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Ionic Liquids as Porogens in the Synthesis of Molecularly Imprinted Polymers

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1. Introduction

Although the information regarding room temperature ionic liquids (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 (Cooper 2004; Pavlova 2006) and molecularly imprinted polymers (MIPs) in particular (Booker et al 2006, 2007; Wang 2006, 2008; He 2008). Herein we examine a model system, cocaine, and will review the properties and performance of imprinted polymers prepared in volatile organic compounds (VOCs) with those prepared in RTILs, and the experimental parameters such as polymerisation temperature, solvent volume, rebinding conditions and template-RTIL combination, which may have a role to play in these systems.

1.1 The design and synthesis of molecularly imprinted polymers

No discussion on the effect of RTILs on the efficacy of MIPs would be complete without a brief discourse on MIP design and synthesis. MIPs are a specialty class of polymers that possess the capacity to selectively sequester a target species from a solution matrix. Selectivity arises through the inclusion of the molecular target in the pre-polymer formulation, which leads to the generation of tailored molecular cavities within the final polymer. MIP synthesis requires four basic ingredients: a template (T); a functional monomer (FM); a crosslinking agent to impart stability and cavity rigidity to the resultant polymer; and a porogen to generate a pore structure within the polymer to aid mass transfer during template rebinding. The typical process for MIP formation is outlined in Figure 1.

With thousands to millions of available template binding pockets, MIPs possess the ability to recognize and bind specific target molecules. Whilst they are the synthetic counterparts to biological receptors and are robust, insoluble materials exhibiting high stability in most media, they generally lack the natural homogeneity of active sites associated with biological receptors. The population of binding sites generated in MIPs typically presents a highly heterogeneous profile because of the influence of the equilibria that govern the T-FM complex formation and the stability and dynamic nature of the growing polymer chains.

Binding site homogeneity and selectivity is a product of the stability of the T-FM cluster during polymerization. Two approaches to cluster formation have been explored; one based on covalent interactions and the other centred around self-assembly (utilising non-covalent binding interactions). With the former, post polymerization template removal requires the destruction of covalent T-FM linkages. The stable nature of this system yields a highly defined cavity (binding site) that complements the size, shape and electronic properties of the template. Non-covalent or self-assembly of the T-FM cluster in the pre-polymer mix relies on combinations of hydrogen bond interactions, electrostatic attraction and associated weak interactions to form, resulting in a distribution of binding site profiles reflecting the stability of the T-FM cluster. Post-polymerisation, the template is removed from the cavity via exhaustive extraction.

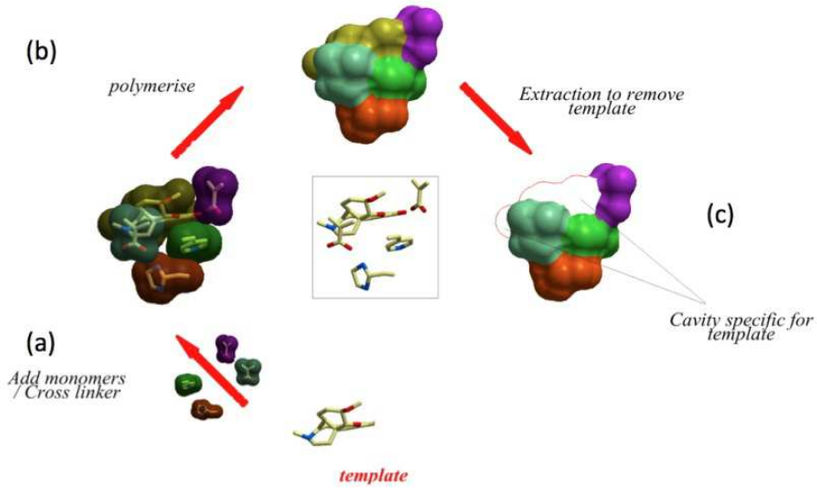


Fig. 1. Development of MIPs. (a) Addition of functional monomers and crosslinker to a solution of the template, followed by prearrangement association of template with functional monomers; (b) resultant preassociation functional monomer / cross linker / template cluster subjected to free radical polymerisation conditions results in cavity formation; and (c) template removal to leave template specific cavity.

MIP technology has been applied in a myriad of areas including, but not limited to, separation and isolation, antibody and receptor mimics, and biosensor style devices (McCluskey et al., 2007). Their shelf stability, robustness and reusability mean that they are highly usable and flexible materials. The variety of molecules 'imprinted' is impressive in both breadth of template and also diversity, highlighting the utility of MIPs. The general MIP area has been extensively reviewed over the past decade. More recently, efforts have focused on garnering a greater understanding of the influence of the template on MIP morphology and function, as well as the role of the porogen in the initial MIP synthesis. In this latter regard, we are one of the few groups who have explored the potential use of room temperature ionic liquids as porogen (Booker et al 2006, 2007; Wang 2006, 2008; He 2008).
The tunable solvating properties of ionic liquids hold considerable potential to facilitate an increase in favorable T-FM interactions, whilst limiting those associated with non-specific binding. An additional attraction of RTILs as porogens is the considerable body of literature pertaining to polymerization rate enhancements (which allows precipitation polymerization

approaches to be applied in a short time frame), and our earlier studies that showed an enhancement of MIP selectivity relative to the identical MIP formulation manufactured in a traditional VOC porogen (Booker et al 2006, 2007).

2. Scope of this study

Prior studies conducted by our group using cocaine as a template molecule have produced data showing that polymers selective for this template could be generated in both a VOC ($CHCl_3$) and RTILs ([bmim]BF_4 and [bmim]PF_6). These results provide a good foundation for the current study (Booker et al 2007). Previous research conducted by our group indicated that a change in the anion of the RTIL used as polymerisation solvent (from BF_4^- to PF_6^-) influences polymer selectivity for the template for both *trans*-aconitic acid and cocaine-imprinted polymers (Booker et al, 2005, 2006, 2008) when prepared under otherwise identical conditions.

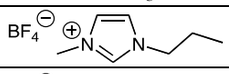
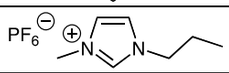
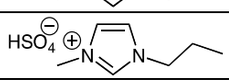
Porogen	Structure	Viscosity at 25°C (mPa s)
Chloroform	$CHCl_3$	0.54
[bmim]BF_4		104.2
[bmim]PF_6		195.9
[bmim]HSO_4		900

Table 1. Chemical structure of RTILs examined in this study and the viscosity of porogens used in the preparation of MIPs and the control polymers, i.e. the non-imprinted polymers (NIPs).

