Efficient preparation and improved sensitivity of molecularly imprinted polymers using room temperature ionic liquids

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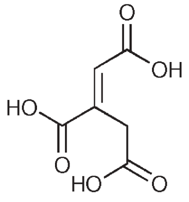
Synthesis of *trans*-aconitic acid molecularly imprinted polymers in [bmim][BF$_4$] and [bmim][PF$_6$] under photochemical (5 °C, AIBN) and thermal (60 °C, AIBN) conditions gave polymer micro-spheres (<200 nm), under bulk and precipitation polymerisation conditions, and higher selectivity indices (100% improvement) relative to the more traditional precipitation polymerisation (CH$_3$CN, high solvent volumes) approach.

Room temperature ionic liquids (RTILs) have received considerable attention from the general chemistry community over the past few years.[1] RTILs have been employed as green solvents for organic chemical reactions, recovery of precious metals, catalysts, separations, electrochemistry and polymerisations. Their ability to be fine tuned to the application/process under evaluation means that they are highly complex solvent systems.[2]

Molecularly imprinted polymers (MIPs), with a memory for the original templating molecule have also attracted considerable attention in general, and especially in environmental and sensor applications.[3] MIPs have been developed for the detection of illicit drugs,[4] environmental pollutants,[5] and in separation science.[6] Multiple methodologies have been implemented to give rise to the most robust and sensitive MIPs.[7] Recent reports have utilised a combination of molecular modelling and NMR titrations to remove the tedium and trial and error approach to the development of MIPs. However, the issue of batch-to-batch reproducibility in particle size and surface area has yet to be satisfactorily resolved. Extensive efforts utilising bulk polymerisation,[8] precipitation polymerisation[9] and emulsion polymerisation methods have been described.[10] Currently, bulk polymers (monolith; the most common approach) require considerable post-polymerisation processing to afford useable MIPs. Typically, bulk polymers undergo a laborious process of grinding and sieving to <38 μm before use, which potentially destroys binding cavities, increases non-specific binding and reduces batch-to-batch reproducibility.[5–8] Whilst each of these approaches has limitations, precipitation polymerisation is known for producing particles of defined size and morphology, but is limited by slow reaction rates, hence long reaction times, and low yields.[11] Notwithstanding this, we felt that the reported enhanced polymerisation rates associated with RTILs would (potentially) facilitate the rapid development of MIPs of defined particle size and morphology.[12]

To the best of our knowledge there have been no reports on the synthesis of MIPs in RTILs. In this report we examined a previously evaluated (precipitation polymerisation based) MIP system that utilised *trans*-aconitic acid as the templating molecule.

As previously reported we typically employ a combination of molecular modelling and NMR titrations to determine the optimal *monomer : template* ratio, and have done so in this instance (data not shown). This combined with existing literature,[13] led us to develop RTIL based MIPs for *trans*-aconitic acid (**1**). We selected methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethylmethacrylate (EGDMA) as the cross linker and *trans*-aconitic acid as the template in a ratio of 4 : 20 : 1, respectively.† We also chose to evaluate MIP generation in two of the most highly evaluated RTIL systems, [bmim][BF$_4$] and [bmim][PF$_6$].‡



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Whilst precipitation polymerisation was the focus of this work we also evaluated the effect of RTILs on bulk polymerisation rates and the effect of temperature. Previous studies on MIP generation have indicated that polymerisation at lower temperature should lead to higher quality MIPs. Accordingly we evaluated our systems at 5 °C (photochemical, AIBN) and 60 °C (thermal, AIBN). Firstly, as can be seen from Table 1 the rates of polymerisation are considerably enhanced relative to CH$_3$CN. In this instance, we have repeated the MIP development reported by Jiang and Tong using CH$_3$CN, differing only in the choice of crosslinker (EGDMA *vs.* TRIM).[13] At 60 °C, thermal (CH$_3$CN) bulk polymerisation requires 6 h; and precipitation polymerisation 18 h for complete reaction (mass conversions 48–91%). At 5 °C (CH$_3$CN) no photochemically initiated polymerisation was observed. Contrast this with the observations with [bmim][BF$_4$]—photochemical initiation (5 °C) both bulk and precipitation polymerisation is complete after 30 min and 2 h, respectively. The corresponding thermal initiation is complete after 2 and 8 h, respectively. Similar observations are made with [bmim][PF$_6$]: photochemical initiation complete after 45 min (bulk) and 2 h (precipitation); and thermal initiation after 2 h (bulk) and

