Modifying the Aggregation Behavior of Poly (N-Isopropylacrylamide) Thermoreversible Gel by a Bile Salt

Anitha C Kumar1, Shilpi Boral2, H B Bohidar2* and Ashok K Mishra1*

1: Department of Chemistry, Indian Institute of Technology Madras, Chennai 600 036, India
2: Polymer and Biophysics Laboratory, School of Physical Sciences, Jawaharlal Nehru University, New Delhi-110 067, India

Email: mishra@iitm.ac.in (Ashok K Mishra); bohi0700@mail.jnu.ac.in (H B Bohidar)

Abstract. One possible method of altering the critical solution temperature (CST) of poly(N-isopropylacrylamide) (PNIPAM) gel is the addition of surfactants. Because of the biological origin of bile salts, their inclusion in PNIPAM could lead to better biocompatibility when the materials are used for biomedical applications. The gelling behaviour of PNIPAM was studied at various concentrations: PNIPAM concentrations in the range of 1% to 12% (w/v) and sodium cholate (NaC) concentrations varied from 0 to 20 mM. From fluorescence, DLS, rheology and turbidity studies it was found that in the presence of NaC, the CST shifts to lower temperature. This effect of the bile salt is in contrast to the effect of conventional surfactants which are known to shift the CST to higher values, due to mutual solubilization. A study of fluorescence spectroscopic parameters like fluorescence anisotropy, spectral shift, intensity and DLS measurements suggest that a NaC-induced aggregation could be responsible for this unusual observation.

1. Introduction
Thermoreversible hydrogels exhibit an LCST-type (lower critical solution temperature) discontinuous first order volume transition phenomenon [1,2]. The current research interest in these hydrogels arises from potential biomedical applications [3-5]. The most widely studied thermoreversible gel, poly(N-isopropylacrylamide) (PNIPAM), is especially interesting as it exhibits the LCST at around 32 °C in water, which is close to the body temperature of homeothermic animals [1,2]. The volume phase transition is reversible with temperature, which leads to some special applications like membranes for molecular separation [3], controlled drug releasing devices [4] and tissue culture substrates [5]. At the phase transition temperature there is a coil to globule transition present in this polymer, which has been extensively studied theoretically and experimentally [6].

The effect of additives such as salts and surfactants on the phase transition temperature of PNIPAM has been actively studied [7,8]. The presences of surfactants drastically change the behavior of PNIPAM, especially the solubility in water, and therefore phase transition. Generally, surfactants promote both inter and intra molecular solubility so that the phase transition temperature increases with the surfactant concentration. Wu et al [8] have reported that the presence of sodium dodecyl sulfate (SDS) and dodecylpyridine bromide (DPB) shifts the phase transition temperature of PNIPAM
to ~ 50°C and ~ 35°C respectively. Schild and Tirrell [9] have been carried out extensive studies of the effects of anionic surfactants on the phase transition temperature of PNIPAM and with surfactants having different chain lengths. They reported that the solubility of PNIPAM can be depressed or enhanced depending on the alkyl chain length and concentration. Surprisingly not much study has been carried out on the interactions of biological surfactants like bile salts with PNIPAM. The phase transition behavior of similar polymer systems N-alkylacrylamide copolymers with methacrylamide derivatives of cholic acid have been studied by Avoce et al [10] and it was found that the LCST decreased with increasing amount of NaC residue. It was shown by Benrebouh et al [11], that the bile acid residues tend to induce the aggregation of PNIPAM copolymers with 1-5%methacrylate derivatives of cholic acid polymers. They found that in the presence of the NaC residue, the aggregation of these copolymers started at very low concentration. They also reported that, NaC modified PNIPAM has no significant effect on the critical micellar concentration (CMC) of pure NaC solutions.

Bile salts are biological compounds synthesized in the liver, stored in gall bladder and released for lipid digestion in the gastro-intestinal tract [12]. They are surfactant molecules possessing 'facial polarity'. The bile salts (figure 1) have a common chemical structure quite different from synthetic surfactants. They have a few hydroxy groups directed toward the concave side (often called the α-plane) of the carbon framework and have the hydrophobic convex side (called the β-plane) [13]. In aqueous solutions, bile salts are known to associate to form aggregates, whose characteristics depend on the experimental conditions. The CMC of sodium cholate (NaC) is reported to be in the range 10 – 15 mM [14]. It is also reported that even at concentrations well below CMC, NaC exists in a dimeric form in aqueous medium, in which the hydrophobic faces come together [14].