This work considers the RTILs [bmim]BF_4, [bmim]PF_6 and [bmim]HSO_4 and compares MIP efficacy to chloroform. Of the three selected RTILs, only [bmim]PF_6 and [bmim]BF_4 have previously been used as polymerisation solvents (Kubisa 2004), [bmim]HSO_4 to the best of our knowledge, has not. Both [bmim]PF_6 and [bmim]BF_4 have been used in the production of MIPs (Booker et al 2006, 2007; Wang et al 2006, 2008; He et al 2008). The work reported herein aims to identify RTILs that reproducibly create well-performing MIPs, and assess factors contributing to variations in polymer performance. Of particular interest is the effect of anion and RTIL viscosity and how they affect polymer morphology. The selected RTILs have viscosities of 104.2, 195.9 and 900 mPa s; substantially more viscous than the VOC chloroform at 0.51 mPa s (Crabtree and O'Brien 1991) (Table 1). Other features that affect polymer morphology, and hence will be evaluated as part of this study, include:
(1) *Polymerisation temperature (0 °C and 60 °C)* as it is has been reported that MIP preparation at low temperature, photoinitiated by UV light, allows for better 'freezing' of the interaction between the template and T-FM pre-polymerisation cluster, leading to an enhanced imprinted framework and improved MIP selectivity (Lu et al 2004). The increased polymerisation rates in RTILs are advantageous here (Andrejewska et al 2009), with some polymerisations failing to proceed in VOCs at 0-5 °C (Booker 2005).
(2) *Polymerisation volume (5 mL and 25 mL),* as this typically differentiates between two of the main imprinting formats, i.e. bulk or precipitation. Bulk (or monolith) polymers are prepared using minimal porogen volumes, where the polymer particles coalesce during

polymerisation to create a monolithic polymer structure (Venn and Goody 1999). By contrast, precipitation polymers are prepared in a large porogen volume (usually >95% of the polymerisation mixture by volume), allowing the polymer to form in solution as discrete particles. In the case of VOCs, minimal amounts of porogen (5 mL) produce monolithic polymers, whereas high porogen volume (25 mL) promotes the formation of polymer nanoparticles (Castell et al 2006). The effect of porogen volume on MIP morphology and selectivity has been documented in both VOCs and RTILs (Booker et al. 2006; Kotrotsiou et al 2009).

In terms of pore size, high porogen volumes increase the pore volume of MIPs, which, in turn, increase the rebinding capacity of the polymers (Kotrotsiou et al 2009). The effect of RTIL porogen volume on MIP morphology, however, is less distinct. Even at 5 mL porogen volume, clusters of irregular shaped nanoparticles have been found to form. Increasing the volume to 25 mL resulted in only minor visible changes, although differences in selectivity were observed over the various template/solvent/polymerisation temperature combinations studied (Booker 2005). 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.

3. Experimental section

3.1 Synthesis of 1-butyl-3-methylimadazolium 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 (Whitehead et al. 2004).

3.2 Synthesis of 1-butyl-3-methylimadazolium tetrafluoroborate ([bmim]BF_4)

[Bmim]Cl (60 g, 0.28 mol) was dissolved in water (60 mL) and cooled in an ice bath. To this chilled solution HBF_4 (60 mL, 0.9 mol) was added over 15 minutes. After complete addition the solution was stirred at room temperature overnight. The aqueous layer was extracted with dichloromethane (3x 50mL), the combined extracts dried over $MgSO_4$ and the solvent removed by rotary evaporation (Whitehead et al., 2004).

3.3 Synthesis of 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6)

[Bmim]Cl (120 g, 0.56 mol) was dissolved in distilled water (100 mL) and HPF_6 (68 mL, 60% aqueous solution, 0.56 mol) slowly added over 20 minutes. The aqueous upper phase was decanted and water added (50mL). The mixture was stirred 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 (Whitehead et al., 2004).

3.4 Synthesis of 1-butyl-3-methylimidazolium hydrogen sulfate ([bmim]HSO_4)

[Bmim]Cl (30 g, 0.14 mol) was dissolved in water (30 mL) and conc. H_2SO_4 (96 mL) 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 (Whitehead et al., 2004).

3.5 Cocaine-imprinted polymers: preparation and rebinding of MIPs

MIPs were prepared following the procedure of Holdsworth with cocaine base (0.14 mmol, 42.6 mg), MAA (0.28 mmol, 24.1 mg) and EGDMA (1.4 mmol, 280 mg), in desired amount of

solvent (Holdsworth et al, 2005). The reaction mixture was degassed with N_2 before AIBN (10 mg) was added and the solution was heated at 60 °C in a Syncore Polyvap Platform (Buchi). Photoinitiated polymerisation was done at 0 ˚C for 3 hours in an ice bath using a UV probe (Acticure). The porogens evaluated were $CHCl_3$ (control), $[bmim]BF_4$, $[bmim]PF_6$ and $[bmim]HSO_4$. Non-imprinted control polymers (NIPs) were prepared using the same method without the addition of the cocaine base.

Template extraction was by exhaustive washing with methanol 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 10 mg of polymer in 2 mL of 25 μM cocaine solution in various porogens for 1 hour. The resulting solution was filtered using a 2 μm 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 (ΔB) was calculated as the difference between the MIP binding and the NIP binding (B_{MIP}-B_{NIP}). Imprinting factors (*I*) were calculated as B_{MIP}/B_{NIP}.

4. Results and discussion

In our hands, the MIP preparation commences with combinatory molecular modelling – NMR titration studies which identified the most favourable FM interactions of cocaine (1) to be with methacrylic acid (MAA, **2**) in a 1:2 (T-FM) ratio (Holdsworth et al, 2005). . Thus, MIP preparation was conducted with a 1:2:10 ratio of cocaine : MAA : EGDMA (ethylene glycol dimethacrylate (EDGMA, **3**)) (Figure 2).

