(_3\) after 6 hours for PNL-imprinted polymers in various solvent systems (single trial).
Figure 4.46 shows the zeta potential data for all the polymer preparations. All polymers prepared using RTILs showed a decrease in the zeta potential compared to the VOC preparation, with the lowest values recorded in the pure [bmim]PF$_6$ preparation. MIP zeta values (-mV) of 38, 24 and 21 and NIP values of 38, 30 and 18 were recorded for polymer prepared in pure VOC, 50:50[bmim]PF$_6$:VOC and pure [bmim]PF$_6$, respectively.

![Zeta potential data for PNL-imprinted polymers prepared in various solvent systems.](image)

**Figure 4.46** Zeta potential data for PNL-imprinted polymers prepared in various solvent systems.

As time-based rebinding studies have already been conducted on the pure VOC and [bmim]PF$_6$ systems, the data for the mixed solvent system only is presented here. Studies were conducted in triplicate using 20mg polymer in 5mL of a 25µM solution of PNL in CHCl$_3$ over a rebinding time of 6 hours; the error bars represent the standard deviation.

The 50:50 [bmim]PF$_6$:VOC preparation system showed good differentiation between the MIP and NIP polymers over the entire 24 hours (see Figure 4.47). The overall uptake of template (MIP$_{[bmim]PF_6} = 46\%$ at equilibrium) is higher than the pure [bmim]PF$_6$ system (31%), indicating that a greater number of binding sites has been generated by the inclusion of the VOC in the polymer preparation mixture.
A difference in binding kinetics was observed in this system, with a significant amount of absorption and desorption of the template occurring until a rebinding time of 8 hours, where equilibrium was reached. Although taking longer to reach equilibrium, the imprinting factor in the mixed solvent system was higher than in the pure [bmim]PF$_6$, increasing from $I = 2.3$ to $I = 2.8$ under equilibrium conditions, although still much lower than for pure VOC ($I = 4.8$) due to the higher levels of NIP binding.

The physical data presented in this section suggests that the effects of [bmim]PF$_6$ in the reaction mixture will predominate over VOC effects. This is highly suggestive of the presence of RTIL domains within the RTIL:VOC mixture where the polymerisation is occurring, limiting the effects of the VOC on polymer formation. Similar effects have been observed in polymerisations conducted in binary mixtures of DMF and the RTIL [Me$_3$NC$_2$-H$_2$OH][ZnCl$_3$], where polymer molecular weights observed were much closer in value to those of the pure RTIL than the pure VOC$^{28}$.
However, in terms of rebinding capacity of the polymers, the values for the mixed solvent systems are closer to those of the VOC-prepared polymers than the [bmim]PF$_6$-prepared polymers. This is a very significant finding as it shows that the advantages of polymerisation in RTILs i.e. faster polymerisation rates and higher polymer yields can be maintained while producing polymers with MIP binding capacities comparable to the VOC polymer preparation and selectivity greater than that observed in pure [bmim]PF$_6$-preparation.

4.8 The Effect of Alkyl Chain Length

One very significant difference between RTILs and conventional solvents is the heterogeneous behaviour displayed by many RTILs in the liquid state. Heterogeneity has been shown to increase as a function of the alkyl chain length of the side chain in imidazolium-based RTILs$^{23}$ due to formation of ordered ‘micellar’ structures within the liquid. The butyl side chain has been reported in a number of studies to be the transitional point between a homogenous composition of the RTIL and one displaying long range order (with the degree of order increasing with the side chain length). As such, it was a reasonable approach to prepare MIPs using RTILs with alkyl side chains on the imidazolium group, which were both shorter and longer than butyl sidechains to see if the heterogeneity of the RTIL had any impact on MIP formation and, ultimately, on selectivity.