![Figure 1 Structure of sodium cholate](image)

The objective of this work was to understand the effect of added sodium cholate, a bio-surfactant, on the phase transition temperature, i.e., gelation temperature of aqueous solution of PNIPAM. Since 10 % (w/v) solution of PNIPAM form a non-flowing gel above 32°C [7], we have used 12% (w/v) (27 mM) PNIPAM solutions for getting a good gel network. The NaC concentrations were varied from 0-20 mM. Fluorescence studies with an extrinsic fluorescent probe 8-Anilinonaphthalene sulphoninic acid (ANS), dynamic light scattering, turbidity and rheology studies have been carried out to understand the bile salt – PNIPAM interactions.

2. Experimental

Poly(N-isopropylacrylamide) (PNIPAM) and 8-Anilinonaphthalene sulphoninic acid (ANS) were purchased from Aldrich and used as such. Sodium cholate (NaC) was purchased from SRL. Triply distilled water was used for sample preparation. Stock solution of NaC (20 x 10⁻³ M) and PNIPAM (33.8 x 10⁻³ M) were prepared in triply distilled water. Final concentration of the PNIPAM in the mixture was 0.027 M (12 % w/v) and NaC concentration was varied from 0 to 20 mM (0, 1, 5, 10, 16 and 20 mM). Stock solution of ANS (10⁻³ M) was prepared in MeOH and 2 x 10⁻³ M was used for the fluorescence measurements. The PNIPAM - NaC mixture was allowed to equilibrate for at least 6 hours prior to the experiments.
2.1 Steady state fluorescence studies
Fluorescence measurements were carried out with a Hitachi F-4500 spectrofluorimeter, with a 150 W Xenon lamp as the light source. The excitation and emission spectra were recorded with slit widths of 5 /5 nm and at the PMT voltage of 700 V. The scan speed was kept at 1200 nm min⁻¹. Temperature dependent experiments were carried out by circulating water through a jacketed cuvette holder from a thermostatted bath (INSREF Ultra Cryostat).

The fluorescence anisotropy (rₐ) values were obtained using the expression

\[ rₐ = \frac{I_\parallel - G I_⊥}{I_\parallel + 2GI_⊥} \]  

where \( I_\parallel \) and \( I_⊥ \) refer to the fluorescence intensities when the emission polarizer is parallel and perpendicular, respectively, to the direction of polarization of the excitation beam. G is the factor that corrects for unequal transmission by the diffraction gratings of vertically and horizontally polarized light.

2.2 Time resolved fluorescence measurements
Fluorescence lifetime measurements were carried out using IBH single-photon counting fluorimeter in a time-correlated single-photon counting arrangement, consisting of ps/fs Ti-Sapphire Laser system (Tsunami Spectra Physics, Bangalore, India). The pulse repetition rate was 82 MHz and the full-width-half-maximum was less than 2ps. The emission was collected at magic angle polarisation (54.7°) to avoid any polarisation in the emission decay. The instrument response time was approximately 50ps. The decay data were further analysed using IBH software. A value of \( \chi^2 \) in the range 0.99 ≤ \( \chi^2 \) ≤ 1.4 was considered a good fit.

2.3 Dynamic light scattering
DLS measurements were carried out with a Photocor instrument having He–Ne laser at 633 nm as light source. Experiments were carried out at scattering angle fixed at 90°. PNIPAM - NaC samples (2 mL) were taken in a 5 mL cylindrical quartz cell and were held inside a home-made temperature controller [15]. This controller provided temperature regulation in the range of 15-75 °C with an accuracy of 0.1 °C. Scattered light from the sample was detected by the photomultiplier tube, and the photocurrent was suitably amplified and digitized before it was fed to a 1024 channel digital photon correlator (Brookhaven BI-9000 AT, U.S.A.). The whole scattering apparatus was installed on a vibration isolation table. The sample was equilibrated for 15 min at the measurement temperature. In the DLS measurement [16], the intensity correlation function has the form

\[ g^{(2)}(\tau) = 1 + \beta |g^{(1)}(\tau)|^2 = 1 + \beta e^{-2\Gamma \tau} \]  

where \( g^{(2)}(\tau) \) is the normalized second – order correlation function, \( \beta \) is the modulation parameter of the system, \( g^{(1)}(\tau) \) is the normalized first order field correlation function , \( \tau \) is the delay time, and \( \Gamma \) is the average characteristic line width. \( g^{(1)}(\tau) \) can be expressed as