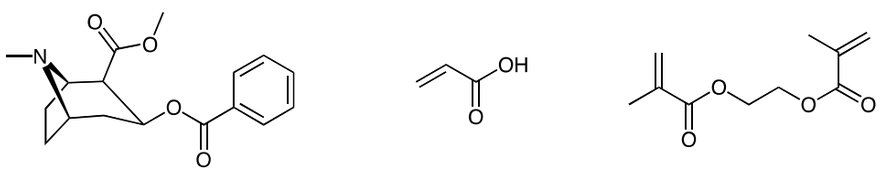


Fig. 2. Chemical structures of cocaine (**1**), methacrylic acid (**2**) and ethylene glycol dimethyl acrylate (**3**).

MIPs corresponding to each RTIL and the VOC ($CHCl_3$) were produced at 60 °C and 0 °C and at 5 mL and 25 mL porogen volume. This resulted in the synthesis of fourteen MIPs (Table 2). The corresponding NIPs were also synthesised (data not shown).

With $CHCl_3$, MIPs were produced at 60 °C and 5 mL and 25 mL porogen volume. MIP preparation in $CHCl_3$ at 0 °C failed. This is the first RTIL / VOC point of differentiation with the rapid polymerisation rates inherent with RTILs facilitating the production of sufficient quantities of MIP for further studies when the equivalent VOC preparation failed. Additionally, the use of RTILs showed marked advantages over $CHCl_3$ in terms of production efficiency. Yields were increased by up to 50% for some RTIL-mediated polymerisations, for example with $BF_{4\text{-}60\text{-}5}$ where a 94% yield was obtained, compared to 46% in $CHCl_{3\text{-}60\text{-}5}$. Reaction times were also markedly reduced in RTILs, with particularly fast reaction times observed in the low temperature photoinitiated polymerisations. At 60 °C in 5 mL solvent, reaction time was reduced from 6 hours in $CHCl_3$ to 2 hours in the RTILs and in 25 mL, from 18 hours in $CHCl_3$ 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 $CHCl_3$ (after a reaction time of 6 hours). The effect of increased reaction rate in RTIL polymerisations has been attributed to a combination of an increased rate of propagation coupled with decreased rates of termination, a function of the high viscosity of the RTILs (Schmidt-Naake et al 2009).

Polymer Code*	**Porogen**	**Temperature (°C)**	**Porogen Volume (mL)**	**Reaction Time (h)**	**Polymer Yield (%)[b]**
$CHCl_{3\text{-}60\text{-}5}$	$CHCl_3$	60	5	6	46
$CHCl_{3\text{-}60\text{-}25}$	$CHCl_3$	60	25	18	40
$BF_{4\text{-}60\text{-}5}$	[bmim]BF_4	60	5	2	94
$BF_{4\text{-}60\text{-}25}$	[bmim]BF_4	60	25	8	86
$BF_{4\text{-}0\text{-}5}$	[bmim]BF_4	0	5	0.75	78
$BF_{4\text{-}0\text{-}25}$	[bmim]BF_4	0	25	2	81
$PF_{6\text{-}60\text{-}5}$	[bmim]PF_6	60	5	2	80
$PF_{6\text{-}60\text{-}25}$	[bmim]PF_6	60	25	8	84
$PF_{6\text{-}0\text{-}5}$	[bmim]PF_6	0	5	0.5	70
$PF_{6\text{-}0\text{-}25}$	[bmim]PF_6	0	25	2	73
$HSO_{4\text{-}60\text{-}5}$	[bmim]HSO_4	60	5	2	69
$HSO_{4\text{-}60\text{-}25}$	[bmim]HSO_4	60	25	8	52
$HSO_{4\text{-}0\text{-}5}$	[bmim]HSO_4	0	5	0.5	55
$HSO_{4\text{-}0\text{-}25}$	[bmim]HSO_4	0	25	2	60

* Polymer codes relate to the conditions used to synthesise each polymer: porogen-temp-volume, thus $CHCl_3$-60-5 identifies the MIP prepared in $CHCl_3$ at 60 °C and 5 mL of porogen. [b] Average of MIP and Values. Yield was determined gravimetrically.

Table 2. Cocaine-imprinted polymer synthesis conditions, reaction time and yield. All polymers were prepared using MAA as functional monomer and EGDMA as crosslinker.

4.1 MIP rebinding

Having successfully prepared the required MIPs (and NIPs), our attention turned to determining the efficacy of cocaine rebinding in each MIP. This was conducted via batch rebinding assays (in triplicate, see Experimental - section 3.5.1.). Typically, rebinding is carried out in the original MIP porogen, however high viscosity of the RTILs means that this is not practical due to mass transport issues significantly hampering ingress and egress of the template to the cocaine specific cavities. The optimal rebinding time was determined to be 60 minutes (data not shown), and as such all analyses were conducted at this time point (see Experimental – section 3.5.1.). Determination of the amount of cocaine rebound to both MIP and NIP allowed the calculation of the *imprinting factor (I)*, where $I = B_{MIP}/B_{NIP}$. These values are shown in bold in Figure 3 below.

A cursory examination of the data presented in Figure 3 reveals a broad range of imprinting values from $I_{HSO4\text{-}60\text{-}25} = 0.4$ (Fig. 3c) to $I_{BF4\text{-}0\text{-}25} = 2.2$ (Fig. 3d). An $I < 1.0$ indicates preferential binding of the template (cocaine) to the control polymer. The $I_{BF4\text{-}0\text{-}25} = 2.2$ value compares very favourably than the best I value noted for $CHCl_3$ (VOC) of $I_{CHCl3\text{-}60\text{-}5} = 1.6$ (Fig. 3a). Interestingly, while none of the $RTIL_{60\text{-}5}$ preparations afforded a selectivity enhancement relative to the $CHCl_3$ preparations, these RTIL-MIPs (and NIPs) displayed a considerably

higher binding capacity (B% up to ca 45%) in all cases. This suggests the formation of a greater number of low specificity binding sites at higher temperature (in keeping with MIP theory that suggests lower temperature favour high specificity binding sites). Regardless, the specificity of these $RTIL_{60\text{-}5}$ MIPs is poor at best with a maximum I = 1.4. Also of note is the HSO_4 systems that returned I = 1.0, that is, no selectivity for the template relative to the NIP. The HSO_4 RTIL displays, by a considerable margin, the highest viscosity of all the systems examined (Table 2), as such the poor specificity was unexpected as the high viscosity should retain the FM and T in close proximity, allowing for a longer interaction lifetime, and thus an enhanced I value. However the lack of specificity may be an artefact of the poor mass transport associated with high viscosity and the short pre-association time (20 minutes) used in the preparation of these MIPs. In the initial MIP pre-association (pre-polymerisation phase), to generate high specificity binding sites, the template and functional monomer must participate in favourable interactions which invariably requires a co-localisation of the FM and T units. The high viscosity of the HSO_4 RTIL may have prevented this association and thus lead to no or little T-FM interactions, in turn leading to no observable specificity.