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Table 1 Polymerisation times and rebinding results for *trans*-aconitic acid with 40 mg MIP prepared in different solvents at various times of contact

Entry	Solvent	Reaction temperature/°C	Bulk polymerisation/h	Precipitation polymerisation/h	Vol. solution/mL	Rebinding time/h	B_{MIP} / mM[a]	B_{NIP} / mM[b]	δB/mM	I[c]
1	CH_3CN	5	—	—	5	—	—	—	—	—
2			—	—	25	—	—	—	—	—
3		60	6	18	25	1	0.58	0.60	−0.02	0.98
4						24	0.34	0.24	0.09	1.40
5	CH_3CN					24	0.47	0.30	0.17	1.56
6	[bmim]PF_6	5	0.75	2	5	1	0.24	0.10	0.13	2.27
7						2	0.36	0.39	−0.02	0.93
8						24	0.32	0.24	0.08	1.33
9					25	1	0.49	0.20	0.29	2.45
10						24	0.23	0.20	0.03	1.15
11		60	2	8	5	1	0.57	0.39	0.18	1.46
12						24	0.45	0.25	0.20	1.80
13					25	1	0.33	0.34	−0.01	1.00
14						24	0.30	0.22	0.08	1.39
15	[bmim][BF_4]	5	0.5	2	5	1	0.23	0.10	0.13	2.30
16						24	0.32	0.21	0.11	1.60
17					25	1	0.41	0.35	0.06	1.17
18						24	0.25	0.16	0.09	2.28
19		60	2	8	5	1	0.55	0.31	0.24	1.76
20						24	0.54	0.25	0.29	2.16
21					25	1	0.41	0.15	0.26	2.74
22						24	0.43	0.26	0.17	1.65

[a] B_{MIP} amount of target rebound to MIP. [b] Amount of target rebound to the control, non-imprinted polymer (NIP). [c] $I = B_{NIP}/B_{MIP}$.

8 h (precipitation). This represents a significant rate enhancement as has been reported by others. Regardless of the rate of reaction, rebinding selectivity and polymer morphology were also crucial features in this study.

To evaluate the effect of RTILs on the selectivity of the synthesised MIPs rebinding studies were conducted. This data is shown in Table 1. In our evaluations we were unable to synthesise the photochemically initiated CH_3CN porogen MIP and as such we have restricted the following discussion to the *trans*-aconitic acid precipitation MIP (Jiang).[13] We observed minor differences in *trans*-aconitic acid selectivity with Jiang reporting a selectivity index, $I = 1.56$ and whilst we observe $I = 1.40$. A ~10% decrease in selectivity, rationalised as a result of our polymer particles being substantially larger (2–3 μM diameter) than that reported by Jiang (0.6–0.7 μM), and the use of different cross linker (TRIM). Presumably the decreased surface area of our MIP adversely affects the degree of specific and non-specific binding. Both specificity values were determined in CH_3CN after 24 h rebinding.

