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DOI: 10.1016/j.molliq.2016.11.060

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# The influence of various alkylammonium-based ionic liquids on the hydration state of temperature-responsive polymer



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## ARTICLE INFO

### Article history:

Received 24 October 2016

Received in revised form 14 November 2016

Accepted 18 November 2016

Available online 19 November 2016

### Keywords:

Poly(*N*-isopropylacrylamide)

Ionic liquids

Lower critical solution temperature

Morphological changes

## ABSTRACT

The influence of different alkylammonium-based ionic liquids (ILs) on the temperature-dependent aqueous poly(*N*-isopropylacrylamide) (PNIPAM) solution is explored by the comprehensive experimental analysis. The co-solvents investigated in the present study included a series of ILs with a fixed (trifluoromethylsulfonyl)imide [NTf<sub>2</sub>]<sup>−</sup> anion and variable ammonium cations such as butyltrimethylammonium (IL-1), methyltrioctylammonium (IL-2), diethylmethyl(2-methoxyethyl)ammonium (IL-3) and ethyldimethylpropylammonium (IL-4). The thermal phase transitions of aqueous solution of thermo-responsive polymer (TRP) in ammonium-based ILs were studied by various experimental techniques such as fluorescence spectroscopy, dynamic light scattering (DLS) and viscosity ( $\eta$ ). We further employed field emission scanning electron microscope (FESEM) to reveal the distinct morphological changes of various self-assembled morphologies. Our results reveal that the lower critical solution temperature (LCST) of the TRP can be significantly altered by the ILs. The phase transition state of aqueous TRP decreases as the concentration of IL increased. We find that IL-4 lowers the LCST of TRP significantly more as compared to the other ILs. Our experimental results reveal that the hydrophobic interactions are predominant between the monomers of PNIPAM and ions of the ILs. This research work highlights new opportunities for the wide applications in engineering of the bio-responsive smart PNIPAM-based devices and appropriate selection of ILs, which should allow for increasing the usage of thermo-responsive phase behaviour of polymers.

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## 1. Introduction

The thermo-responsive polymers (TRPs) are inspired to alter their structures and activity in response to surrounding environmental stimuli. These TRPs are composed of a hydrophobic group, grafted with hydrophilic chains. The thermo-responsive properties are governed by the balance between hydrophobic and hydrophilic moieties [1]. Thermo-responsive water soluble polymers have attracted considerable interest as they often display large, reversible conformational changes in response to small external stimuli. Poly(*N*-isopropylacrylamide) (PNIPAM) is one of the most efficiently and essentially exploited TRPs from the whole scientific world over the decades [1–6]. The most important advantage for PNIPAM is fully reversible phase transition in aqueous solution. PNIPAM aqueous solution is known to exhibit demixing at physiological temperatures, which can be interesting towards its wide range of chemical and biological applications such as controlled drug release and aggregation behaviour, nanoparticle

synthesis and gene delivery [7–11]. The phase separation behaviour of aqueous PNIPAM solution is characterized by a lower critical solution temperature (LCST), which lies at ~33 °C and has been thoroughly explored by a wide variety of biophysical techniques, especially in dilute conditions, due to its external stimuli responsive properties [12,13].

Naturally, PNIPAM exists as solvated random coils below the LCST whereas tightly packed globular particles above the LCST, which leads to a coil-globule phase transition and aggregation of the polymer [14,15]. This phase transition is associated with the temperature-dependent molecular interactions, mainly hydrogen bonding and hydrophobic association.<sup>15</sup> A great effort has been investigated in understanding the phase transition behaviour and the parameters affecting the phase transition temperature of PNIPAM. The temperature-sensitive phase transition of PNIPAM aqueous solution undergoes extensive conformational changes in the presence of various environmental variations (such as co-solvents, surfactants and additives) [8,9,16–19]. In recent years, the study of interactions between polymer and the solvent particles have attracted considerable interest from both fundamental and applied research areas. The presence of additives can modulate the properties of polymers and make their performance better under certain experimental conditions, which are attracting special attention because of versatile practical applications across the diverse scientific fields. In spite of numerous studies on the influence of

Abbreviations: PNIPAM, poly(*N*-isopropylacrylamide); ILs, ionic liquids; LCST, lower critical solution temperature.