The 0 °C, 5 mL MIP preparation (Figure 3c) were effectively non-selective. We do note, however, the highest levels of cocaine re-binding across the board in these systems. Given that this is a non-specific effect, it is most likely due to an increased surface area / surface binding rather than the development of template specific cavities.

These findings appear to contradict the conventional MIP theory, which stipulates that the template selectivity of MIPs is enhanced by low temperature polymerisation due to lower system internal energies. At present, with the limited number of RTIL-MIP studies, there appears to be no fixed correlation between polymerisation temperature and MIP efficacy. It is important to note here that these findings are limited to the $RTIL_{0\text{-}5}$ systems. These systems strongly favour the formation of a bulk (monolithic) MIP. Under these conditions we believe that polymer precipitation induces a high level of template to co-precipitate, effectively destroying the T-FM association crucial for high specificity MIPs. Thus, increasing the lifetime of a homogeneous system would be expected to result in the preparation of a higher specificity MIP (see below). Another possible explanation for this is that the generation of selective sites is limited by the fast polymerisation kinetics at low temperatures in the RTILs. Fast reaction kinetics make the overall polymerisation times very short compared to the polymers prepared at 60 °C. Although this is generally seen as an advantage of the RTIL-based system (due to vastly improved efficiency), short polymerisation times have been linked with decreased selectivity of polymers for the template (Piletska et al 2009). Shorter polymerisation times limit the thermally driven equilibrium between functional monomers and template required for the generation of selective sites. These reduced polymerisation times will also reduce the level of crosslinking in the polymer which inturn may reduce cavity specificity.

The rebinding performance for the 60 °C, 25 mL polymer systems (Fig. 3c) showed retention of selectivity with $CHCl_{3\text{-}60\text{-}25}$ and $PF_{6\text{-}60\text{-}25}$ (I = 1.5), with enhanced specificity for $BF_{4\text{-}60\text{-}25}$ (I = 1.9). Selectivity has been derived at the cost of binding capacity with a decrease in binding noted for $BF_{4\text{-}60\text{-}25}$ (B = 24%; c.f. $BF_{4\text{-}60\text{-}5}$ B = 39%). The VOC system, $CHCl_{3\text{-}60\text{-}25}$ showed increased levels of total binding (B = 28%) relative to the corresponding $CHCl_{3\text{-}60\text{-}5}$ (B = 16%) with no change in specificity. Again, the HSO_4-prepared polymer system showed different behaviour to other RTILs; in $HSO_{4\text{-}60\text{-}25}$, NIP binding was higher (B = 33%) and MIP binding was lower (B = 13%) than in $HSO_{4\text{-}60\text{-}5}$. In the $MIP_{0\text{-}25}$ systems (Fig. 3d), there was increased specificity with the BF_4 (I = 2.2) and HSO_4 (I = 1.9) systems. This was due to both an increase in MIP binding

and a reduction in NIP binding compared to both other systems. The selectivity of $PF_{6\text{-}0\text{-}25}$, however was adversely affected with I = 0.8 and total binding levels significantly lower than in all other [bmim]PF_6 systems.

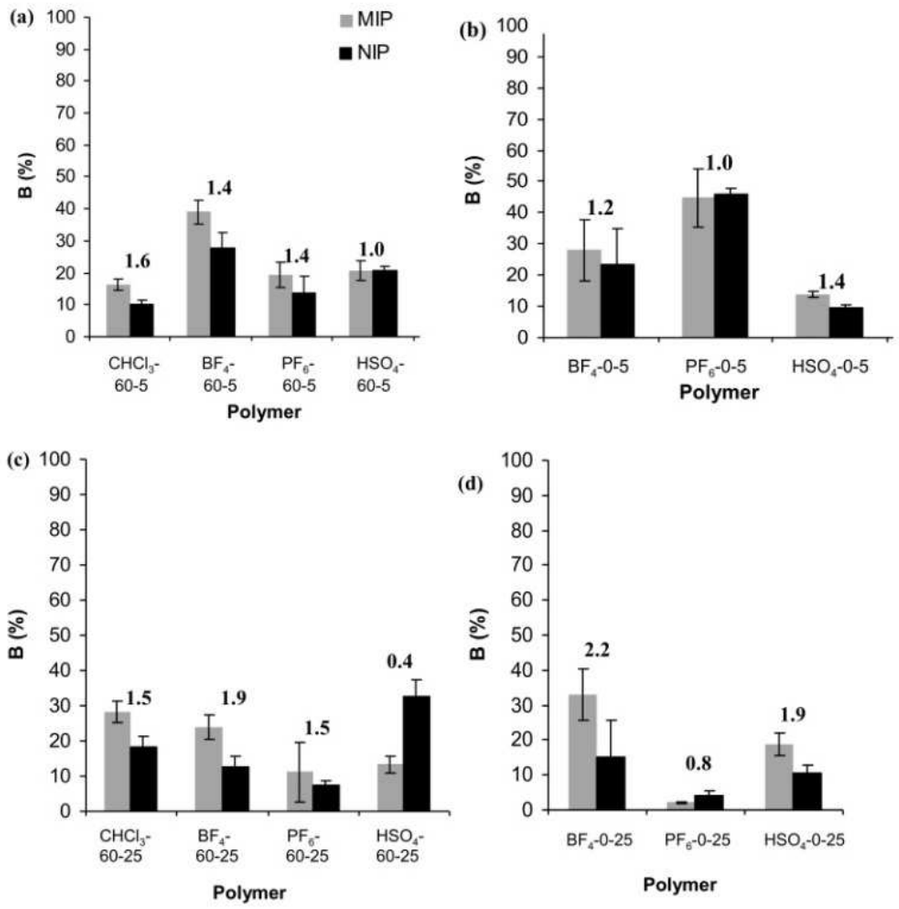


Fig. 3. Rebinding data (percentage cocaine rebound MIP and NIP, and I values shown in bold over the MIP/NIP graph columns) for cocaine-imprinted polymers prepared in $CHCl_3$, [bmim]BF_4, [bmim]PF_6 and [bmim]HSO_4. MIPs were prepared at temperatures and porogen volumes of: (a) 60 °C, 5 mL; (b) 0 °C, 5 mL; (c) 60 °C, 25 mL; and (d) 0°C, 25 mL. All rebinding studies were conducted in $CHCl_3$. The error bars represent the standard deviation after 3 measurements.

