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Molecularly Imprinted Polymers and Room Temperature Ionic Liquids: Impact of Template on Polymer Morphology

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Molecularly imprinted polymers (MIPs) were generated for *trans*-aconitic acid **1** and cocaine **2** in a variety of porogens (CH_3CN, $CHCl_3$, [bmim][BF_4], and [bmim][PF_6]). MIP synthesis in either [bmim][BF_4] or [bmim][PF_6] resulted in significant acceleration of polymerization rates and, in the case of low temperature polymerizations, reactions were complete in less than 2 h, while no product was observed in the corresponding volatile organic carbon (VOC) porogen. In all instances, MIPs generated in [bmim][BF_4] or [bmim][PF_6] returned imprinting selectivities (I values) on par with or better than the corresponding MIP generated in VOCs. Imprinting values ranged between $I = 1$ and 2.9, with rebinding limited to 1 h. MIP synthesis conducted at low temperature (5°C) afforded the highest I values.

Scanning electron microscopy examination of MIP morphology highlighted an unexpected template effect with MIP structure varying between discrete nanoparticles and robust monoliths. This template–monomer interaction was also observed in the rates of polymerizations with differences noted in reaction times for **1** and **2** MIPs, thus providing indirect conformation of our previously proposed use of molecular modelling–nuclear magnetic resonance titrations (the MM-NMR method) in the design phase of MIP generation. In addition, considerable batch-to-batch rebinding selectivities were observed.

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Introduction

The illegal importation of illicit drugs is a growing concern for the Australian Federal Police and Australian Customs services and is placing an increasing demand on already stretched resources at major Australian border entry points. While canine detection and intelligence led policing has been highly successful, both have considerable drawbacks, the latter has considerable resource implications and the former is limited by the expense associated with the handling and training of dogs, their narrow attention span, and the limited amount of reliable scientific information obtained.[1,2] While some instrumental trace-level detection methods are commercially available for use (gas chromatography with chemiluminescence, electron capture, or surface acoustic waves detectors and, of particular note, ion mobility spectrometers, and biosensors) and are continually improving, they generally still suffer from selectivity and sensitivity problems, limited mobility/tracking ability, high level of intrusiveness, high rate of false positive results, short shelf-lives (e.g., immunosensors), and high cost.[1,3,4] These existing technologies also often require expert training in their use and data analysis, and may be too complex and specialized to allow a lay jury to fully comprehend the significance of the data generated.

Over the past decade, our group has been developing methodologies that have the potential to allow passive sensing of target molecules, for example, flavour contaminants in wine and selected illicit drugs[5–9] in a wide range of domestic settings. Pivotal to our approach is the rapid development of specific recognition elements that have the ability to respond (generate a signal) in the presence of a chemical vapor.[10] Most transduction methods are already well developed but the design of a suitable chemical recognition element remains a challenge to date. Techniques that have been widely used to impart recognition to sensing devices for trace levels of explosives and narcotics are based on the interactions of the substrate with biological molecules (such as host–guest and antigen–antibody interactions),[4,5] which are unstable and prone to saturation and decomposition.

Over the past five years, we have developed considerable expertise in the field of molecular imprinting (a 'biomimetic' process) and have applied the technology to the extraction, detection, and measurement of specific chemical components.[5–9] Specific recognition sites created during the molecular imprinting process hold considerable potential as highly specific chemical recognition elements. Our group,[5–9] and others,[10–12] have expended considerable effort in developing rational approaches to such elements. The application of the molecular modelling–nuclear magnetic resonance titration (MM-NMR) approach by us has resulted in the rapid and rational design of a wide variety of molecularly imprinted polymers (MIPs). Notably, we have

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obtained excellent results even with the more recalcitrant of templates, namely those lacking in hydrogen-bonding or electrostatic interactions. Notwithstanding our successes, post-MIP manipulation (to uniform particle size) is laborious and often destroys the specific cavities that were developed during the templating process. Precipitation polymerization approaches, excepting their slow reaction rates and hence extended reaction times and high porogen requirements, are ideally disposed to circumvent this issue.

