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.

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

1-Butene-4-methylpyridinium bromide (C₄PBr). 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°C for 24h. After 24h, acetonitrile was evaporated under vacuum, resulting in a yellow viscous liquid. TLC in 90% CHCl₃:MeOH showed that the product was pure (16 g, 94% yield). ¹H-NMR (CDCl₃, 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); ¹³C-NMR (CDCl₃, 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 (C₈PBr) and 1-undecene-4-methylpyridinium bromide (C₁₁PBr) were synthesized following the same procedure mentioned above and characterized by ¹H-NMR, ¹³C-NMR, ESI/MS spectroscopy.

1-Octene-4-methylpyridinium bromide (C₈PBr). ¹H-NMR (CDCl₃, 400 MHz) (C₈PBr) : 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); ¹³C-NMR (CDCl₃, 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\textsubscript{11}PBr). \(^1\)H-NMR (CDCl\textsubscript{3}, 400 MHz) (C\textsubscript{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\textsubscript{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\textsuperscript{+}).

**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 \(S_b = \alpha [\text{Total surfactant}]\)

In this equation, \(I_0\) is the fluorescence intensity of the protein in the absence of ILs (C\textsubscript{4}PBr, C\textsubscript{8}PBr or C\textsubscript{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, \(\nu\), is defined as \(\alpha [\text{Total surfactant}]/[\text{Total protein}]\) and the concentration of free surfactant (c) was obtained from \([\text{Total surfactant}](1-\alpha)\). Each linear portion of a Scatchard plot (\(\nu/c vs \nu\)) 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 C₄PBr, C₆PBr, C₁₁PBr (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 C₄PBr (A), C₈PBr (B) in association with BSA (10 uM), determined by steady state fluorescence (λₑₓ =295 nm, 25°C).

Fig. S4  Scatchard plots of BSA with (A) C₄PBr, (A) C₈PBr, (B). The inset expands the low concentration regions of the corresponding plots.