This rebinding data highlights the complex nature of the RTIL effects on MIP systems. Overall, the best performing RTIL-MIP system was [bmim]BF_4. The [bmim]BF_4-MIPs retained and at times showed enhanced selectivity across all temperature and porogen volumes studied. The highest imprinting factor was achieved in $BF_{4\text{-}0\text{-}25}$ with an imprinting factor of I = 2.2. This represents an improvement over the VOC-$CHCl_{3\text{-}60\text{-}5}$ with I = 1.6. While MIPs have been prepared using RTILs that show selectivity comparable to, and in some cases better than, polymers prepared in $CHCl_3$, this has been shown to be dependent on the RTIL composition and the synthesis conditions. These effects are not directly linked to RTIL viscosity. Various factors related to the physical characteristics of the polymers, such as swelling and surface charge, were found to be contributing factors driving this selectivity (Rampey et al 2004).

4.2 Polymer morphology

The MIPs herein have been generated as monolithic (5 mL porogen) and precipitation polymers (25 mL porogen), with the precipitation approach displaying the highest *I* values. This suggests that the final polymer morphology affects the selectivity of template rebinding. Our initial study of the physical characteristics of MIPs commenced with evaluation of the polymer surfaces by scanning electron microscopy (SEM). SEM images of cocaine-imprinted polymers prepared in both $CHCl_3$ and RTILs are shown in Fig. 4 below.

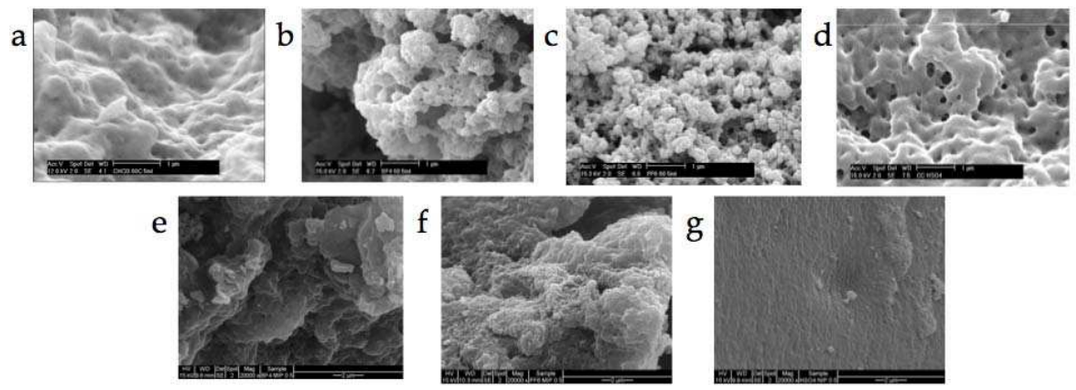


Fig. 4. SEM images of cocaine-imprinted polymers prepared in 5 mL porogen at 60 and 0 °C. (a) $CHCl_{3\text{-}60\text{-}5}$; (b) [bmim]$BF_{4\text{-}60\text{-}5}$; (c) [bmim]$PF_{6\text{-}60\text{-}5}$; (d) [bmim]$HSO_{4\text{-}60\text{-}5}$; (e) [bmim]$BF_{4\text{-}0\text{-}5}$; (f) [bmim]$PF_{6\text{-}0\text{-}5}$; (g) [bmim]$HSO_{4\text{-}0\text{-}5}$.

There are marked differences between the MIP-$CHCl_3$, which is clearly amorphous monolithic in nature, typical of polymers synthesised under similar conditions (Sun & Fung, 2006). However the RTIL preparations show much greater structural definition and are typically composed of nano-particulate clusters (< 300 nm), which result in greatly enhanced surface area. This morphology difference is attributed to increased polymer solubility in the RTIL, promoting a much later phase separation, yielding the observed cluster formation. The $HSO_{4\text{-}60\text{-}5}$ system forms a space filled gel (according to Stover's naming system (Goh & Stover, 2002)) (Fig. 4d).

Examination of these polymers now at 0 ^oC and 5 mL porogen, leads to a marked decrease in the visible surface area (Fig. 4e-g). Presumably this results from an increase in RTIL viscosity with lower temperatures (note that the $CHCl_3$ 0 ^oC preparation failed), decreasing polymer solvation, decreasing diffusion resulting in an increasing monolithic polymer characterisation.

Typically, the MIPs prepared at lower temperature displayed much smoother surfaces. This was anticipated, as it is known that polymerisation temperature has a direct influence on the polymer particle size and porosity (Lu et al., 2004; Kotrotsiou et al., 2009; Li et al., 2007; Rajaram & Hudson, 1996). Interestingly, no fixed correlation between polymer surface area and porosity has been reported for polymers formed in $CHCl_3$, with character dependent upon the nature of the template, functional monomer and cross linker used (Li et al., 2007; Kotrotsiou et al., 2009).

The effect of increasing porogen volume (5 mL to 25 mL) was next examined. Of particular note is the now particulate character (~300 nm) of the $CHCl_3$ MIP (cf. Fig. 4a ($CHCl_{3\text{-}60\text{-}5}$) and Fig. 5a ($CHCl_{3\text{-}60\text{-}25}$)). In all instances, there was little difference noted between the 5 mL and 25 mL RTIL preparations. Both the BF_4 and PF_6 systems afforded nano-particulate clusters (Fig. 5 a and b), with the HSO_4 system, again, the outlier with a gel structure lacking any

obvious structural features (Fig. 5d). Reducing the polymerisation temperature to 0 °C, resulted in a similar reduction in the observable surface area (as with the 60-5 and 0-5 mL preparations above). All RTIL polymers adopt an amorphous surface morphology. As before, this is most likely due to high RTIL viscosity and low polymer solvation.