In order to discount any possible effect of the anion on these systems, a common anion (PF$_6$) was selected for all RTILs. The ideal situation would be to investigate imidazolium-based RTILs with ethyl, butyl and hexyl side chains to examine RTILs in both the homogeneous and heterogeneous states. However, studies using [emim]PF$_6$ could not be performed due to
insolubility of the crosslinker. Therefore, instead of investigating the difference between the heterogenous and homogenous RTILs, the study investigated the effect of the increase in long range order. RTILs with butyl, hexyl and octyl imidazolium side chains ([bmim], [hmim] and [omim], respectively) were selected for analysis.

Polymers were prepared as in Section 4.3, using 30mL [hmim]PF₆ and [omim]PF₆ as polymerisation solvents. SEM images of the polymers obtained (Figure 4.48) showed the effect of the alkyl side chain length on the morphology of the polymer particles. There was little observable difference between the [bmim] and [hmim] based polymer preparations, with particle sizes of 300-500nm visible. However in the [omim]PF₆ polymer preparation, there appeared to be a greater degree of agglomeration and an increase in particle size (500-1000nm). This phenomenon may be related to the viscosity of the RTIL, as the viscosity increased with the alkyl chain length, which may promote aggregation of the growing polymer chains during phase separation, similar to what is observed in polymers when minimal volumes of solvent are used. Reported viscosities of these RTILs are given in Table 4.5.

<table>
<thead>
<tr>
<th>RTIL</th>
<th>Viscosity at 25°C (cP)</th>
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<tbody>
<tr>
<td>[bmim]PF₆</td>
<td>450</td>
</tr>
<tr>
<td>[hmim]PF₆</td>
<td>585</td>
</tr>
<tr>
<td>[omim]PF₆</td>
<td>682</td>
</tr>
</tbody>
</table>
An alternative explanation of this effect is that the formation of increasingly large domains within the RTIL structure as alkyl chain length increases results in the formation of larger particles. This has been reported in the literature in the synthesis of polypyrroles, where increased particle size was found to correspond with an increase in the length of the imidazolium side chains (from butyl to octyl to decyl) due to this domain effect.

The effect of alkyl chain length on the thermal stability of the polymers was examined using TGA. MIP and NIP polymers behaved similarly so only the MIP data is presented here (Figure 4.49).

All three polymer preparations showed a similar onset of decomposition at approximately 270°C and they all appeared to go through two decomposition phases. The thermal stability of
the polymers increased with increasing alkyl chain length through the initial decomposition phase. There were slight differences in the secondary stage of decomposition, with thermal stability increasing in the order of [hmim] > [omim] > [bmim].

![TGA traces of [bmim], [hmim] and [omim]PF₆- prepared MIPs.](image)

**Figure 4.49** TGA traces of [bmim], [hmim] and [omim]PF₆- prepared MIPs.

The swelling data for the polymers in CHCl₃ is given in Figure 4.50. The RTIL-prepared polymers all exhibited significantly lower swelling percentages than in the VOC. There appeared to be little difference between the [bmim] and [hmim]-based systems, however the [omim]PF₆-prepared polymer swelled to a lesser degree.
Figure 4.50  Swelling analysis measured in CHCl₃ after 6 hours for polymers prepared in VOC and RTIL of varying alkyl chain length (single trial).

Rebinding analysis was conducted in triplicate on the [hmim]PF₆ and [omim]PF₆-prepared polymers; the error bars represent the standard deviation of the measurements. Polymer weight saturation studies (Figure 4.51) again indicated a polymer mass of 20mg would be optimum for rebinding of 5mL of a 25µM PNL solution in CHCl₃. Both polymer preparations showed differentiation between MIP and NIP at these polymer masses as well as substantial increase in binding capacities compared to the [bmim]PF₆-prepared polymer. At a polymer mass of 20mg the [bmim]PF₆-prepared MIP bound 26% of the template, compared to 61% and 76% in the [hmim]PF₆ and [omim]PF₆-prepared polymers respectively. These represent higher binding capacities of these polymers than MIPₖ, which bound 49% of the PNL solution.
Figure 4.51  Template rebinding by polymer weight in 25μM solution of PNL over 6 hours for (a) [hmim]PF₆ and (b) [omim]PF₆-prepared polymers.
Time-based rebinding studies were conducted and showed good selectivity for the template in both solvent preparation systems (Figure 4.52). The [hmim]PF₆ polymer preparation (image a, Figure 4.52) showed higher template uptake than that was observed in the [bmim]PF₆-prepared polymer, however the imprinting factor was reduced from $I = 2.3$ to $I = 1.3$ at 6 hours.

