# Supporting Information

## Purification of lipase using ionic liquids as adjuvants in aqueous two-phase systems

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**Table A.1:** The volume ratio (*R*v) with the respective standard deviations and mass fraction compositions of the ATPS for CaLB purification at 25 (± 0.1) ºC and atmospheric pressure**.**

| ATPS | | Mass Fraction Composition / (wt%) ± $\sigma$ | | | | $R$v ± $\sigma$ |
|---|---|---|---|---|---|---|
| | | PEG | Salt | IL | CalB | |
| PEG 1500 | no IL | 15.02 ± 0.02 | 14.95 ± 0.04 | | 5.15 ± 0.05 | 0.93 ± 0.03 |
| | [$C_2$mim]Cl | 14.96 ± 0.03 | 14.62 ± 0.64 | 5.03 ± 0.05 | 5.16 ± 0.05 | 1.79 ± 0.02 |
| | [$C_4$mim]Cl | 15.05 ± 0.04 | 14.90 ± 0.10 | 5.05 ± 0.04 | 5.06 ± 0.10 | 1.83 ± 0.06 |
| | [$C_6$mim]Cl | 15.09 ± 0.04 | 15.15 ± 0.22 | 5.00 ± 0.01 | 5.14 ± 0.17 | 1.87 ± 0.01 |
| | [$C_8$mim]Cl | 15.05 ± 0.06 | 15.02 ± 0.06 | 5.03 ± 0.01 | 5.11 ± 0.15 | 1.54 ± 0.06 |
| PEG 4000 | no IL | 10.04 ± 0.04 | 13.01 ± 0.01 | | 5.16 ± 0.08 | 0.65 ± 0.03 |
| | [$C_2$mim]Cl | 10.05 ±0.06 | 13.04 ± 0.03 | 5.05 ± 0.06 | 5.11 ± 0.03 | 0.86 ± 0.02 |
| | [$C_4$mim]Cl | 10.04 ± 0.05 | 12.98 ± 0.04 | 5.10 ± 0.03 | 5.02 ± 0.02 | 0.85 ± 0.06 |
| | [$C_6$mim]Cl | 10.00 ± 0.03 | 12.99 ± 0.03 | 5.03 ± 0.04 | 5.19 ± 0.13 | 1.53 ± 0.04 |
| | [$C_8$mim]Cl | 10.01 ± 0.01 | 13.05 ± 0.07 | 5.04 ± 0.04 | 5.12 ± 0.03 | 0.82 ± 0.02 |
| PEG 6000 | no IL | 10.03 ± 0.03 | 12.03 ± 0.03 | | 5.07 ± 0.04 | 0.69 ± 0.01 |
| | [$C_2$mim]Cl | 10.00 ± 0.02 | 12.01 ± 0.01 | 5.05 ± 0.04 | 5.03 ± 0.02 | 0.73 ± 0.02 |
| | [$C_4$mim]Cl | 10.07 ± 0.09 | 12.06 ± 0.08 | 5.09 ± 0.05 | 5.06 ± 0.09 | 0.92 ± 0.01 |
| | [$C_6$mim]Cl | 10.04 ± 0.04 | 12.04 ± 0.03 | 5.08 ± 0.04 | 5.11 ± 0.12 | 1.16 ± 0.03 |
| | [$C_8$mim]Cl | 10.09 ± 0.05 | 12.06 ± 0.11 | 5.08 ± 0.09 | 5.08 ± 0.09 | 0.92 ± 0.02 |
| PEG 8000 | no IL | 10.07 ± 0.08 | 12.02 ± 0.06 | | 5.09 ± 0.09 | 0.63 ± 0.01 |
| | [$C_2$mim]Cl | 10.07 ± 0.08 | 12.03 ± 0.04 | 5.06 ± 0.05 | 5.05 ± 0.07 | 0.65 ± 002 |
| | [$C_4$mim]Cl | 10.05 ± 0.05 | 12.03 ± 0.01 | 5.12 ± 0.02 | 5.14 ± 0.09 | 0.85 ± 0.02 |
| | [$C_6$mim]Cl | 10.07 ± 0.06 | 12.05 ± 0.06 | 5.02 ± 0.01 | 5.03 ± 0.02 | 1.06 ± 0.03 |
| | [$C_8$mim]Cl | 10.08 ± 0.11 | 12.07 ± 0.04 | 5.09 ± 0.11 | 5.06 ± 0.08 | 0.79 ± 0.04 |