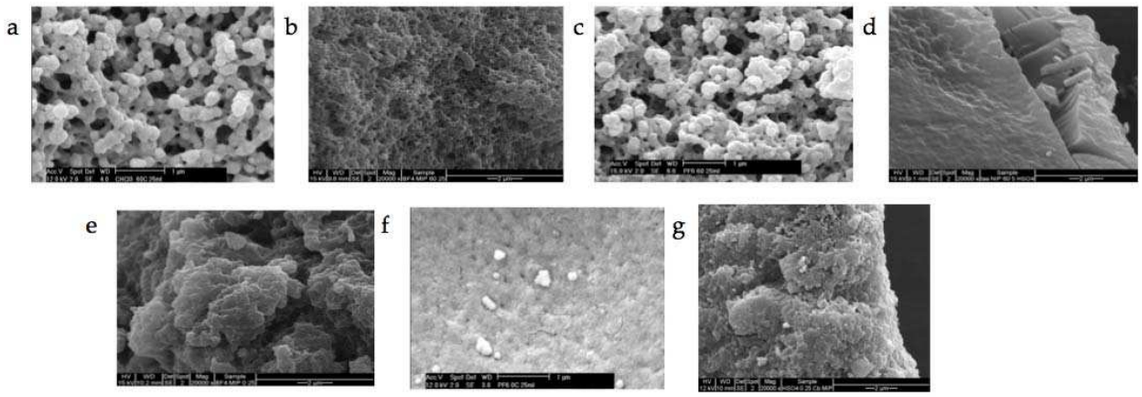


Fig. 5. SEM images of cocaine-imprinted polymers prepared in 25 mL porogen at 60 and 0 °C. (a) $CHCl_{3\text{-}60\text{-}25}$; (b) [bmim]$BF_{4\text{-}60\text{-}25}$; (c) [bmim]$PF_{6\text{-}60\text{-}25}$; (d) [bmim]$HSO_{4\text{-}60\text{-}25}$; (e) [bmim]$BF_{4\text{-}0\text{-}25}$; (f) [bmim]$PF_{6\text{-}0\text{-}25}$; (g) [bmim]$HSO_{4\text{-}0\text{-}25}$.

4.3 MIP swelling

Swelling behaviour is known to impact on MIP performance in two ways. Some degree of swelling facilitates template access to the specific binding cavity within the MIP. However, high levels of swelling can lead to cavity deformation reducing the efficacy of the template-cavity interaction (Spivak, 2005). Typically polymers prepared in solvating porogens will experience a greater dynamic swelling range due to a decreased crosslinking network (Sellergren, 2001). This results from later phase separation of the growing polymer chains, resulting in a lower degree of aggregation of the polymer particles. Generally, RTIL prepared MIPs and NIPs have been observed to swell less (Booker et al., 2006). It is important to note here though, that the absence of polymer swelling does not preclude the development of high specificity, high capacity MIPs.

MIP	**60 °C 5 mL**	**0 °C 5 mL**	**60 °C 25 mL**	**0 °C 25 mL**
$CHCl_3$	100%	-	250%	-
[bmim]BF_4	20%	33%	100%	170%
[bmim]PF_6	70%	160%	285%	80%
[bmim]HSO_4	35%	50%	35%	10%

Table 2. Degree of swelling exhibited by MIPs prepared at 60 °C, 5 mL porogen; 0 °C, 5 mL porogen; 60 °C, 25 mL porogen; and 0 °C 25 mL porogen, in $CHCl_3$.

The degree of MIP swelling was determined in $CHCl_3$, the batch rebinding solvent. Minimal differences were noted between MIPs and NIPs. In the 60 °C, 5 mL polymer preparation $CHCl_{3\text{-}60\text{-}5}$ MIP showed approximately 100% swelling, significantly higher than the $BF_{4\text{-}60\text{-}5}$ (20%), $PF_{6\text{-}60\text{-}5}$ (70%), and $HSO_{4\text{-}60\text{-}5}$ (35%) MIPs (Table 2). The MIPs prepared at 0 °C in 5 mL solvent showed a similar pattern of swelling behaviour to the 60 °C preparation

across the solvent range, with an increase in MIP swelling compared to the 60 °C, 5 mL polymer preparation was observed in most cases: $BF_{4\text{-}0\text{-}5}$ (33%), $PF_{6\text{-}0\text{-}5}$ (160%) and $HSO_{4\text{-}0\text{-}5}$ (50%). This is most likely attributable to the decreased crosslinking density when prepared under photoinitiated conditions (Sellergren, 2001).

Short polymerisation times may also result in lower degrees of crosslinking, which limits the formation of well-defined rebinding cavities, which in turn can reduce selectivity due to decreased polymer rigidity. This could also serve as an explanation of the increased swelling and template uptake, as a more flexible polymer will facilitate mass transfer through the polymer and allow for more binding to take place. The associated cavity deformation has a substantial impact on the reproducibility of results and on selectivity for the template (Spivak, 2005).

The porogen volume in the initial synthesis also influenced the polymer 'swellability'. Polymers prepared at 60 °C in 25 mL porogen (Table 2) showed enhanced swelling compared to their 5 mL counterparts for most polymer preparations; $CHCl_{3\text{-}60\text{-}25}$ (250%), $BF_{4\text{-}60\text{-}25}$ (100%) and $PF_{6\text{-}60\text{-}25}$ (285%). This is not the case in $HSO_{4\text{-}60\text{-}25}$ where the degree of swelling remained constant. Again, this effect may be attributed to a general decrease in crosslinking density as a result of the dilution of the polymerisation components. This had no effect on the [bmim]HSO_4-prepared polymer where the high viscosity (900 mPa s) promotes high levels of crosslinking (due to very slow diffusion). When prepared at 0 °C in 25 mL porogen, a different trend was observed. The $BF_{4\text{-}0\text{-}25}$ prepared polymer showed enhanced swelling (170%) compared to the 0 °C, 5 mL preparation (33%), while in $PF_{6\text{-}0\text{-}25}$ (80%) and $HSO_{4\text{-}0\text{-}25}$, swelling was reduced (10%). We speculate, and are currently investigating, that the lower viscosity of these solvents, which will be increased at lower temperatures, prevents polymer diffusion, thereby increasing crosslink density.