Examination of the corresponding *I* values in RTILs reveals a significant difference both in the level of specificity and also in the optimal re-binding time. Firstly, polymerisation in 5 mL of solvent affords the bulk polymer and polymerisation in 25 mL the corresponding precipitation polymer, according to literature precedent. Examination of the 'bulk' photochemical polymerisation results shows a maximum $I_{[bmim][PF6]} = 2.27$ (Table 1, entry 6) after 1 h, decreasing to 0.93 (Table 1, entry 7) after 24 h. The corresponding data $I_{[bmim][BF4]} = 2.30$ (Table 1, entry 15) after 1 h decreasing to 1.60 (Table 1, entry 16) after 24 h. The 1 h $I_{[bmim][PF6]}$ rebinding represents an improvement (+62%), with $I_{[bmim][BF4]}$ a (+64%) improvement over the MIP_{CH3CN} precipitation result (Table 1, entry 4).

Conducting polymerisations under photochemical initiation and precipitation conditions (5 °C and 25 mL) saw an increase in selectivity with $I_{[bmim][PF6]} = 2.45$ (Table 1, entry 9); and $I_{[bmim][BF4]} = 2.74$ (Table 1, entry 21), (+75 and +96%, respectively). No change in the rate at which optimal binding was achieved was noted.

Thermally initiated bulk polymerisations resulted in a drop in selectivity in both RTILs examined, approaching the levels evidenced for MIP_{CH3CN}. Of note also is the reversal in optimal binding with 24 h giving the highest *I* values, $I_{[bmim][PF6]} = 1.46$ (Table 1, entry 11) and $I_{[bmim][BF4]} = 1.76$ (Table 1, entry 19). In addition to being a significant decrease in selectivity relative to the photochemically synthesised MIPs the optimal binding is achieved after 24 h rather than 1 h. Previous workers in this field have noted that low temperature MIPs give higher *I* values, presumably a result of higher levels of functional monomer to template preassociation during the polymerisation process. Our NMR titration studies confirm an increased preassociation at 5 °C (data not shown).

Counterintuitively, we note that under thermally induced precipitation polymerisation that only $I_{[bmim][BF4]} = 2.74$ (Table 1, entry 21) changes significantly, and that the optimal binding is now recorded after 1 h rather than 24 h as noted in the thermally bulk MIPs. The $MIP_{[bmim][PF6]}$ generated at 60 °C in returns $I = 1.46$, essentially identical to MIP_{CH3CN}.

Each of the synthesised MIPs was examined by scanning electron microscopy (SEM). The results of these examinations are show in Fig. 1. Panel 1a shows the micro-spheres generated in this study using Jiang's conditions.[13] Particles are typically 2–3 μm in diameter, larger than Jiang's 0.6–0.7 μm, the differences in size is presumably due to use of trimethyl-propane trimethyl acrylate (TRIM) as the cross-linker. Of greater interest is the obviously smaller particles associated with all RTILs 20–50 times smaller with particles of <200 nm (Fig. 1b–i). This holds true for those synthesised under conditions normally associated with bulk polymerisations, excepting the case of polymerisations conducted at 5 °C in [bmim][BF_4]. These polymers were monolithic in nature (Fig. 1i).

