Supporting Information

Surface Active Ionic Liquid Induced Conformational Transition In Aqueous Medium of Hemoglobin

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Annexure S1

1. **Synthesis of 1-dodecyl-3-methyl imidazolium chloride**

   Synthesis of [C12mim][Cl] involves the alkylation of 1-methylimidazole with the 1-chlorododecane in two necked RBF and the mixture kept under reflux conditions for 3 h. Reaction was monitored with TLC. After completion of reaction the product was washed with ethyl acetate three times. 1-dodecyl-3-methyl imidazolium chloride thus obtained was dried and characterized using 1H NMR

2. **Synthesis of 1-hexyl-3-methylimidazolium dodecylsulfate**

   An equimolar mixture of alkyl imidazolium chloride and sodium dodecyl sulfate salt was dissolved in water and kept at 60 °C for 4 h. Reaction was monitored with TLC. Water was removed from the reaction mixture after completion of reaction using a rotary evaporator. The product was extracted with dichloromethane (DCM), and solid salt (NaCl) was filtered off. The product was washed with water several times until complete removal of chloride ions (monitored with acidic solution of AgNO$_3$) and completely dried prior to use. The 1H NMR spectrum of pure [C$_6$mim][SDS] is given in figure S1.

   Annexure SII

   1. Γ_{max} is calculated following the Gibbs adsorption equation:

   $$\tau_{max} = -\frac{1}{2.303\ nRT}\left(\frac{\partial\gamma}{\partial\log C}\right)$$

Where $\left(\dfrac{\partial\gamma}{\partial\log C}\right)$ has been taken as maximum slope from γ vs. log of total surfactant concentration plot, R is universal gas constant (R = 8.314 JK^{-1}mol^{-1}), N$_A$ is Avogadro's number, T is temperature, γ$_o$ and γ$_{cmc}$ are surface tension values of pure solvent and mixtures at cmc respectively and n is the number of chemical species whose concentration at the interface changes with bulk phase concentration. In this work the value of n has been chosen is n as the work is performed in buffer.

   2. A_{min} was determined from following relation:

$$A_{min} = \frac{10^{20}}{\left(N_A \, \tau_{max}\right)}$$

3. The surface pressure (Π_{cmc}) is calculated using following equation:

$$\pi_{cmc} = \gamma_o - \gamma_{cmc}$$

Here γ_o and γ_{cmc} are surface tension of pure solvent and mixture at cmc respectively

Annexure SIII

Job's Plot

To obtain Job's plot, various volume fractions of equimolar solution of Hb and SAILs were prepared and mixed. The absorption spectra of both Hb and SAILs were recorded at concentration 0.05 mM of each in the range of 200-800 nm where SAILs has no absorbance. Hb exhibit intense peak at 406 nm and another peak at 265 nm (which later disappear on the addition of SAILs). The Job's plot method involves the measurement of absorbances of various samples and the corrected absorbance (ΔA) of these mixtures are plotted against the volume fraction of SAIL solution. According to Beer-Lambert law, if there is no interaction between Hb and SAILs then the total absobance of the mixture (A_{theo}) is equal to the sum of their individual absorbances as per equation

$$A_{theo} = \varepsilon_S \, C_S^{\circ} \, X_S + \varepsilon_{Hb} \, C_{Hb}^{\circ} \left(1 - X_S\right)$$

Where ε_S and ε_{Hb} are the molar extinction coefficients and C_S° and C_{Hb}° are the concentrations of SAILs and Hb respectively, while X_S is the volume fraction of SAIL. But the presence of interactions among Hb and SAILs leads to the formation of Hb-SAIL complex which makes the solution absorbance to satisfy the equation

$$A_{exp} = \varepsilon_S \, C_S + \varepsilon_{Hb} \, C_{Hb} + \varepsilon_{Hb-S} \, C_{Hb-S}$$

