Electronic Supplementary Material (ESI) for RSC Advances.


# Supporting Information

## Ionic liquids as buffer additives in ionic liquid-polyacrylamide gel electrophoresis separation of mixture of low and high molecular weight proteins

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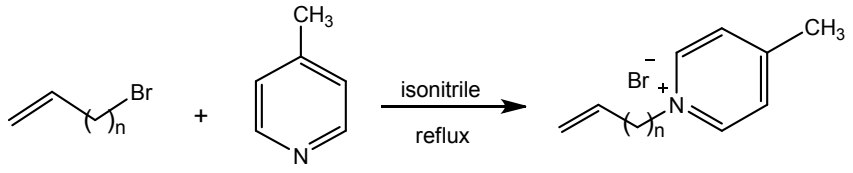
## Synthesis and characterization of ionic liquids.




n= 2, 6, 9

n=2: 1-Butene-4-methylpyridiniumbromide ($C_4$PBr)

n=6: 1-Octene-4-methylpyridiniumbromide ($C_8$PBr)

n=9: 1-Undecen-4-methylpyridiniumbromide($C_{11}$PBr)

**Fig. S1** Synthesis of Pyridinium based ionic liquids

**1-Butene-4-methylpyridiniumbromide ($C_4$PBr).** To acetonitrile, was added 4-bromo-1-butene (10g, 0.074M) and 4-methylpyridine (1.02 eq., 7g, 0.075M), and the mixture was refluxed at 80$^o$C for 24h. After 24h, acetonitrile was evaporated under vacuum, resulting in a yellow viscous liquid. TLC in 90% $CHCl_3$:MeOH showed that the product was pure (16 g, 94% yield). $^1$H-NMR ($CDCl_3$, 400 MHz): δ 9.33 (d, J= 6.52, 2H), 8.36 (d, J= 6.62 Hz, 2H), 5.82 (m, 1H), 4.97 (m, 1H), 4.84 (m, 2H), 2.71 (m, 2H), 2.26 (s, 3H); $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ 158.4, 144.1, 140.5, 128.3, 119.8, 59.4, 35.5, 22.2; (MS, ESI) m/z 148 ($M^+$).

Other ILs, 1-octene-4-methylpyridinium bromide ($C_8$PBr) and 1-undecene-4-methylpyridinium bromide ($C_{11}$PBr) were synthesized following the same procedure mentioned above and characterized by $^1$H-NMR, $^{13}$C-NMR, ESI/MS spectroscopy.

**1-Octene-4-methylpyridiniumbromide ($C_8$PBr).** $^1$H-NMR ($CDCl_3$, 400 MHz) ($C_8$PBr) : δ 9.43(d, J =6.68Hz, 2H), 7.98(d, J=6.28Hz,2H), 5.79(m, 1H), 5.75(m,1H), 5.72(m,1H), 4.90(m, 2H), 2.86(s, 3H), 2.39(m, 2H), 2.0(m,2H), 1.36(m,6H); $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ 158.5, 144.1, 138.4, 128.7, 114.2, 60.7, 33.2, 31.5, 28.2, 25.6, 22.06; (MS, ESI) m/z 204 ($M^+$).

**1-Undecene-4-methylpyridiniumbromide ($C_{11}$PBr).** $^{1}$H-NMR ($CDCl_3$, 400 MHz) ($C_{11}$PBr) : δ9.30(d, J =6.6Hz, 2H), 7.89(d, J=6.28Hz,2H), 5.77(m, 1H), 5.73(m,1H), 5.71(m, 1H), 4.80 (m, 1H), 2.63(s, 3H), 2.0(m,2H), 1.96(m,2H), 1.29(m, 10H); $^{13}$C-NMR ($CDCl_3$, 100 MHz): δ 158.7, 144.2, 139.09, 128.8, 114.1, 61.1, 31.8, 29.2, 29, 28.9, 28.8, 26, 22.2; (MS, ESI) m/z 246 ($M^+$).

**Binding parameters for Scatchard analysis.** The various parameters characteristic of such analyses were determined as mentioned below:

Fraction of surfactant bound, $\alpha = (I-I_0) / (I_m-I_0)$

The concentration of the bound surfactant $S_b = \alpha$ [Total surfactant]

In this equation, $I_0$ is the fluorescence intensity of the protein in the absence of ILs ($C_4$PBr, $C_8$PBr or $C_{11}$PBr), I is the fluorescence intensity at different concentrations of ILs and $I_m$ is the fluorescence intensity when the protein is saturated with ILs. The concentration (in M) of free IL was determined using 1-[bound-IL]. The parameter, *v*, is defined as α [Total surfactant]/[Total protein] and the concentration of free surfactant (c) was obtained from [Total surfactant](1-α). Each linear portion of a Scatchard plot (*v/c vs v*) was given a linear fit and the equilibrium binding constant (*K*) and number of binding sites (*n*) for a particular concentration region were obtained from the respective slope and intercept.