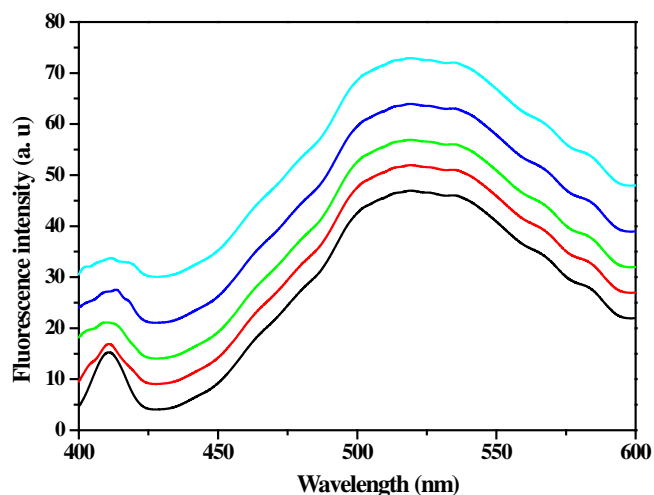
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E-mail addresses: [venkatesup@hotmail.com](mailto:venkatesup@hotmail.com), [pvenkatesu@chemistry.du.ac.in](mailto:pvenkatesu@chemistry.du.ac.in) (P. Venkatesu).

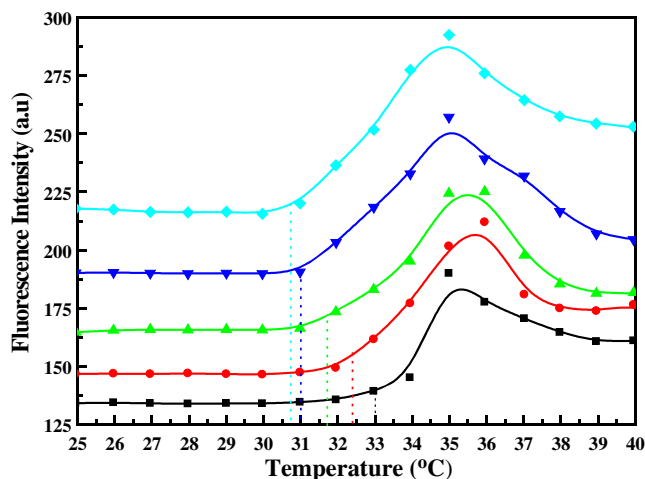
various additives on the temperature-sensitive property of PNIPAM aqueous solution, it is still obscure of the effect of the additives on the nature of interactions between the monomers of polymer and the solvent particles.

Ionic liquids (ILs) exhibit tunable and unique physical and chemical properties including negligible vapour pressure, non-flammability, and good ability to dissolve in chemical compounds [20–24]. The utilization and applications of ILs have been extensively expanded in all scientific studies by several researchers [20–30]. ILs are consisting of cation and anion and are often in liquid state at ambient temperature. The appropriate modification of cation and anion can tune their hydrophobicity/hydrophilicity, thus making it possible to be used according to the need and demand of the reaction conditions [31–33]. ILs have been used as co-solvents/additives for biochemical processes as well as polymerization processes. The combination of temperature-sensitive polymers with ILs produces controllable morphology, improved separation properties which are more useful as biomaterials for controlled drug delivery. Apparently, the phase transition behaviour of polymers in ILs remains poorly understood because of the difficulties in obtaining the structural information of polymers in the presence of the ions of ILs [34–37].