Where ε_{Hb-S} is the molar extinction coefficient of Hb-SAIL complex, C_S, C_{Hb}, and C_{Hb-S} are the concentrations of species in the mixture. The corrected absorbance (ΔA) represents the difference among measured absorbance (A_{exp}) and theoretical absorbance (A_{theo}) and is given as:

$$\Delta A = A_{exp} - A_{theo} \tag{14}$$

The plot of corrected absorbance (ΔA) versus the volume fraction of SAIL solution gives either minima or maxima which corresponds to the stoichiometry ratio of Hb-SAIL complex. As shown in figure S6, the presence of minima for Hb-SAIL complexes at $X_{SAIL} = 0.5$ implies 1:1 stoichiometry.
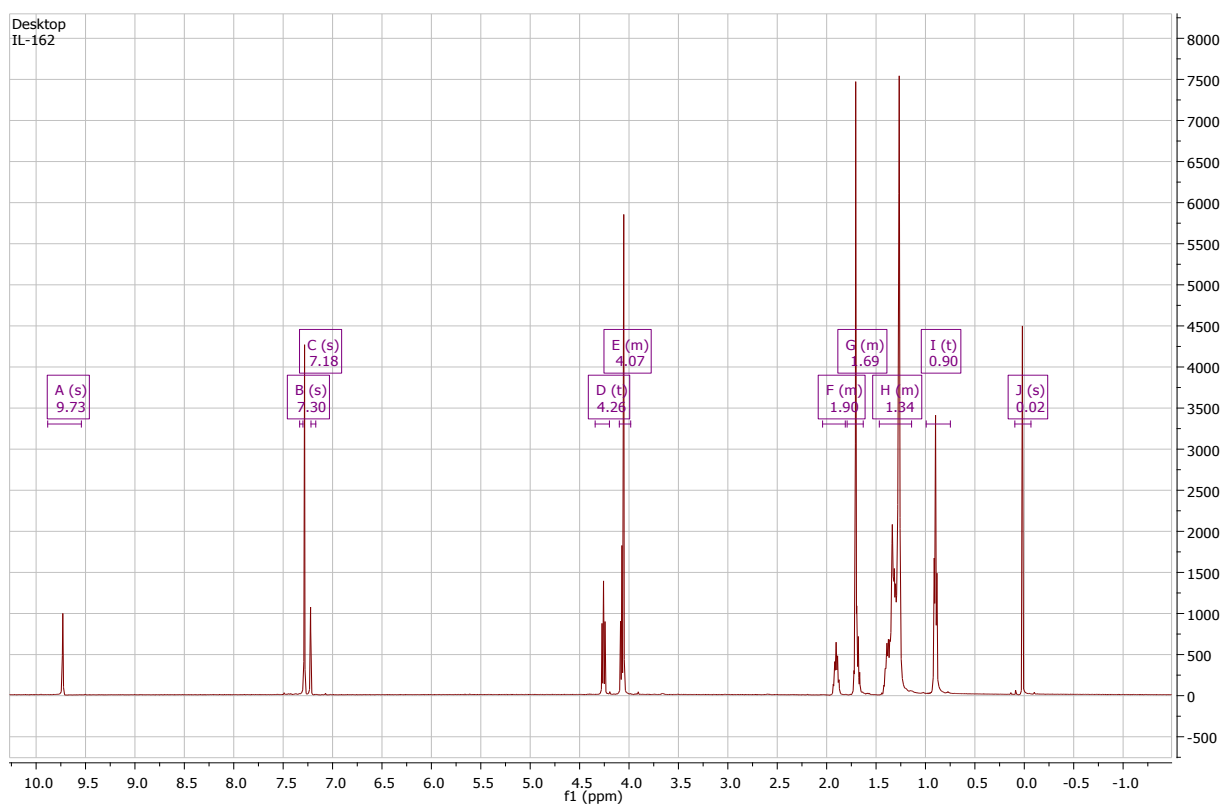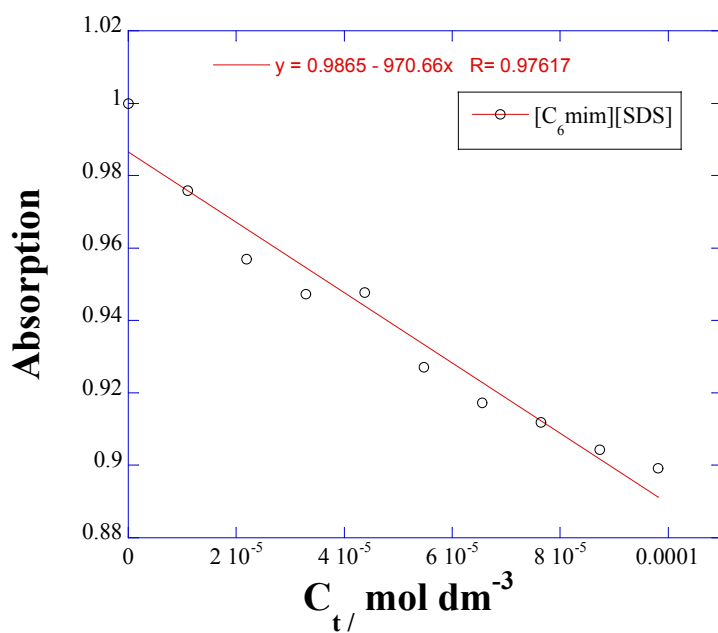