Recently, room temperature ionic liquids (RTILs) have been shown to accelerate polymerization rates.[13] The magnitude of the propagation rate constant for these reactions has been shown to increase with increasing mole fraction of the ionic liquid in the reaction mixture. The unusual solvation properties displayed by RTILs have been partially attributed to the maintenance of a supramolecular structure in the liquid phase, which leads to the formation of discrete polar and non-polar microenvironments.[14] Enzymes have been reported to exhibit enhanced thermal stability when immersed in RTIL.[15]

Not surprisingly we believed that this might alleviate the protracted reaction time associated with traditional approaches to precipitation polymerizations. Our preliminary findings using arguably the two most highly evaluated RTILs, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) and 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]), are reported herein.

Results and Discussion

We have previously detailed the procedure whereby we identified the most appropriate ratio of template to functional monomer by our MM-NMR approach.[6–9] Herein we evaluate two template systems and the effect of volatile organic compounds (VOCs) (CH_3CN and $CHCl_3$), [bmim][BF_4], and [bmim][PF_6] on the rebinding efficacy and polymer morphology relative to the analogous non-imprinted polymers (NIPs). In this study our data (not shown) indicated that with *trans*-aconitic acid and cocaine (Fig. 1), the most favourable template to monomer ratio was 2:1 (methacrylic acid to template).[8,9] Ethylene glycol dimethacrylate (EGDMA) was used as the polymer crosslinker.

In the initial templating process of MIP generation, the template and monomer interact to form a transient cluster. As the internal energy of the system increases (e.g., by heating) the transitory nature of the template–monomer interaction also increases, and should decrease the strength of the pre-polymerization cluster to theoretically yield lower imprinting values. As the temperature of the pre-polymerization mixture is decreased the stability of the template–monomer cluster increases. Thus in a low temperature system we anticipate larger template–monomer interactions being reflected in the ultimate imprinting value. Indeed the change in 'cluster nature' should be evident from NMR analysis.[12] To evaluate the impact of temperature, we conducted polymerizations at 5°C (photochemical initiation, azobisisobutyronitrile (AIBN)) and at 60°C (thermal initiation, AIBN). Thus in addition to evaluating the porogens' impact on selectivity as a function of bulk versus precipitation polymerization, we were also keen to evaluate the effect of temperature.

In essence, two series of studies were conducted; the first under bulk conditions (5 mL of porogen at 5 and 60°C) and the second under precipitation conditions (25 mL porogen at 5 and 60°C). Each template and porogen combination in turn was subjected to our standard polymerization conditions (for both bulk and precipitation). The outcomes of these reactions are shown in Table 1.

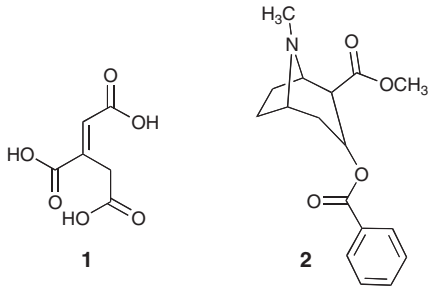

Fig. 1. *Trans*-aconitic acid and cocaine.

Table 1. Polymerization times for 1 and 2

Porogen	Reaction temperature [°C]	Volume porogen [mL]	Reaction time [h] 1	2
CH_3CN	5	5	—[A]	—[B]
		25	—[A]	—[B]
	60	5	6	—[B]
		25	18	—[B]
$CHCl_3$	5	5	—[B]	—[A]
		25	—[B]	—[A]
	60	5	—[B]	6
		25	—[B]	18
[bmim][BF_4]	5	5	0.75	0.5
		25	2	2
	60	5	2	2
		25	8	4
[bmim][PF_6]	5	5	0.5	0.75
		25	2	2
	60	5	2	2
		25	8	4

[A]No reaction. [B]Not performed.