\[ g^{(1)}(\tau) = \int G(\Gamma)e^{-\Gamma \tau} d\Gamma \]  

where \( G(\Gamma) \) is the relaxation time distribution function \( \Gamma \) purely originating from Brownian motion. The autocorrelation function was analyzed using the CONTIN method [17], which yields the mean relaxation frequency, \( \Gamma \) and the variance (polydispersity). The z - averaged diffusion coefficient, D, was obtained using \( \Gamma = Dq^2 \). The hydrodynamic radius, \( R_h \), was calculated using the Stokes- Einstein equation \( R_h = k_B T/6\pi\eta D \), where \( \eta \), \( k_B \) and T are solvent viscosity, Boltzmann constant, and absolute temperature respectively. All the DLS measurements were carried out at \( \theta = 90° \).

2.4 Rheology measurements
Rheology measurements were carried out on an AR-500 model stress controlled rheometer (T.A. Instruments, UK) with the objective to inter-relate the stiffness and thermal stability of the network in
flow mode. The dynamic rheology of the solutions was measured using stainless steel cone-plate geometry of radius 60 mm and angle 2° with truncation gap 50 µm. Silicon oil was used as solvent trap to prevent loss of solvent because of evaporation.

2.5 Turbidity measurements

Turbidity measurements were done under Brikmann PC 910 calorimeter (Brikmann instruments, USA), operating at a wavelength of 450 nm, details of which are given elsewhere [18]. The inflection point in the turbidity curves was taken for the value of LCST of the polymer. The polymer concentration was at 12% w/v (27 mM) in distilled water. The concentration of NaC was varied from 0 to 16mM.

3. Results & Discussion

3.1 Fluorescence studies

It is known that ANS in water gives very weak fluorescence, and when incorporated to an organized media it shows an increase in fluorescence intensity and a blue shift [19]. The position of emission maximum gives an idea of the local polarity of the fluorophore [20]. The general features observed upon incorporation in an organized medium are a blue shift in the $\lambda_{\text{max}}$ arising out of decreased local polarity, and increase in the fluorescence lifetime as well as quantum yield arising out of restricted mobility of the fluorophore [20]. ANS is an anionic fluorescent probe and is expected to interact negligibly with the anionic NaC due to electrostatic repulsion. Thus the probe is expected to report primarily from the PNIPAM nano-environment. The response of ANS fluorescence intensity to increasing concentration of NaC in aqueous media at 20 °C is shown in figure 2. In the absence of PNIPAM, the intensity enhancement is insignificant even at the CMC (16 mM) of NaC. On the other hand, in aqueous 12% PNIPAM in its sol phase (20 °C) without NaC, the intensity enhancement is clearly seen. These results verify that the extrinsic probe ANS is essentially present in a PNIPAM environment. A large enhancement of ANS fluorescence intensity was observed in 12% PNIPAM - 16mM NaC medium (20 °C) which indicates NaC-induced structural changes in PNIPAM even in the sol state. ANS is thus seen to be a particularly suitable fluorescent probe for this system.

![Figure 2 Variation of fluorescence intensity of NaC in water and PNIPAM at 20 °C](image)

ANS fluorescence spectra in 12% (27mM) PNIPAM solutions and 0 - 20 mM solutions of bile salt at 20 °C are shown in figure 3. The ANS concentration was maintained at a low level (2x10^{-5}M) so that the effect of ANS on the aggregation process is negligible.
With increasing concentration of NaC there was an increase in the fluorescence emission intensity and a blue shift in the $\lambda_{\text{max}}$. The blue shift of the band indicates that the ANS is solubilized in a hydrophobic nanoenvironment. For NaC, the reported onset of micellisation is in the concentration range of 10 - 15 mM [14]. In the present study an increase in intensity was observed even after addition of 1mM NaC, clearly indicating that micellisation of NaC is not a prerequisite for PNIPAM - NaC interactions. Modification of polymer chain arrangement gets initiated even at sub-micellar concentrations of NaC. The increase in intensity of ANS in PNIPAM solutions might be due to the rigid packing of PNIPAM aggregates in presence of sodium cholate.

Temperature dependent experiments have been done by changing the temperature from 20°C to 40°C and results are shown in figure 4.