The fact that MIP and NIP polymers do not vary greatly indicates that it is the preparation solvent, not the presence of the template, which has the greatest effect on the polymer swelling behaviour (data not shown).

4.4 MIP zeta potential

Zeta potential analysis can give valuable data regarding the surface charge of the polymers. This indicates whether or not the polymer is colloidally stable in solution whilst also giving some indication as to the nature and relative density of functional groups that may be present at the polymer surface. The zeta potential of polymers of this type can be related to the number of surface accessible MAA carboxylic acid groups (Perez-Moral & Mayes, 2004). These carboxylic acid groups on the polymer surface will influence the degree of surface binding, i.e. a larger zeta value would be expected to correlate with higher levels of surface binding, assuming uniform distribution of MAA units.

While all the RTIL samples examined by SEM were nano-particulate in nature, no sample showed high levels of dispersion, instead showing a high degree of polymer aggregation (Fig. 4 and 5). Pilot studies showed that sonication time had no effect on the measured zeta potential (data not shown). With polymers prepared at 60°C, 5mL, most measurements fell within zeta values of -18 mV and -22 mV, with readings obtained that were within the same error range across the various solvent preparations. The major outliers were clearly $BF_{4\text{-}60\text{-}5}$ MIP (-28mV) and $HSO_{4\text{-}60\text{-}5}$ NIP (-14 mV). The increased surface charge of $BF_{4\text{-}60\text{-}5}$ indicates a higher concentration of MAA present on the surface of the polymer. This could have an impact on the rebinding behaviour of the polymer as it would increase the levels of template

rebound to the polymer surface (Perez-Moral & Mayes, 2004). The decrease in zeta potential observed in the $HSO_{4\text{-}60\text{-}5}$ NIP further emphasises the difference in the nature of this polymer, as the TGA indicated (see section 4.5).

As the SEM data showed a significant decrease in the visible surface area of the polymers at low polymerisation temperatures, it follows that there may be some differences in zeta potential. Image **b**, Figure 3.6 shows the zeta potential data for the polymers prepared at 0 °C in 5 mL solvent. Overall, the polymers showed a slight decrease in the average zeta values compared to the 60 °C-prepared system (approximately 2mV lower in most cases), corresponding to the decrease in visible surface area observed via SEM. Again, the effect of solvent choice for the polymerisation process appeared to have little effect on the observed zeta values. The $HSO_{4\text{-}0\text{-}5}$, however, showed a significant drop in the zeta potential of the NIP (-12 mV compared to -18 mV in the MIP), as was observed for this RTIL in the 60 °C system.

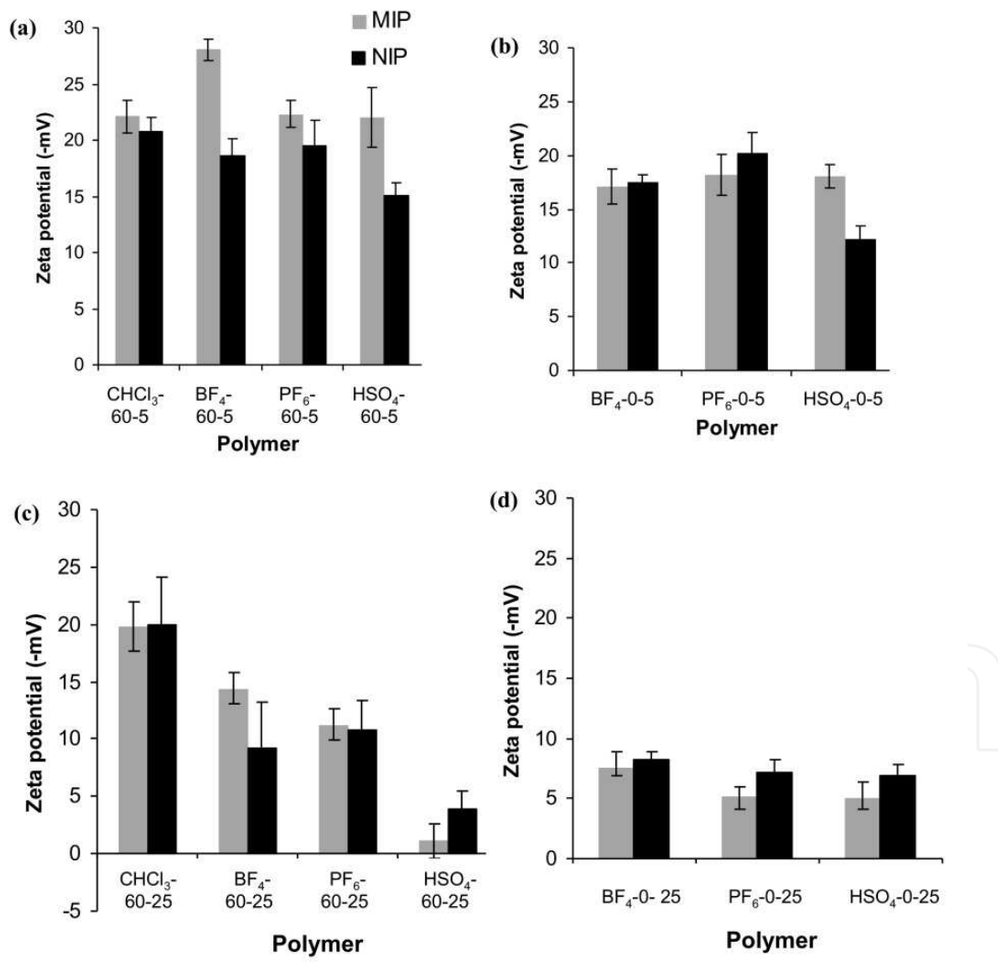


Fig. 6. Zeta potential data for cocaine-imprinted polymers prepared in various solvents. Polymers were prepared at temperatures and solvent volumes of (a) 60 °C, 5 mL, (b) 0 °C, 5 mL, (c) 60 °C, 25 mL and (d) 0 °C, 25 mL. The error bars represent standard deviation after 10 measurements.

4.5 MIP stability

All MIPs showed good thermal stability up to 260 °C, as did the corresponding NIPs (data not shown). In the 0 °C polymer preparations (Figure 7), the polymers were again observed to be stable up to 260 °C. The TGA traces show little variation between the polymers, with the exception of [bmim]PF_6 which showed enhanced thermal stability. There is one very distinct decomposition phase, indicating a more homogeneous composition than the 60 °C-prepared polymers.