Figure S1 1H NMR spectra for pure [C$_6$mim][SDS]

1H NMR chemical shift range in δ (ppm)
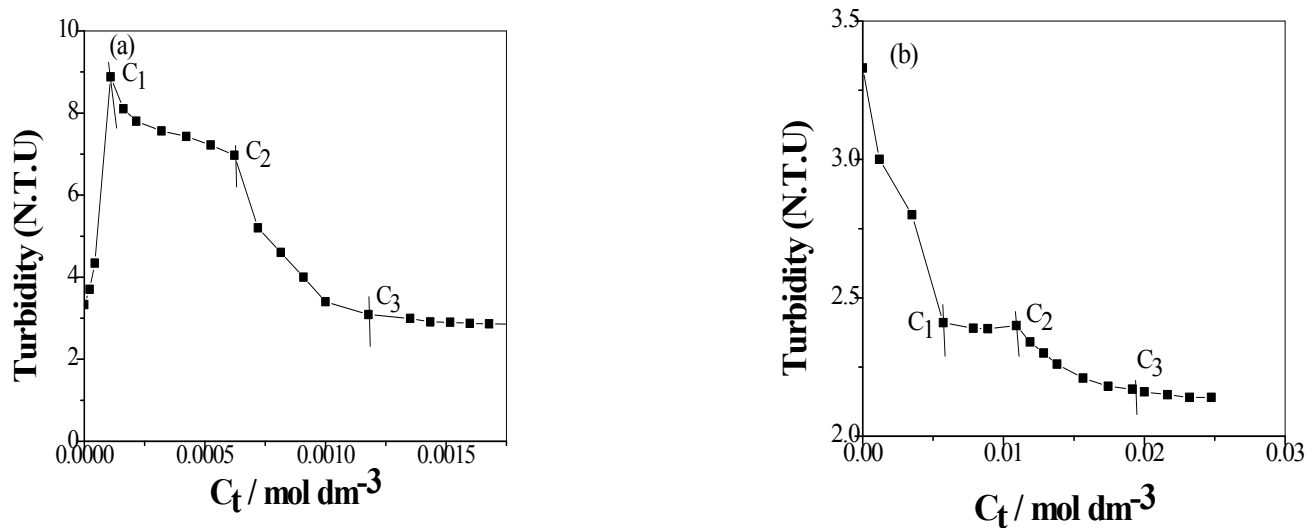δH (ppm) 0.90 (t, 6H; CH3), 1.34 (m, 24H; alkyl chain of anion), 1.69 (m, 2H; CH2), 1.90 (m, 2H; CH2), 4.07 (m, 2H, CH2), 4.26 (t, 2H; CH2), 7.18 (S, 1H; CH), 7.30 (S, 1H; CH), 9.73 (S, 1H;CH).
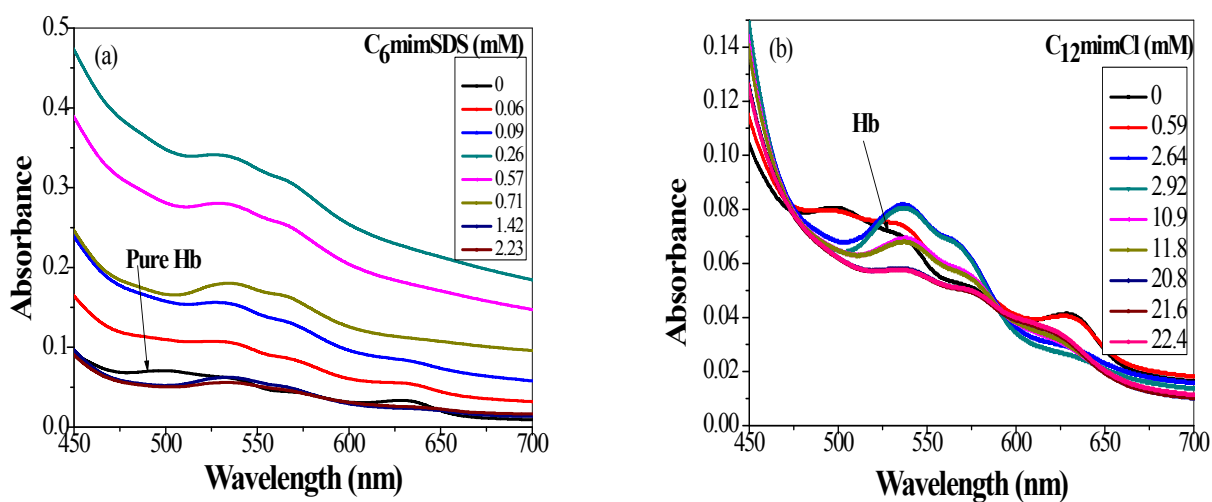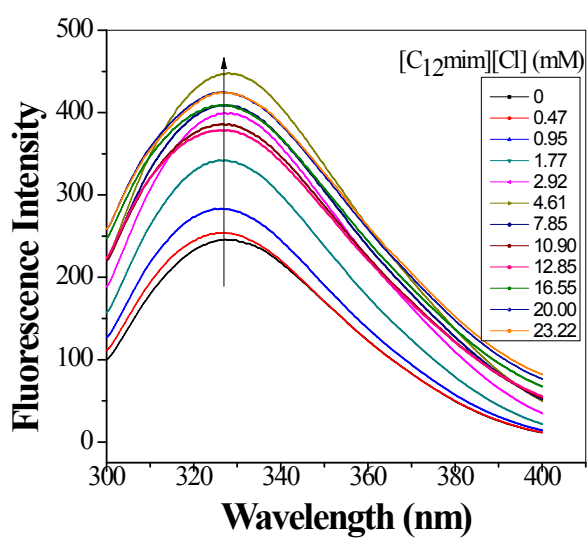
Figure S2 Absorption spectra of Hb (5 µM) in the range of 450-700 nm with increasing concentration of (a) C_6mimSDS and (b) C_{12}mimCl