As can be seen from Table 1 traditional VOCs, i.e., CH_3CN and $CHCl_3$, give no polymerization at low temperature under either bulk or precipitation conditions, whereas both RTILs, [bmim][BF_4] and [bmim][PF_6], examined at 5°C gave excellent yields of polymer, and also at 60°C. Interestingly, although the monomer-to-template ratio was consistent, and the same functional monomer unit (methacrylic acid) was used in preparing MIPs from **1** and **2**, there is an observable difference in the rates of reaction observed. At both 5 and 60°C, polymerization is more rapid with **2** under bulk conditions (5°C, 30 versus 45 min; 60°C, 4 versus 8 h) in [bmim][BF_4]. The rate of reaction is reversed when the reactions are conducted in [bmim][PF_6], but only at low temperature (5°C, 45 versus 30 min). Perhaps the most surprising observation in this sequence is the extended polymerization intervals required at higher temperatures. At 60°C the rate of reaction attains consistency with cocaine MIPs generated twice as fast, but requiring 4 to 8 h for completion. Obviously, at lower temperatures the RTILs have a pronounced effect on the stability and reactivity of the radicals generated during polymerization and at low temperatures affect a cage-like structure, which is negated by the decrease in viscosity as the reaction temperature increases. Notwithstanding this, polymerization still occurs more rapidly in RTILs than in the traditional VOCs examined.

Table 2. Rebinding results (after 1 h) for MIPs generated from 1 and 2 in porogens: CH_3CN, $CHCl_3$, [bmim][BF_4], and [bmim][PF_6]

Porogen	Reaction temp. [°C]	Volume [mL]	*I* of **1**[A]	*I* of **2**[A]		
				Batch 1[B]	Batch 2[B]	Ave.
CH_3CN	60	25	0.98 (1.6)[B]	—	—	
$CHCl_3$	60	5	—	1.2[C]	2.9	2.0
		25	—	1.1	1.3	1.2
[bmim][BF_4]	5	5	2.3	1.2	1.7	1.5
		25	1.2 (2.3)[B]	1.1	2.8	2.0
	60	5	1.8 (2.2)[B]	1.3	1.2	1.2
		25	2.7	1	2.1	1.6
[bmim][PF_6]	5	5	2.3	1	1.2	1.1
		25	2.5	1	1.2	1.1
	60	5	1.5 (1.8)[B]	2.2	1.2	1.7
		25	1.0 (1.4)[B]	1.6	2.3	1.9

[A] $I = B_{MIP}/B_{NIP}$. [B] 24 h rebinding time. [C] $I = 3.26$ (from McCluskey et al.,[8] T:M ratio 1:2) and 1 h rebinding.

The inability to form polymers in CH_3CN at low temperature could have been caused by the presence of significant free radicals capable of inhibiting polymer chain growth brought about by photochemically induced homolytic cleavage of the C–H bond.[16] Conversely, chloroform is a recognized chain-transfer agent in free radical polymerization reactions, its C–Cl bond can be easily cleaved to generate free radicals. While chain transfer to these solvents is also expected at 60°C, the effect is more pronounced at lower temperature (5°C), where diffusion is hindered. Hence chain propagation can be very slow, and free radicals generated by the solvents abound.

With the required polymers in hand, we set about examining their ability to rebind the original template. As our objective is the development of a convenient test for the detection of illicit drugs, we restricted rebinding times to 1 h. These data are presented in Table 2.