With increasing temperature there is an increase in fluorescence intensity. Since PNIPAM forms a turbid gel above its phase transition temperature, the fluorescence intensity decreases due to scattering. So the inflection point can be taken as the phase transition temperature. It is clear from the figure that the phase transition temperature decreases with increasing concentration of NaC. In the absence of NaC, PNIPAM shows a phase transition temperature of 32°C same as the reported value. In presence of 16 mM NaC the phase transition temperature shifted to 27°C. This decrease in CST is in sharp contrast to the effect of other surfactants which are known to increase the CST [8]. Further studies involving other fluorescence parameters as well as other techniques like DLS, rheology and turbidity has been done to confirm the observation and the results presented later in the manuscript.
A possible explanation of the fluorescence changes could be as follows. At the gelation temperature, because of the coil to globule transition, there is an increase in hydrophobicity on the globule surface [6]. The enhanced surface hydrophobicity would induce partitioning of the ANS molecules onto the hydrophobic - hydrophilic interfaces, where they would experience reduced mobility due to the hydrophobic structuring of water present in the system. ANS is known to preferentially distribute on to hydrophobic hydrophilic interfaces, where they show a blue shifted emission [21]. The quantum yield of fluorescence ($\phi$) is given as

$$\phi = \frac{k_f}{k_f + \sum k_{nr}}$$  \hspace{1cm} (4)

where $k_f$ is the emissive rate of fluorescence and $\Sigma k_{nr}$ is the rate of all other nonradiative decay processes. The reduced mobility of ANS molecules would result in a reduction of $\Sigma k_{nr}$, thereby resulting in an increase of $\phi$. The hydrophobicity of ANS environment would result in a hypsochromic shift of the fluorescence spectrum.

Figure 5 shows the variation of emission frequency in presence of NaC. The emission frequency increases with increasing concentration of NaC and temperature which clearly indicates the increased hydrophobicity of the aggregated gel environment. A blue shift of about 459 cm$^{-1}$ was observed in presence of 10 mM NaC.

![Figure 5 Change in fluorescence emission frequency of ANS in PNIPAM - NaC samples with increasing temperature.](image)

Table-1 shows the fluorescence lifetime values of 0 and 16 mM NaC added PNIPAM samples at 15°C. Fluorescence lifetime ($\tau$) is defined by the average time the molecule spends in the excited state prior to the return to the ground state and is given by the expression

$$\tau = 1/(k_f + \sum k_{nr})$$  \hspace{1cm} (5)

The fluorescence decay profiles were measured by exciting at 360 nm. The decay profile recorded for ANS followed tri-exponential kinetics in these three samples. The average lifetime of ANS in water is reported to be 0.25 ns [22].

The weighted average lifetime ($\tau_{avg}$) of multi-component decay is given by

$$\tau_{avg} = \frac{\tau_1(\alpha_1) + \tau_2(\alpha_2) + \tau_3(\alpha_3)}{\alpha_1 + \alpha_2 + \alpha_3}$$  \hspace{1cm} (6)

where $\alpha$’s are the amplitudes of each component in %.
\( \tau_{\text{avg}} \) of ANS was shifted slightly from 4.90 ns in pure PNIPAM solution to 5.16 ns in presence of 16 mM NaC. The slight enhancement of average fluorescence lifetime does not correspond to the rather large increase in the fluorescence intensity. A possible reason for this lack of correspondence could be that the ANS that is still present in a pure aqueous environment emitting with extremely low intensity is not reflected in the decay curve.

**Table 1** Fluorescence lifetime of ANS in NaC added PNIPAM solutions at 15 °C.

| [NaC]  | 0mM  | 16mM |
|--------|------|------|
| \( \tau (\text{ns}) \) |
| \( \tau_1 (\alpha_1) \) | 0.85(20) | 0.16(7) |
| \( \tau_2 (\alpha_2) \) | 3.5(43) | 1.64(24) |
| \( \tau_3 (\alpha_3) \) | 8.8(37) | 6.89(69) |
| \( \chi^2 \) | 1.16 | 1.03 |
| \( \tau_{\text{avg}} \) | 4.90 | 5.16 |

Figure 6 shows the emission frequency of ANS in different solvents and PNIPAM in the presence and absence of NaC as a function of solvent polarity (\( Z \)). The \( Z \) scale, a UV/vis spectroscopic solvent polarity scale was set up by Kosower in 1958, using the intermolecular charge-transfer (CT) solvent-sensitive absorption process of 1-ethyl-4(methoxycarbonyl)pyridinium iodide [23].