**Table A.2:** The volume ratio (*R*v) with the respective standard deviations and mass fraction compositions of the ATPS for purification of lipase from *Bacillus* sp. ITP-001, at 25 (± 0.1) ºC and atmospheric pressure**.**

| Route | ATPS | | Mass Fraction Composition / (wt%) ± $\sigma$ | | | | $R$v ± $\sigma$ |
|---|---|---|---|---|---|---|---|
| | | | PEG | Salt | IL | Solution of *Bacillus* | |
| ***i*** | PEG 1500 | no IL | 15.02 ± 0.02 | 14.95 ± 0.04 | | 70.03 ± 0.06 | 1.26 ± 0.01 |
| | | [$C_2$mim]Cl | 14.96 ± 0.03 | 14.62 ± 0.64 | 5.03 ± 0.05 | 65.40 ± 0.56 | 1.10 ± 0.01 |
| | | [$C_4$mim]Cl | 15.05 ± 0.04 | 14.90 ± 0.10 | 5.05 ± 0.04 | 65.01 ± 0.10 | 1.26 ± 0.08 |
| | | [$C_6$mim]Cl | 15.09 ± 0.04 | 15.15 ± 0.22 | 5.00 ± 0.01 | 64.76 ± 0.16 | 1.29 ± 0.04 |
| | | [$C_8$mim]Cl | 15.05 ± 0.06 | 15.02 ± 0.06 | 5.03 ± 0.01 | 64.90 ± 0.01 | 1.00 ± 0.00 |
| ***ii*** | PEG 1500 | no IL | 15.02 ± 0.03 | 15.07 ± 0.07 | | 69.05 | 1.09 ± 0.03 |
| | | [$C_6$mim]Cl | 15.10 ± 0.09 | 15.15 ± 0.16 | 5.16 ± 0.07 | 64.87 | 1.90 ± 0.05 |
| ***i*** | PEG 6000 | [$C_2$mim]Cl | 15.04 ± 0.02 | 15.05 ± 0.02 | 5.17 ± 0.01 | 64.77 ± 0.18 | 0.83 ± 0.02 |
| | | [$C_6$mim]Cl | 15.06 ± 0.02 | 15.02 ± 0.02 | 5.14 ± 0.04 | 64.98 ± 0.14 | 1.09 ± 0.02 |
| ***i*** | PEG 8000 | [$C_2$mim]Cl | 15.08 ± 0.08 | 15.13 ± 0.20 | 5.01 ± 0.01 | 64.79 ± 0.28 | 0.86 ± 0.02 |
| | | [$C_6$mim]Cl | 15.05 ± 0.07 | 15.08 ± 0.13 | 5.05 ± 0.04 | 64.90 ± 0.10 | 1.05 ± 0.06 |

**Table A.3:** The enzyme recovered in the bottom phase ($R_{EB}$), the contaminating protein recovered in the top phase ($R_{PT}$), partition coefficient for contaminant protein ($K_P$), and the enzyme ($K_E$), plus the purification factor (PF) with the respective standard deviations ($\sigma$). All ATPS were constituted with PEG + $K_2HPO_4$/$KH_2PO_4$ + IL (when present) + water + CaLB, at 25 (± 0.1) ºC and atmospheric pressure.

| ATPS | | $R_{EB} \pm \sigma$ | $R_{PT} \pm \sigma$ | $K_E \pm \sigma$ | $K_P \pm \sigma$ | PF ± $\sigma$ |
|---|---|---|---|---|---|---|
| PEG 1500 | no IL | 67.13 ± 0.08 | 78.39 ± 3.00 | 0.52 ± 0.01 | 3.96 ± 0.85 | 3.25 ± 0.51 |
| | [$C_2$mim]Cl | 60.12 ± 1.27 | 81.66 ± 2.22 | 0.37 ± 0.02 | 2.49 ± 0.44 | 2.55 ± 0.35 |
| | [$C_4$mim]Cl | 56.53 ± 0.86 | 87.61 ± 0.005 | 0.42 ± 0.07 | 3.90 ± 0.14 | 3.45 ± 0.08 |
| | [$C_6$mim]Cl | 51.68 ± 0.003 | 92.49 ± 0.83 | 0.50 ± 0.05 | 6.79 ± 0.99 | 5.22 ± 0.65 |
| | [$C_8$mim]Cl | 67.33 ± 3.02 | 72.21 ± 7.97 | 0.38 ± 0.04 | 2.08 ± 0.31 | 2.24 ± 0.29 |
| PEG 4000 | no IL | 72.62 ± 1.49 | 66.02 ± 0.79 | 0.58 ± 0.02 | 2.96 ± 0.08 | 2.51 ± 0.02 |
| | [$C_2$mim]Cl | 65.34 ± 2.58 | 68.56 ± 0.01 | 0.62 ± 0.07 | 2.54 ± 0.01 | 2.19 ± 0.09 |
| | [$C_4$mim]Cl | 65.91 ± 3.87 | 62.00 ± 0.57 | 0.61 ± 0.09 | 1.85 ± 0.02 | 1.78 ± 0.09 |
| | [$C_6$mim]Cl | 56.50 ± 1.92 | 70.17 ± 0.01 | 0.50 ± 0.04 | 1.53 ± 0.01 | 1.69 ± 0.04 |
| | [$C_8$mim]Cl | 69.83 ± 0.29 | 27.51 ± 0.49 | 0.53 ± 0.02 | 0.46 ± 0.01 | 0.96 ± 0.01 |
| PEG 6000 | no IL | 72.24 ± 0.48 | 65.19 ± 0.60 | 0.55 ± 0.01 | 2.66 ± 0.07 | 2.38 ± 0.04 |
| | [$C_2$mim]Cl | 72.07 ± 0.65 | 55.35 ± 4.96 | 0.54 ± 0.02 | 1.71 ± 0.33 | 1.63 ± 0.03 |
| | [$C_4$mim]Cl | 63.89 ± 2.72 | 48.79 ± 0.15 | 0.57 ± 0.07 | 1.04 ± 0.01 | 1.26 ± 0.06 |
| | [$C_6$mim]Cl | 63.77 ± 1.87 | 47.60 ± 0.65 | 0.47 ± 0.02 | 0.78 ± 0.01 | 1.20 ± 0.02 |
| | [$C_8$mim]Cl | 72.01 ± 2.67 | 23.62 ± 0.09 | 0.39 ± 0.05 | 0.34 ± 0.01 | 0.94 ± 0.04 |
| PEG 8000 | no IL | 77.13 ± 3.19 | 56.96 ± 0.60 | 0.48 ± 0.10 | 2.09 ± 0.01 | 2.10 ± 0.15 |
| | [$C_2$mim]Cl | 76.61 ± 0.48 | 69.84 ± 0.75 | 0.46 ± 0.01 | 3.54 ± 0.03 | 3.12 ± 0.01 |
| | [$C_4$mim]Cl | 69.95 ± 0.39 | 61.73 ± 0.58 | 0.51 ± 0.02 | 1.91 ± 0.02 | 1.93 ± 0.03 |
| | [$C_6$mim]Cl | 67.72 ± 0.59 | 25.38 ± 0.44 | 0.45 ± 0.01 | 0.32 ± 0.05 | 0.91 ± 0.02 |
| | [$C_8$mim]Cl | 77.23 ± 3.53 | 12.23 ± 0.62 | 0.38 ± 0.06 | 0.18 ± 0.01 | 0.85 ± 0.04 |