First, examination of the data obtained with *trans*-aconitic acid templated MIPs clearly reveals that $MIPs_{RTIL}$ are significantly superior to the corresponding MIP_{CH_3CN}. Rebinding values, *I*, increase from I_{CH_3CN} 0.98 (no specificity relative to NIP) to a maximum of $I_{[bmim][BF_4]}$ 2.7. At low temperatures, which should favour template monomer interaction, $MIP_{[bmim][BF_4]}$ exhibits maximal rebinding ($I_{[bmim][BF_4]}$ 2.3) under bulk polymerizations conditions, not under precipitation conditions. Whereas with $MIP_{[bmim][PF_6]}$, maximal $I_{[bmim][PF_6]}$ 2.5 is obtained under precipitation conditions, but also at low temperature. While at first glance the $MIP_{[bmim][BF_4]}$ result appears counterintuitive (precipitation polymerization generates discrete particles of uniform size while bulk polymerization yields a monolith that requires post-imprinting manipulation (grinding and sieving to $< 38 \times 10^{-6}$ m)), this is not the case with *trans*-aconitic acid MIP_{RTIL}. Both precipitation and bulk polymerization approaches give nanometer-sized polymer particles (Figs 2b–2i). In this instance the higher $I_{[bmim][BF_4]}$ values are presumably a result of a tighter template–monomer cluster as a function of the higher reaction concentration (all other things being equal). To further explore these findings we also evaluated this MIP series after 24 h rebinding (the values in parentheses within Table 2) and note that when diffusion and contact times increase it is the precipitation-based MIPs that exhibit the highest *I* values. This is presumably an artifact of accessing well-defined cavities within the polymeric matrix, and a slight difference in surface morphology (Fig. 2). Porogen viscosity undoubtedly also affects the outcome of rebinding studies and there is, uniformly, a decrease in $I_{[bmim][BF_4]}$ and $I_{[bmim][PF_6]}$ under thermal initiation conditions. This is most notable with $MIP_{[bmim][PF_6]}$ at 60°C, which gives no selective rebinding after 1 h.

With cocaine-based MIPs our initial results were extremely disappointing and led us to question the utility of RTIL-based porogens in MIP preparation. In previous work, we had observed an $I_{CHCl_3} = 1.17$ for a cocaine MIP (and I_{CHCl_3} 3.26;[8] with template/monomer ratios of 1:2), but we obtained a maximal rebinding from RTIL-based MIPs of $I_{[bmim][BF_4]}$ 2.17. Indeed the other data presented are strongly suggestive of no specific binding being obtained, with *I* values ranging from 1 (no specificity) to 1.60 (modest specificity). Perplexed, we generated a second batch of cocaine MIPs using both RTILs and $CHCl_3$, and noted an across the board improvement in *I* values on an equal footing with I_{CHCl_3} 2.86 and $I_{[bmim][BF_4]}$ 2.83. Again both RTILs displayed divergent outcomes with the maximal $I_{[bmim][BF_4]}$ 2.83 observed at low temperature and precipitation polymerization conditions, whereas the maximal $I_{[bmim][PF_6]}$ 2.27 was observed under thermal precipitation conditions. We are thus not yet able to predict which RTIL and/or combination of polymerization conditions will afford the most responsive MIP.

Scrutiny of the scanning electron microscopy (SEM) images in Fig. 2 shows striking, template-dependant contrasts in polymer morphology. With traditional VOCs there is an obvious transition from precipitation micro-polymer spheres (Fig. 2a) to a monolithic structure (Fig. 2b). While it is conceivable that the change from MIP_{CH_3CN}-**1** to MIP_{CHCl_3}-**2** is the sole rational for a change of this magnitude, our findings with MIP_{RTILs} suggest that this is not the case. Rather, it strongly suggests that the imprinting template influences the polymerization process and is a major determinant of the type of polymer material isolated. In addition, different RTILs affect template-induced polymer morphology changes in different manners. With $MIP_{[bmim][BF_4]}$-**2**, a greater degree of monolithicity compared with $MIP_{[bmim][BF_4]}$-**1** is evident, and differences in surface structure and particle sizing is also noted when comparing the corresponding SEM images, e.g., Fig. 2c versus 2l, 2d versus 2m, and 2e versus 2n. Similar but less distinct trends are evident with $MIP_{[bmim][PF_6]}$-**2** compared with $MIP_{[bmim][PF_6]}$-**1** with the major variations appearing to be a difference in particle size, with most $MIP_{[bmim][PF_6]}$ examined showing an apparent hybrid of monoliths and discrete particles. SEM was not able to provide suitable resolution to determine a statistical distribution of each (monolith versus polymer sphere).