The \( Z \) - value is defined by

\[
Z = 2.859 \times 10^4/\lambda \tag{7}
\]

where \( Z \) is in kcal mol\(^{-1}\) and \( \lambda \) is in nm.

Pure 12% PNIPAM shows the polarity of ethyl glycol, which is shifted close to ethanol by the addition of 16 mM NaC.

Figure 6 Change in emission frequency as a function of solvent polarity of ANS in different solvents and PNIPAM.

Fluorescence anisotropy (\( r_{\text{ss}} \)) is an independent parameter offered by fluorescence spectroscopy. It is a measure of the degree of rotational diffusion of the fluorophore during the lifetime of the excited molecule, which reveals the average angular displacement of the molecule. Thus \( r_{\text{ss}} \) is expected to be a useful parameter in obtaining information on the efficiency of rotational
motion of the fluorophore molecule. The value of $r_{ss}$ varies between the maximum of 0.4 for a completely restricted fluorophore and the minimum of 0.0 for a completely free molecule [24]. The rotational diffusive motion of the molecule is dependent of the viscosity of the medium. The reported steady state fluorescence anisotropy of ANS in water is 0.03, and in 95% glycerol at 0 °C it is 0.32 [25]. The variation of fluorescence anisotropy values of PNIPAM – NaC samples are shown in figure 7. Fluorescence anisotropy values are reasonably high even in the absence of NaC. The anisotropy slightly increases with NaC and temperature. Because PNIPAM forms a turbid gel at the phase transition temperature, there is a sudden drop in the fluorescence anisotropy due to the scattering. The inflection point in the anisotropy data were taken as the phase transition temperature of the sample. The phase transition temperature changes from 32 °C to 27 °C in the presence of 16mM NaC.

![Figure 7](image1)

Figure 7 Change in fluorescence anisotropy of ANS in PNIPAM - NaC samples with temperature.

Thus all the fluorescence parameters: the fluorescence spectral blue shift, intensity enhancement, increases in fluorescence lifetime and increase in fluorescence anisotropy clearly confirm the increased hydrophobicity of the aggregated gel environment. The gelation temperature reported by these parameters are also fairly consistent: changing from 32 °C in pure PNIPAM (12% w/v) to 27 °C in PNIPAM -16 mM NaC.

### 3.2 Dynamic Light Scattering Studies

![Figure 8](image2)

Figure 8 DLS data of 12% PNIPAM with different concentration of NaC

DLS measurements are very useful to study the structure of macromolecules and molecular assemblies.
With increasing concentration of NaC there is an increase in the size of the aggregates. The $R_h$ of these aggregates increases with temperature. Increase of $R_h$ is accompanied by an increase of scattered light that clearly indicates the aggregation of PNIPAM chains in water. Above LCST, DLS measurement is impossible because of turbidity. In the absence of NaC, below phase transition temperature the $R_h$ is very small which may be due to the individual PNIPAM chains present in it. In the presence of NaC, PNIPAM chains start to aggregate forming large particles and with increasing temperature the size of the aggregates further increases due to the hydrophobic interaction of PNIPAM chains (figure 8). After phase transition, further increase in temperature has no effect on $R_h$ [26]. With increasing NaC concentration and temperature the size of the aggregates are increasing. $R_h$ can be converted to $D$ using the Stokes – Einstein equation:

$$R_h = \frac{k_B T}{\pi \eta D}$$

where $\eta$, $k_B$, and $T$ are the solvent viscosity, the Boltzmann constant, and the absolute temperature respectively. For a typical polymer gels, $D$ is of the order of $10^{-7}$ to $10^{-6}$ cm$^2$/s, depending on the polymer concentration [26].

### 3.3 Rheology studies

#### 3.3.1 Shear rate data:

Rheology experiments were performed using an AR-500 model stress controlled rheometer (T.A. Instruments, UK) with the objective to inter-relate the stiffness and thermal stability of the network in flow mode. The dynamic rheology of the solutions was measured using a stainless steel cone-plate geometry of radius 60 mm and angle 2° with truncation gap 50 µm. The truncation gap was deliberately chosen to be more than the length scales existing in coacervates. Silicon oil was used as solvent trap to prevent loss of solvent because of evaporation. The shear induced flow behavior of coacervates samples were quite revealing. The shear rate, $\gamma$ dependent viscosity, $\eta(\gamma)$ data of these samples is plotted in figure 9, which reveals the non-Newtonian yield [27].