Very few reports exist on the study of the effects of imidazolium-based ILs on the LCST of PNIPAM aqueous solution [10,13,38–41] and obviously, very little is known about the influence of ammonium-based ILs on the phase transition of PNIPAM aqueous solution [42]. In 2011, the first experimental evidence of the existence of the modulation LCST of PNIPAM aqueous solution was provided on the basis of biophysical techniques by some of us [13]. Later, in 2012, the role of ILs such as 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF<sub>4</sub>]) [38] or a hydrophobic IL (1-ethyl-3-methylimidazolium bis(trifluoromethyl sulfonyl)imide [C<sub>2</sub>mim][NTf<sub>2</sub>]) [39] on the phase transition behaviour of PNIPAM aqueous solution have explored in detail. Furthermore, Debeljuh et al. [42] quantified the phase transition of PNIPAM in aqueous protic ILs. In 2014, we reported results of a comprehensive analysis of biophysical techniques for the influence of ILs containing the same cation, 1-butyl-3-methylimidazolium [Bmim]<sup>+</sup> and commonly used anions such as [SCN]<sup>-</sup>, [BF<sub>4</sub>]<sup>-</sup>, [I]<sup>-</sup>, [Br]<sup>-</sup>, [Cl]<sup>-</sup>, [CH<sub>3</sub>COO]<sup>-</sup> and [HSO<sub>4</sub>]<sup>-</sup> on phase transition temperature of PNIPAM aqueous solution and found that the extent of diminishment in LCST is more pronounced in the case of kosmotropes than that of chaotropes at all studied concentrations [42]. The research studies in the field of PNIPAM-IL interaction have remained a prospective avenue of investigation for the phase transition of PNIPAM. Particularly, the studies related to the LCST of TRP in the presence of ILs are still in its early stages.



**Fig. 1.** Fluorescence emission spectra of ANS in PNIPAM aqueous solution (black line), PNIPAM in (the concentration IL is 5  $\mu$ L/mL) IL-1 (red line), IL-2 (green line), IL-3 (blue line) and IL-4 (cyan line) at 25  $^{\circ}$ C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** Thermal Fluorescence emission spectra of ANS in PNIPAM aqueous solution (black line), PNIPAM in (the concentration IL is 5  $\mu$ L/mL) IL-1 (red line), IL-2 (green line), IL-3 (blue line) and IL-4 (cyan line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Many reports [43–46] have characterized the protein folding/unfolding in the presence of ammonium-based ILs, however, obviously, no studies have reported on the influence of ammonium-based ILs on the phase transition of aqueous PNIPAM solution, which is often considered as a model protein. A lack of sufficient knowledge regarding the behaviour of ammonium-based ILs on TRP hinders the potential use of responsive polymers as biomaterials and medical devices. Therefore, there are numerous approaches based on experimental results to elucidate the phase transition behaviour, yet such studies related to polymer-IL interactions mainly focusing on cation variation of ammonium-based ILs are still lacking. However, no conclusive experimental results have been systematically explored the influence of ammonium-based ILs on the phase behaviour of PNIPAM.

Therefore, the main goal of this work is to experimentally explore the polymer behaviour in aqueous medium under novel kind of ammonium-based ILs through phase separation process. In this work, fluorescence spectroscopy, dynamic light scattering (DLS) and viscosity ( $\eta$ ) as a function of temperature and field-emission scanning electron microscopy (FESEM) at room temperature were employed to understand the role of ammonium-based ILs on the phase transition temperature of PNIPAM aqueous solution. The results of the present study provide knowledge in elucidating and identifying the TRP behaviour during a phase separation process using water-immiscible ILs.