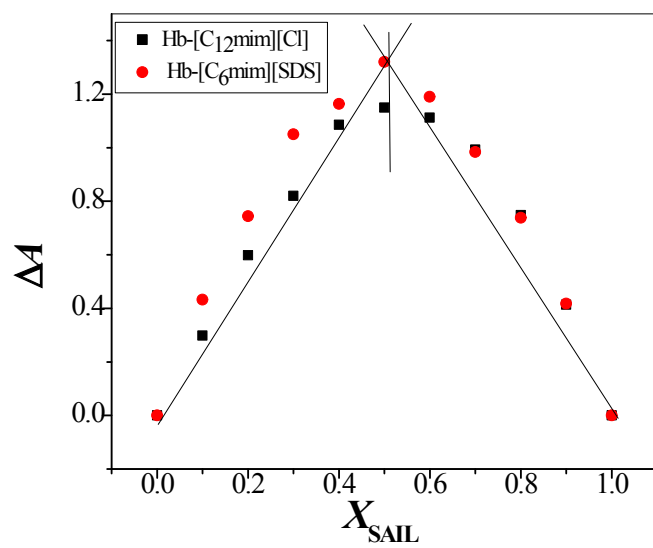

Figure S3: Variation of absorbance as a function of [C_6mim][SDS] concentration in the range of 0 to 0.1 mM

Figure S4 Variation of turbidity of Hb as a function of concentration of (a) $[C_6mim][SDS]$ and (b) $[C_{12}mim][Cl]$

Figure S5: Fluorescence emission spectra of Hb (0.005 mM) in the presence of increasing concentration of $[C_{12}mim][Cl]$

Figure S6: Job's plot depicting 1:1 stoichiometry of Hb-SAIL complexes.