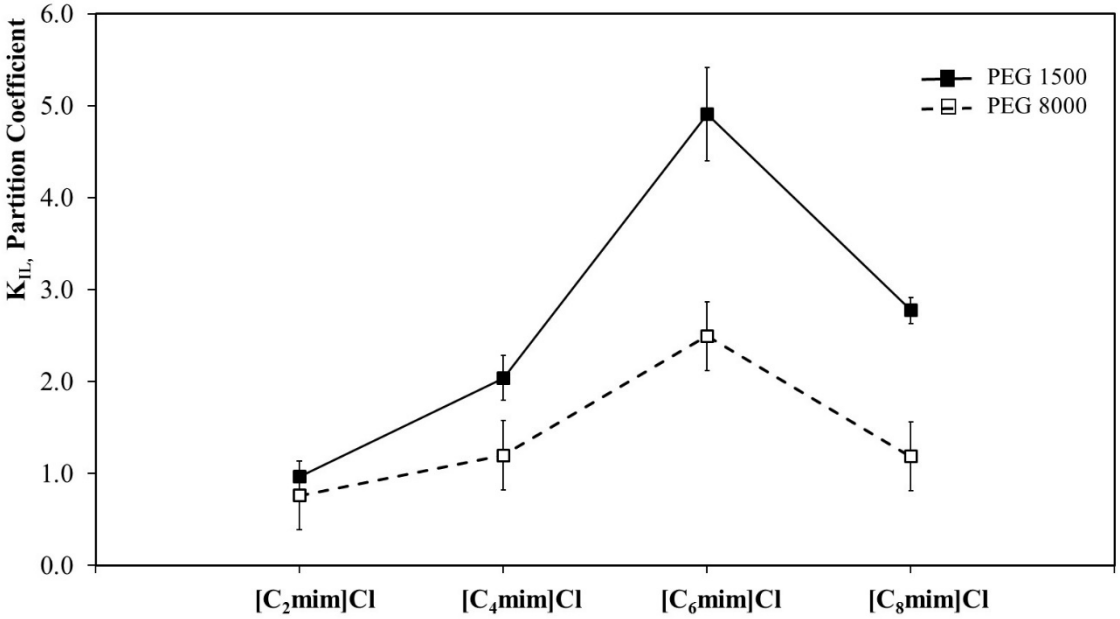


**Figure A.1:** ILs distribution between the two phases in terms of their partition coefficient ($K_{IL}$) and their respective standard deviations, for systems based in 15 wt% of PEG (1500 and 8000) + 5 wt% of [$C_n$mim]Cl + water + 15 wt% of $K_2HPO_4/KH_2PO_4$ (pH 7) [1].

## Reference

1. R. L. Souza, V. C. Campos, S. P. M. Ventura, C. M. F. Soares, J. A. P. Coutinho and Á. S. Lima, *Fluid Phase Equilib.*, 2014, 375, 30-36.