The [omim]PF₆-prepared polymer (image b, Figure 4.52) again showed a significant increase in binding capacity (over 6x higher than the [bmim]PF₆-prepared polymer in the MIP). However, the imprinting factor at 6 hours was $I = 1.4$, substantially less than the [bmim]PF₆ polymer preparation. The time-based rebinding data also showed the increase in rebinding kinetics observed in the [omim]PF₆-prepared polymer system. Whilst other systems showed an initial increase in the amount of template bound until an equilibrium is reached (at approximately 6 hours in most systems studied here), the [omim]PF₆-based system appeared to reach an equilibrium in 1 hour.

This data is consistent with an increase in surface area of these polymer preparations. This would account for the increase in template uptake as well as the rapid rebinding kinetics observed and would not necessarily be expected to correlate with an increase in selectivity (which has not been observed here). This effect would correspond with increasing size of the RTIL domains responsible for pore formation, although this effect on porosity remains to be confirmed via BET and PALS analysis.
Figure 4.52  Time-based rebinding data using a 25µM solution of PNL over 6 hours for (a) [hmim]PF₆ and (b) [omim]PF₆-prepared polymers.
4.9 Conclusions

The utility of RTILs as molecular imprinting solvents has been studied here in some detail in regards to PNL as template molecule. The impact that RTILs have on the polymerisation process and the resultant polymer properties (compared to polymers prepared using VOCs) has been thoroughly assessed.

Molecular modelling and NMR studies showed that although there is some interaction observed between the MAA monomer and PNL template with the RTIL itself, monomer/template associations were still observed in [bmim]PF₆. This is a significant finding as the monomer/template associations leading to the creation of template specific cavities are a crucial aspect of MIP preparation.

Subsequent synthesis of the polymer in [bmim]PF₆ and VOC showed the improved reaction efficiency in [bmim]PF₆ (as has been the case in all other RTIL-prepared polymerisations conducted in this study) and the resultant polymers show the small particle size, good thermal stability and low swelling properties which are becoming synonymous with the RTIL-prepared polymers.

The synthesised polymers displayed a good level of selectivity for PNL in both VOC and [bmim]PF₆ preparations with imprinting factors of \( I = 4.8 \) and \( I = 2.3 \), respectively, while cross-reactive studies with other compounds confirmed the ability of both polymer preparations to distinguish the template from both structurally analogous and non-analogous compounds. The lower binding capacity for PNL observed in the [bmim]PF₆-prepared polymers is attributed to a decrease in specific surface area due to the existence of a lower number of pores (although larger in size) than in the VOC polymer preparation. Selectivity for the template was achieved in [bmim]PF₆-prepared polymers even when the volume of solvent used for polymerisation was
substantially reduced ($I = 2.6$), representing a significant improvement in efficiency and cost-effectiveness of the [bmim]PF$_6$-mediated system.

Both template uptake and imprinting factors for the [bmim]PF$_6$-prepared polymers were enhanced through variations in rebinding and synthesis conditions. MeOH was shown to be the optimal rebinding solvent for the [bmim]PF$_6$-prepared polymers, with enhanced levels of MIP rebinding observed compared to all other solvent systems as well as the highest imprinting factor of $I = 3.8$.