The shear induced flow behavior of the samples was quite revealing. The shear rate dependent viscosity data of these samples are plotted in fig-9, which reveals the non-Newtonian yield. In addition, shear-thinning behavior was exhibited by these samples. The data presented in figure 9 were fitted to Carreau model function that adequately described the relation.

$$\eta(\gamma) \sim (\gamma)^k$$

with $k = 0.25\pm0.05$ for samples having NaC concentration in the range of 5mM to 16 mM. For 0 mM NaC samples, the value of $k = 0.17$ at 20 °C that increased to $k = 0.29$ at 38 °C implying significant structural change induced by temperature. In fact, $k$ reveals the viscous response of the samples to applied shear: $k = 0$ gives Newtonian, $k<0$ indicates shear thickening and $k>0$ implies shear-thinning behavior. The $k$ value remained close to 0.35±0.05 invariant of temperature for 1mM NaC sample. Thus, shear-thinning features are clearly manifested in all these samples. The sudden drop in the viscosity values on addition of NaC could correspond to the rupture of the weak network structure present in the system due to the intermolecular binding of PNIPAM molecules to NaC.

The shear rate dependent viscosity data reveals two things: (i) the viscous response is non-Newtonian and (ii) the material exhibits shear thinning behavior. The lower viscosity at higher shear rate may owe its origin to the possible alignment of the network structure, which is shear induced. Another interesting feature pertains to the observation that for a given shear rate the viscosity increases with temperature unlike what is normally seen. Increase in NaC concentration did not change the viscosity significantly which could be possibly ascribed to the fact that the internal structure is not much altered during this process. Detailed analysis of the internal structures requires a more extensive study, which is in progress.
3.4 Turbidity Measurements

The simplicity and sensitivity of turbidimetric titration method as applied to a phase separating system is based on the fact that turbidity is proportional to both the molecular weight and the number density of particles present in dispersion. The turbidimetric titration experiments were performed using a colorimeter (Model-910, Brinkmann Instruments, USA) operating at a wavelength = 450nm, details are given elsewhere [17].
The turbidity of PNIPAM - NaC samples were studied as a function of temperature and the data were shown in fig-11. The phase transition temperature is changing from 32 °C to 27 °C in presence of 16 mM NaC. The phase transition temperatures are clearly seen from this figure, which correlates well with the DLS data and fluorescence measurements.

As was observed earlier in the manuscript, the observed lowering of CST in the PNIPAM-NaC system is in contrast to the effect of other surfactants that cause an increase of CST [7]. Thus the PNIPAM-NaC interactions appear to be different from the ‘solubilizing’ interaction of other surfactants. There is some similarity of NaC and PNIPAM in their ‘facial’ nature of polarity. NaC possesses distinct polar and nonpolar faces. Similarly, depending on the temperature, PNIPAM presents a hydrophilic (at <CST) or hydrophobic (at >CST) surface to the aqueous media, which makes them thermoreversible gels with lower CST. The results in this study shows that even in the sol phase of PNIPAM and even at < CMC of NaC, there is a NaC – induced aggregation of PNIPAM. The coming together of hydrophilic faces of PNIPAM and NaC at < CST temperatures could be a reason for this observation. The pre-aggregated PNIPAM can then undergo gelation at relatively lower temperatures.

4. Conclusion

The effect of added sodium cholate on the aggregation behavior of PNIPAM was studied by different techniques. With increasing concentration of NaC, an increase in fluorescence intensity, fluorescence anisotropy, fluorescence lifetime and a blue shift in $\lambda_{\text{max}}$ were observed which indicates the increase in hydrophobicity of the medium. From DLS data it was clear that the size of the PNIPAM particles increases with NaC concentration and temperature. Rheology experiments suggest that for a given shear rate the viscosity increases with temperature unlike what is normally seen. Increase in NaC concentration did not change the viscosity significantly which could be possibly ascribed to the fact that the internal structure is not much altered during this process. It was found from the fluorescence, DLS and turbidity measurements that the phase transition temperature of PNIPAM solution changes from 32 °C to 27 °C in presence of 16 mM NaC. The PNIPAM-NaC interactions appear to be different from the solubilizing interaction of other surfactants. The ‘facial polarity’ nature of the NaC bile salt appears to be the factor that induces PNIPAM aggregation in the sol state, which results in lowering of CST.
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