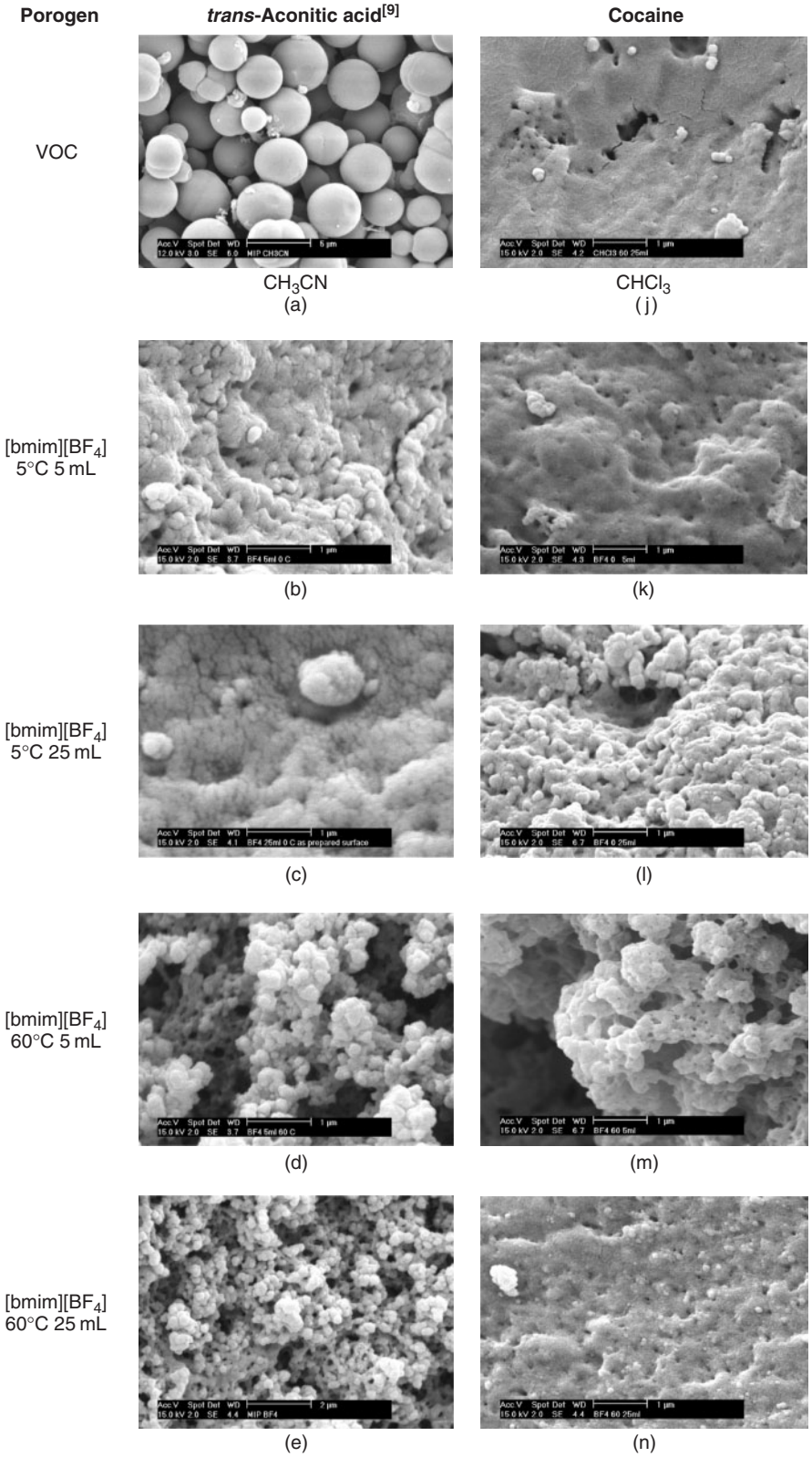

Fig. 2. SEM images of MIPs prepared using different porogens, temperatures, and volumes. (a)–(i) *trans*-aconitic acid MIPs, (j)–(r) cocaine MIPs.

The effect of template–monomer interaction was also observed in the rates of polymerizations with differences noted in reaction times for **1** and **2** MIPs. This provided indirect conformation of our previously proposed use of MM-NMR titrations in the design phase of MIP generation.