Other means of increasing template uptake for the RTIL-based system include the use of mixed RTIL:VOC systems for polymerisation or using RTILs with long imidazolium side chains. The use of a 50:50 [bmim]PF$_6$:VOC polymerisation solvent increased MIP rebinding to 42% (from 31% in the pure [bmim]PF$_6$), presumably due to an increase in the number of available bindings sites. Binding capacity was found to increase with the length of the imidazolium alkyl side chain, a phenomenon attributed to the hypothesised increase in pore size due to the presence of larger RTIL domains. However, while these synthesis conditions enhanced template uptake, NIP rebinding increased along with MIP rebinding, resulting in no improvement in terms of polymer selectivity for the template.

Through the analysis of PNL as a model template system, RTIL-mediated polymerisations have been shown to be a viable alternative to conventional VOC systems for MIP production. In addition to improving reaction efficiency and polymer yields, the RTIL-prepared imprinted polymers showed high levels of selectivity for the template. Through adjustment of synthesis and rebinding parameters, the template uptake and selectivity of the polymers was further improved.
4.10 References


Chapter 5

Conclusions and Future Work

5.1 Conclusions

The study conducted here has yielded significant information regarding the use of Room Temperature Ionic Liquids (RTILs) as a polymerisation medium for the preparation of Molecularly Imprinted Polymers (MIPs).

There are significant benefits associated with the use of RTILs as polymerisation solvents in many areas of chemistry. These can be taken advantage of in the production of MIPs, resulting in improved reaction efficiency and high polymer yields. There is also the possibility of recycling and reusing the RTIL, reducing both costs and environmental footprint compared to traditional organic solvents, although this was not conducted in this current study.

Examination of the cocaine-imprinted system showed that RTILs can be used to synthesise MIPs which have improved template selectivity over their VOC-prepared counterparts, with imprinting factors as high as 2.2 observed (for the polymer BF$_4$-0-25), compared to the best result obtained for CHCl$_3$-prepared polymers ($I = 1.6$) when conducted under identical rebinding conditions. The selectivity of the polymers for the template was found to be influenced by a variety of factors related to polymer synthesis, including polymerisation temperature, solvent volume and the choice of RTIL. These resulted in variations of the physical characteristics of the polymers which ultimately impacted on selectivity. Rebinding conditions
were also found to be an important factor in MIP selectivity, and polymer performance was enhanced through the use of DCM as rebinding solvent, increasing the total uptake of the template by the polymer, as well as raising imprinting factors from 1.4 to 1.9 in the case of BF$_4$-60-5 and from 1.6 to 2.3 for CHCl$_3$-60-5.

Some general trends between synthesis parameters and selectivity were observed. For example, polymers showed increased binding capacity when prepared at lower temperatures (due to a decrease in crosslinking density), although ultimately the effects were found to be highly RTIL-dependant. A high degree of error was observed in many of the rebinding analyses which was attributable to the heterogeneous nature of binding sites in the polymers and made interpretation of the data a difficult process. Due to the complicated nature of the interactions between the template, monomer, crosslinker and solvent that were observed under the various synthesis parameters, investigations then shifted to a model MIP system. This was conducted in order to provide a better understanding of the effects of RTILs and validate their use as VOC replacements in the preparation of MIPs.

The model MIP system (using PNL as template and [bmim]PF$_6$ as RTIL) involved molecular modelling and NMR studies, which showed that although there was some interaction between the MAA monomer and PNL template with the RTIL itself, monomer/template associations could still be observed in [bmim]PF$_6$. This was a significant finding as the monomer/template associations leading to the creation of template specific cavities are essential in the production of high-performance MIPs.