**Preparation of sample and running buffers.** Standard 10× running buffer (RB) stock solution contained 25 mM Tris and 192 mM Glycine (pH 8.4). The RB solution was prepared by pipeting an appropriate amount of ILs (or SDS) into a volumetric flask, dissolving it with 50 mL of running buffer stock solution, and diluting it to a final volume of 500 mL with ultrapure water (18.2 MΩ). Concentrations of 0.0125%, 0.025%, 0.05%, 0.05% and 0.5% w/v ILs were used in the running buffer for optimization and validation of separations. The sample buffer (SB) was prepared in 2 mL Eppendorf tubes by combining appropriate amounts of ultrapure water, 50 mM Tris HCl (pH 6.8), glycerol, bromophenol blue, and 0.025%, 0.05%, 0.25%, 0.5%, 1% w/v ILs (or SDS). Proteins from stock solution (3mg/mL) were added in SB at protein:SB ratio of 1:20. The reducing agent, β-mercaptoethanol, was added at 5% v/v of the SB.

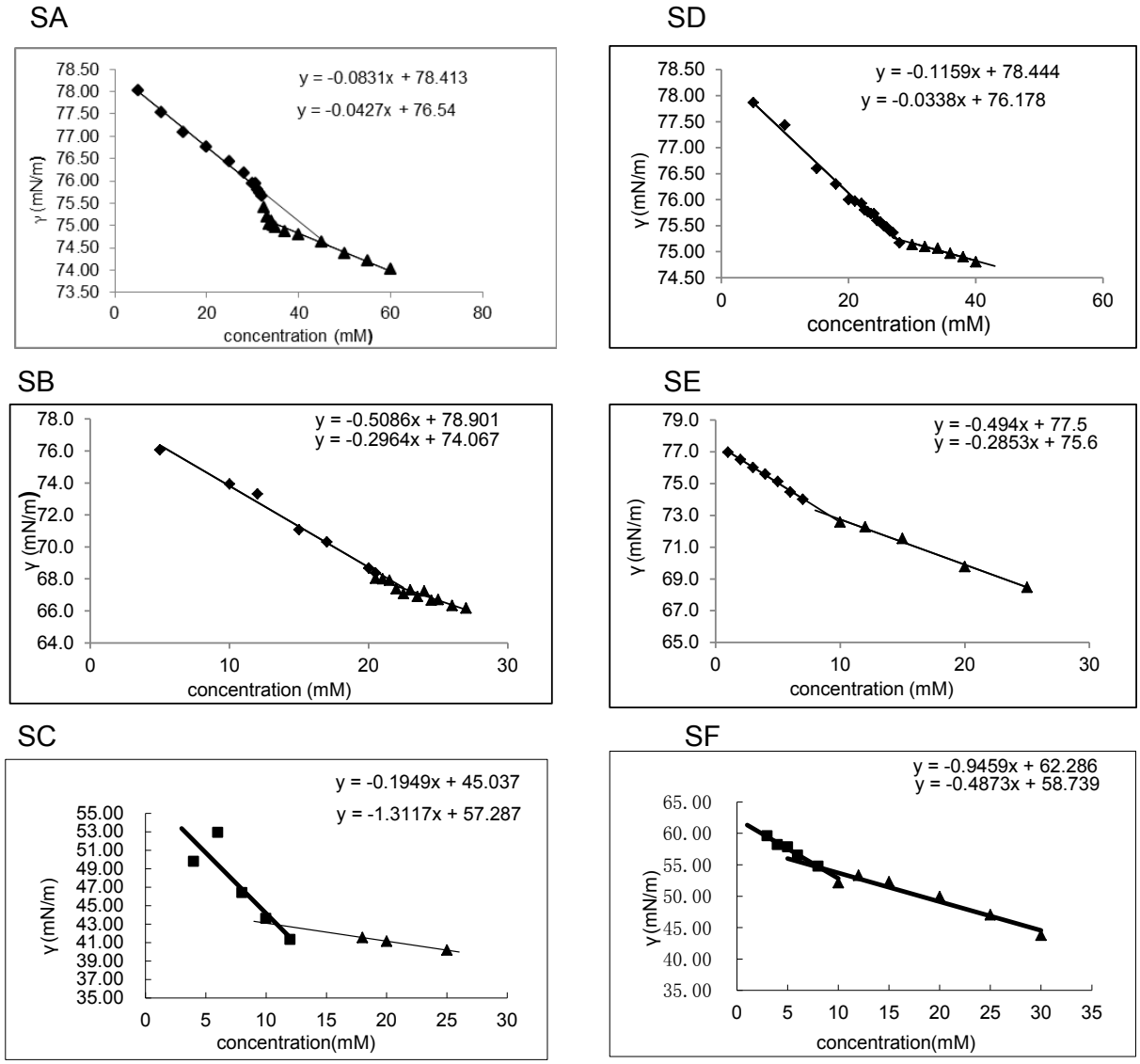



**Fig. S2** Critical micelle concentration of $C_4$PBr, $C_8$PBr, $C_{11}$PBr (SA-SC) in water and (SD-SF) in 25 mM Tris/glycine buffer respectively.

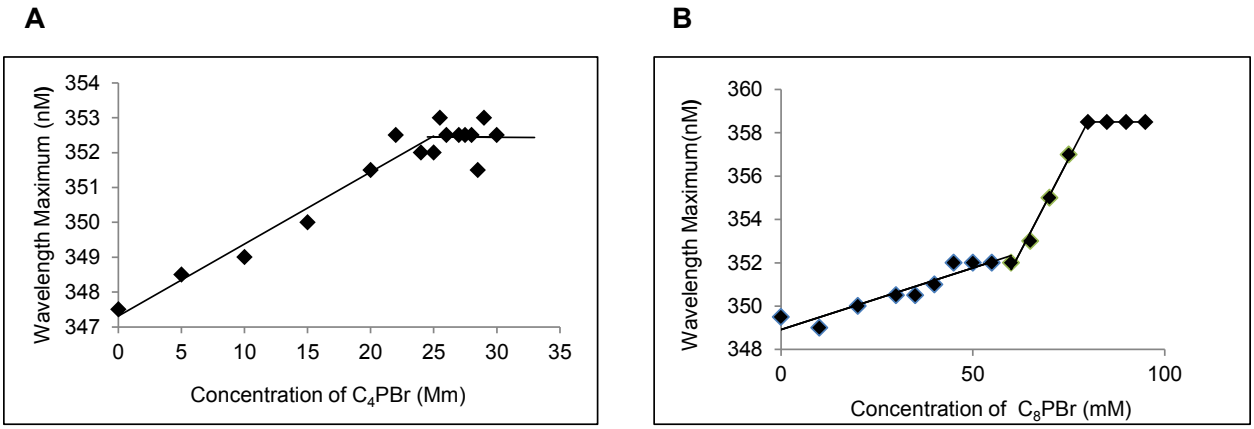



**Fig. S3** Fluorescence wavelength maxima shift of Trp in the presence of increasing IL concentration of $C_4$PBr (A), $C_8$PBr (B) in association with BSA (10 uM), determined by steady state fluorescence ( $\lambda_{ex}$ =295 nm, 25$^{o}$C).

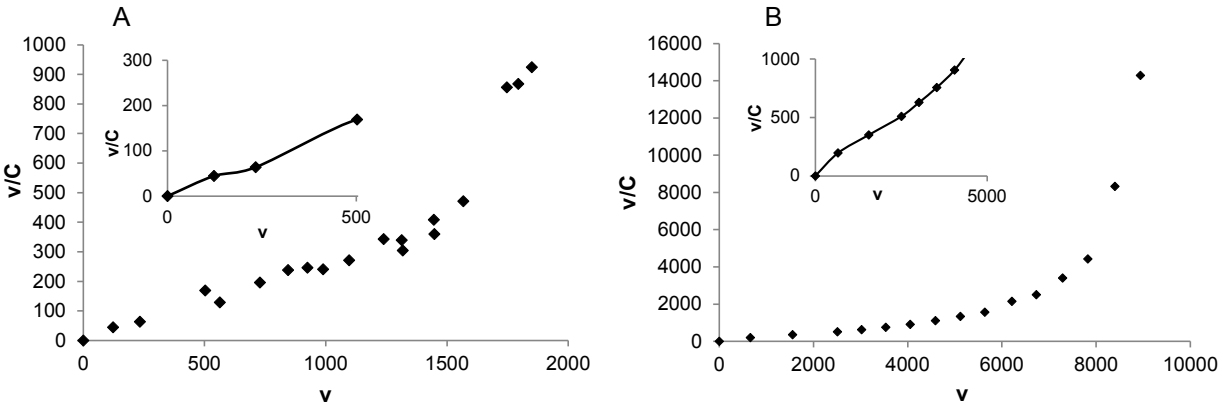



**Fig. S4** Scatchard plots of BSA with (A) $C_4$PBr, (A) $C_8$PBr, (B). The inset expands the low concentration regions of the corresponding plots.