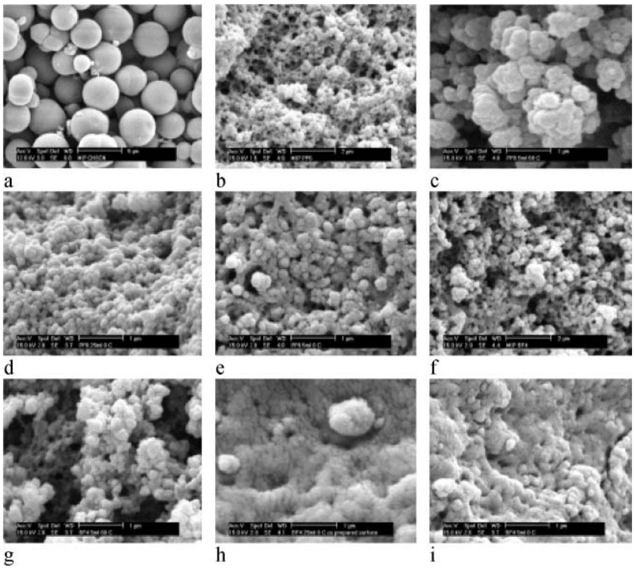

Fig. 1 SEM micrographs of (a) CH_3CN, 60 °C, 25 mL, average particle size 2–3 µm; (b) [bmim][PF_6], 60 °C, 25 mL; (c) [bmim][PF_6], 60 °C, 5 mL; (d) [bmim][PF_6] 0 °C 25 mL; (e) [bmim][PF_6] 0 °C 5 mL; (f) [bmim][BF_4] 60 °C 25 mL, (g) [bmim][BF_4], 60 °C 5 mL, (h) [bmim][BF_4], 0 °C 25 mL (i) [bmim][BF_4], 0 °C 5 mL.

Prior work from our and other laboratories has indicated that the degree of polymer swelling can have an impact on the selectivity measurements. Accordingly, we conducted a series of swelling experiments and found that the MIP_{CH3CN} exhibited a 7% swelling, all $MIP_{[bmim][BF4]}$ and $MIP_{[bmim][PF6]}$ exhibited negligible swelling (0.5%).

In summary, the ionic liquids [bmim][BF_4] and [bmim][PF_6] allowed the rapid (accelerated) synthesis of polymer micro-spheres more typical of those produced under precipitation polymerisation conditions. In addition to an increased ease of production, polymer micro-spheres are <200 nm in size, and afforded MIPs with improved selectivities. Differences in polymerisation methods (thermal *vs.* photochemical) resulted in differences in the rate at which optimal re-binding of the *trans*-aconitic acid template was observed. The best selectivity was noted for $MIP_{[bmim][BF4]}$ prepared under thermal conditions. $MIP_{[bmim][BF4]}$ was 2 times more selective than the corresponding MIP generated under traditional precipitation conditions. Additional potential advantages in the MIP field relate to the tuneable nature of RTILs, which, potentially, overcome some of the solubility issues in the initial MIP generation, especially in the field of illicit drugs with heroin and other opiates displaying low levels of solubility (at the limit required for MIP generation) in traditional organic solvents. We have also observed that the addition of VOC co-solvents allows fine-tuning of polymer micro-sphere dimensions.[14] Conceivably RTIL–MIP systems offer the possibility of direct electrochemical observation of template uptake, given the known electrochemical properties of common RTILs. The combination of RTILs and MIPs demands further examination and development, we will report on our findings in due course. Thus, RTILs have the potential to increase the rate of micro-sphere polymer production, increase MIP specificity and decrease the time required for optimal rebinding.§

Notes and references

† *Trans*-aconitic acid (0.25 mmol), EGDMA (1 mmol) and MAA (0.8 mmol) were dissolved in CH_3CN. Either 25 mL or 5 mL porogen (CH_3CN (control), [bmim][BF_4] [bmim][PF_6]) was added for precipitation and bulk polymerisations, respectively, degassed (N_2), AIBN (10 mg) was added before the tube was sealed. Thermally initiated polymerisations were conducted at 60 °C, and photoinitiated polymerisation conducted at 5 °C using an Acticure UV probe. NIPs were prepared using the same procedure but without the addition of *trans*-aconitic acid. Reaction durations are listed in Table 1. Synthesised polymers were filtered, and the templates extracted by washing (MeOH, 5 × 30 mL; 5 × 30 mL 10% acetic acid/MeOH). Both NIP and MIP were treated in an identical fashion, and dried under vacuum ~24 h at 0.01 mmHg.

‡ Synthesised according to J. G. Huddleston, H. D. Willauer, R. P. Swatloski, A. E. Visser and R. D. Rogers, *Chem. Commun.*, 1998, 1765–1766.

§ Rebinding studies, in each porogen—CH_3CN, [bmim][PF_6] and [bmim][BF_4]—were conducted as previously described.[5]

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