Both [bmim]PF$_6$ and VOC-prepared polymers showed good levels of selectivity for PNL in CHCl$_3$, with imprinting factors of $I = 4.8$ and $I = 2.3$, respectively. Additionally, cross-reactivity studies showed higher affinity for PNL than EDP and CAFF for both polymer preparations. The [bmim]PF$_6$-prepared polymers showed a lower rebinding capacity than the VOC-prepared polymers, which was attributed to a decrease in specific surface area. This was
confirmed by PALS and BET analysis which indicated a reduced pore density compared with
the VOC polymer preparation. Polymers prepared when using very low volumes of [bmim]PF₆
as polymerisation solvent also showed good selectivity for the template ($I = 2.6$), increasing the
efficiency of the [bmim]PF₆-mediated system.

Variation in rebinding conditions allowed for the optimisation of template selectivity for the
[bmim]PF₆-prepared polymers. MeOH was shown to be the rebinding solvent of choice both in
terms of template uptake and selectivity, with an imprinting factor of $I = 3.8$ observed.

Template uptake (although not selectivity) was enhanced through the use of mixed RTIL:VOC
systems for polymerisation and using RTILs with longer imidazolium side chains. The use of a
50:50 [bmim]PF₆:VOC polymerisation solvent increased MIP rebinding to 42% (from 31% in
the pure [bmim]PF₆), while polymers produced using [hmim]PF₆ and [omim]PF₆ as
polymerisation solvent increased MIP rebinding to levels higher than those observed using the
VOCs. The increased binding capacity was attributed to the hypothesised increase in pore size
due to the presence of RTIL domains.

The use of RTIL-mediated polymerisations has been shown to be a viable alternative to
conventional VOC systems. They provide a means to improve reaction efficiency and polymer
yields, as well as maintaining high levels of selectivity for the template. This selectivity can be
optimised for any given system through the adjustment of both synthesis and rebinding
parameters. The extensive range of RTILs provides an enormous number of anion-cation
permutations. This means that the scope for RTIL:MIP investigations is almost limitless and
there are undoubtedly many other systems apart from those investigated here where RTILs may
prove a beneficial substitute for VOCs.

5.2 Future Work
All the polymerisations conducted in this study have been carried out using free radical polymerisation. One of the drawbacks of this type of polymerisation is the heterogeneity of the polymers it produces\textsuperscript{4}. RTILs have been successfully utilised in many other types of polymerisations and it is hypothesised that controlled polymerisations could be utilised in the production of RTIL-MIPs which may lead to the formation of more ordered, homogeneous polymers exhibiting improved selectivity and a higher batch-to-batch reproducibility. Another potential polymerisation technique which could be explored is that of microwave polymerisation, which may further reduce polymerisation times and improve template binding efficiencies\textsuperscript{5}.

As the type of crosslinker was shown in this study to have a large impact on the thermal behaviour of the polymers, different aspects of this experimental parameter (including the types and percentage of crosslinker present in the polymerisation mixture) could also be investigated.

As the BET analysis and the PALS analysis techniques provided valuable information regarding the porosity and surface area of the PNL-imprinted polymers prepared in [bmim]PF\textsubscript{6} and VOC, it would be beneficial to extend these studies to all of the polymers, including the cocaine-imprinted polymers, to more fully gauge the effects of RTIL structure, polymerisation temperature and solvent volume on polymer surface area and porosity. This would be a particularly important study in the case of polymers prepared using RTILs with differing imidazolium alkyl side chain lengths, as it has been hypothesised that the length of the alkyl chain will have a direct relationship to the pore size.

Molecular modelling and NMR analysis indicated interactions between the template and RTIL anions. Although template/monomer associations were found to occur regardless, the elimination of this interaction may still lead to enhanced selectivity of the polymer for the template. There are extensive reports of polymerisations conducted where the RTIL itself is

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used as a monomer\textsuperscript{6-8}. These studies could be extended to molecular imprinting to potentially produce polymers with a better defined imprinted cavity.

The sheer number of available RTILs provides enormous scope for the extension of this work. Based on the data generated in this study, this can now be attempted with a greater understanding of the processes involved and the role of the RTILs in MIP systems.

5.3 References