Conclusions

This work has identified RTILs as potentially viable porogens for the synthesis of molecularly imprinted polymers with benefits, in this limited study, that include a decrease in reaction duration, the ability to generate MIPs at low temperature with

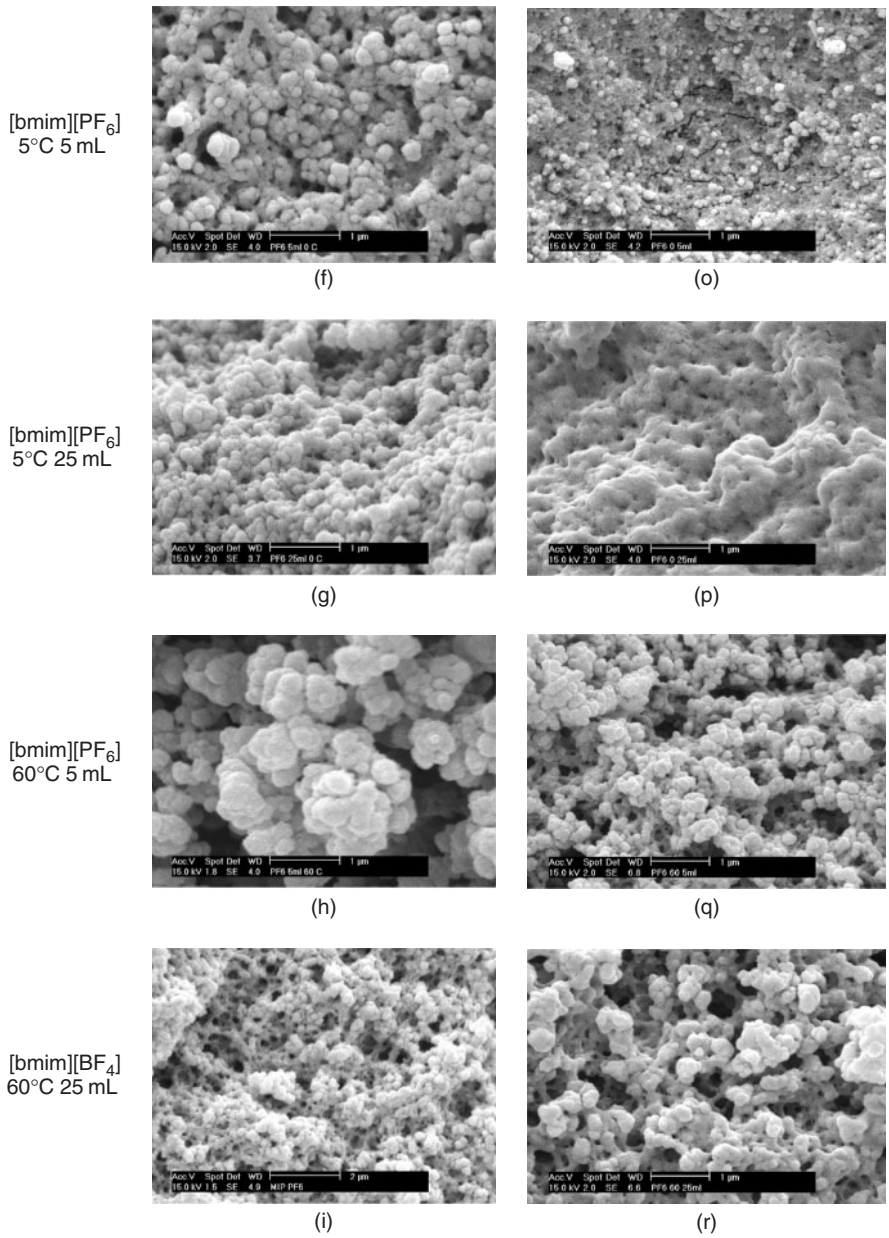

Fig. 2. Continued

a concomitant increase in imprinting specificity and selectivity, and in selected instances, control over particle size. Thus RTILs hold considerable promise to accelerate the synthesis of a wide variety of MIPs, and in the examples studied here deliver selectivities at least on par with, if not better than, those observed with traditional VOC-based porogens. Notwithstanding this, a more expansive range of both RTILs and templates must be evaluated before concluding that MIP_{RTIL}s are uniformly better than MIP_{VOC}s. The effect of template on polymer morphology was unexpected, but confirms a template–monomer interaction during the polymerization process, but it further complicates the issue of how to predict which RTIL and what conditions are best suited to the development of highly specific and robust MIPs. A final note of caution is the observed batch-to-batch variation of selectivity observed when MIPs are prepared under identical conditions. MIP design and synthesis have evolved over the past decade or so, but there are still issues to be resolved before their synthesis and utility can be described as routine. MIPs and RTILs are thus kindred cousins, they have immense untapped potential, but are unable to enter mainstream chemistry while their lack of predictability remains. We are continuing to evaluate the effect of template and RTILs on MIP generation and selectivity, and will report our findings in due course.

Experimental

Cocaine MIPs were prepared with a cocaine base (0.14 mmol, 42.6 mg), methacrylic acid (MAA, 0.28 mmol, 24.1 mg) and EGDMA (1.4 mmol, 280 mg), following the procedure of Holdsworth et al.,[8] in 5 mL (bulk condition) and 25 mL (precipitation condition) of porogen at both 60°C (thermal initiation) and 5°C (photochemical initiation, UV irradiation at 365 nm). The reaction mixture was degassed with N_2 before AIBN (10 mg) was added. The porogens used were $CHCl_3$ (control), [bmim][BF_4], and [bmim][PF_6]. NIPs were prepared using the same method but without the addition of the cocaine base.