MIPs prepared under precipitation polymerisation conditions (25 mL progen) showed similar levels of thermal stability to those prepared in 5 mL porogen (data not shown), with most polymers stable to 260°C. The exception to this was polymer $HSO_{4\text{-}60\text{-}25}$, which showed substantial mass losses (>20%) at temperatures below 260 °C and high char yields, again indicating that the ionic liquid may not have been completely removed from solution. Polymers prepared at low temperature again showed a more distinct decomposition phase, indicating a more homogeneous composition. This decomposition data shows that a good level of thermal stability can be achieved in polymers prepared in RTILs, which is, in most cases, comparable to polymers prepared in VOCs. These results are in line with literature for linear polymers prepared in RTILs, which showed similar thermal stability to that of VOC-prepared polymers (Cheng et al., 2004).

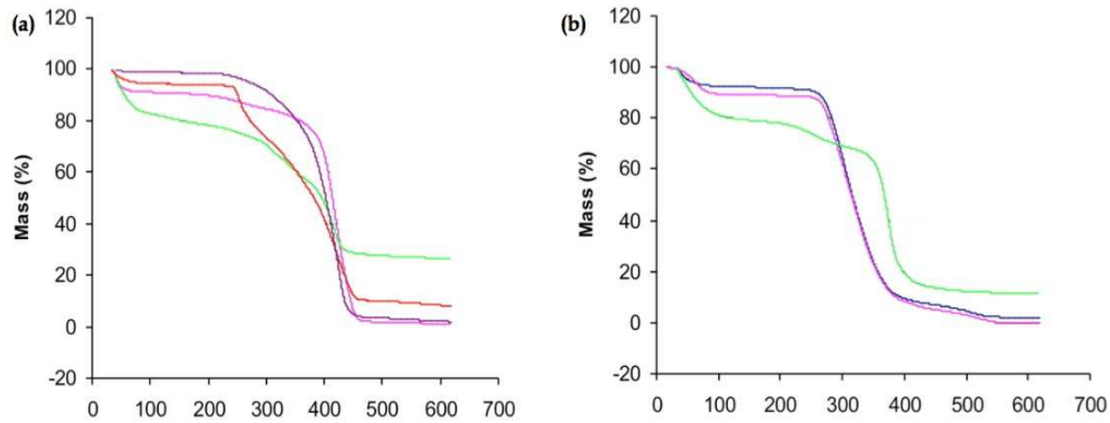


Fig. 7. TGA spectra for cocaine-imprinted MIPs prepared in: —— $CHCl_3$; —— [bmim]PF_6; —— [bmim]BF_4; —— [bmim]HSO_4 at (**a**) 60 °C, 25 mL and (**b**) 0 °C, 25 mL.

5. Conclusions

This chapter has highlighted the many different factors related to using RTILs as polymerisation solvents, which can influence polymer characteristics and rebinding performance. In depth analysis of the previously studied cocaine-imprinted system showed that the porogen used for polymerisation, temperature and solvent volume all have significant effects on polymer properties. Some general trends could be observed, such as the increased binding capacity of polymers when prepared at lower temperatures, due to a decrease in crosslinking density. It showed that extremely high levels of crosslinking, whilst producing very thermally stable polymers, results in decreased uptake of the template and a loss of selectivity for both VOC and RTIL-prepared polymers. Overall, using [bmim]BF_4 as

polymerisation solvent gave the best results in terms of polymer selectivity from all the RTILs studied. Imprinting factors >1 were achieved across all temperature ranges and solvent volumes with better imprinting factors achieved than in the VOC-prepared polymers (the highest *I* value in [bmim]BF_4 was 2.2 compared to 1.6 in the $CHCl_3$).

The RTIL-prepared polymers show promise as VOC alternatives in MIP systems, with numerous advantages (such as improved reaction efficiency, the elimination of the need to grind the polymers, the ability to polymerise at low temperatures and the improved selectivity for the template in some instances). There is also the possibility of recycling and reusing the RTIL for numerous reactions, although this was not examined in the current study. However, the data presented thus far has highlighted one of the problems associated with MIP synthesis, which was the lack of reproducibility observed in rebinding studies. This is presumably a result of heterogenous binding sites present in the polymers. In order to achieve good selectivity for the template, it is necessary to create the right balance between the different synthesis and rebinding conditions. The fact that this is further affected by the choice of RTIL makes the process even more complex. The prediction of polymer properties in any given RTIL-MIP system has been shown to be challenging, which places limitations on the utility of these systems. A more comprehensive study is required, to include analysis such as molecular modelling and NMR studies right through to rebinding isotherms on a well studied, 'model' MIP template. This may assist in furthering our understanding of the role of RTILs in MIP polymerisations and enhance the scope of RTILs in MIP applications.

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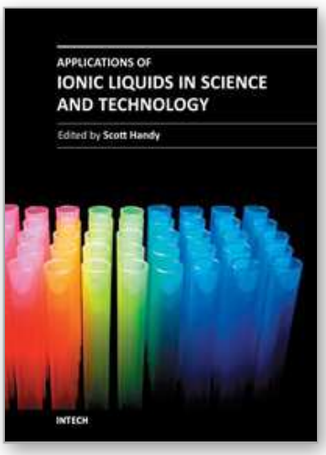

Applications of Ionic Liquids in Science and Technology

Edited by Prof. Scott Handy

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This volume, of a two volume set on ionic liquids, focuses on the applications of ionic liquids in a growing range of areas. Throughout the 1990s, it seemed that most of the attention in the area of ionic liquids applications was directed toward their use as solvents for organic and transition-metal-catalyzed reactions. Certainly, this interest continues on to the present date, but the most innovative uses of ionic liquids span a much more diverse field than just synthesis. Some of the main topics of coverage include the application of RTILs in various electronic applications (batteries, capacitors, and light-emitting materials), polymers (synthesis and functionalization), nanomaterials (synthesis and stabilization), and separations. More unusual applications can be noted in the fields of biomass utilization, spectroscopy, optics, lubricants, fuels, and refrigerants. It is hoped that the diversity of this volume will serve as an inspiration for even further advances in the use of RTILs.

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