Extraction of the template was achieved by five washings in methanol (30 mL) until no cocaine peak was registered by GCMS analysis.

Rebinding was carried out using 10–40 mg of polymer suspended in a 3–4 ppm solution of cocaine in $CHCl_3$ for various time periods. The resulting solution was filtered and analyzed using GC-MS. Cocaine (retention time 9.33 min) was quantified using an external calibration method with a linear curve where $R^2 = 0.998$ at the concentration range of 3–30 ppm.

The concentration of rebound cocaine 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 ($B_{MIP} - B_{NIP}$). The results are expressed as an imprinting factor (I) of B_{MIP}/B_{NIP}.

Trans-aconitic acid imprinted polymers were prepared using the template (0.25 mmol), EGDMA (1 mmol), and MAA (0.75 mmol) with CH_3CN as a control solvent. Polymer preparation, extraction, and rebinding were conducted in line with the cocaine imprinted polymer method. However, after rebinding and filtering the filtrate was evaporated and the aconitic acid residue was derivatized with 0.5 mL of 13% BF_3 in methanol by heating overnight at 65°C to form the aconitic acid ester for quantitation using GC-MS.

Trans-aconitic acid tris methyl ester (retention time 15.78 min) was quantified against octadecane (1.44 M solution in ethyl acetate, retention time 10.53 min), as an internal standard.

General GCMS

All GCMS experiments were conducted in a Shimadzu GCMS-QP2010 attached to an AOC-20 autosampler/autoinjector using a split ratio of 5:1 and injection temperature of 250°C. High purity helium was used as the carrier gas. The column used was a ZB-5MS capillary column, 30 m × 0.25 mm. Column was set to a flow rate of 1.00 mL min^{-1}, total flow of 9.0 mL min^{-1}, linear velocity of 37.2 cm s^{-1}, and a pressure of 73.0 kPa. The injection source and interface temperatures of the MS were both set as 250°C.

Cocaine Quantification

The column was set at an initial temperature of 100°C which was raised at 20°C min^{-1} over 10 min, reaching a final temperature of 300°C.

Trans-Aconitic Acid Quantification

The column was set at an initial temperature of 84°C, followed by an increase of 8°C min^{-1} to 200°C, hold 2 min, then increased by 10°C min^{-1} to 300°C and hold 15 min.

Acknowledgments

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