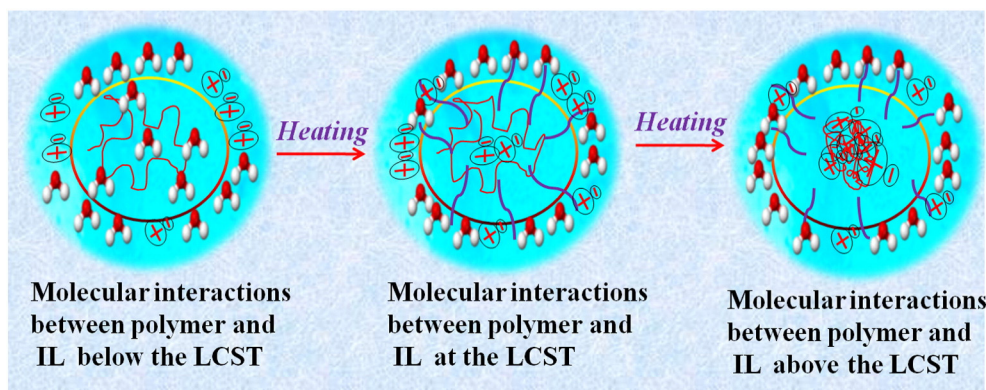
## 2. Experimental section

### 2.1. Materials

PNIPAM ( $M_n = 20,000$ – $25,000$ ), 8-anilino-1-naphthalenesulfonic acid (ANS), butyltrimethylammonium (trifluoromethylsulfonyl)imide (IL-1) ( $\leq 0.2\%$  water), methyl-trioctylammonium (trifluoromethylsulfonyl)imide (IL-2) ( $\leq 300$  ppm water), diethylmethyl(2-methoxyethyl)ammonium (trifluoromethylsulfonyl)imide (IL-3) ( $\leq 500$  ppm water) and ethyldimethylpropylammonium (trifluoromethylsulfonyl)imide (IL-4) ( $\leq 1.0\%$  water) were purchased from Sigma Aldrich chemical company (USA) and used without further purification. Double distilled deionized water (Ultra 370 series, Riens India, India) with 18.3 M $\Omega$  resistivity was used for the sample preparation.

### 2.2. Sample preparation

All the sample solutions were prepared gravimetrically by using Mettler Toledo balance with a precision of  $\pm 0.0001$  g and the required



**Scheme 1.** Schematic depiction of molecular interactions between polymer aqueous solution and ILs.

amount of ANS and PNIPAM was weighed for stock solutions. Aliquots of these solutions were mixed to prepare the sample solutions at desired concentration of polymer. The polymer concentration for all measurements was maintained 7 mg/mL. The weighed amounts of ILs at various concentrations (5, 10, 15, 20 & 25  $\mu\text{L}/\text{mL}$ ) were added directly to the aqueous polymer solution. All the polymer-IL samples were kept at room temperature for few hours to equilibrate the samples for the proper incorporation of polymer and IL aqueous solution. The polymer samples were stored in cool place and kept in a closed container tightly to prevent absorption. In fluorescence measurements, the concentration of the probe was kept at  $2 \times 10^{-5}$  M to avoid the probe interference in the measurements. Prior to the measurements, each sample was filtered with 0.45  $\mu\text{m}$  disposable filters (Millipore, Millex-GS) through a syringe.

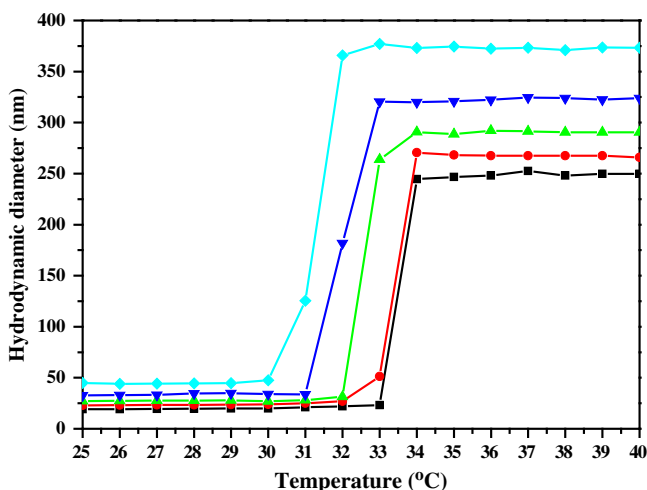
### 2.3. Measurements

Fluorescence intensity measurements of ANS in aqueous PNIPAM solution in the absence and in the presence of ILs were measured using a Cary Eclipse fluorescence spectrophotometer (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia) with an intense Xenon flash lamp as light source. Dynamic light scattering (DLS), Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK), equipped with He-Ne (4 mW, 632.8 nm) was used for hydrodynamic radius ( $R_h$ ) of all samples. The viscosities ( $\eta$ ) of all the samples are measured using a sine-wave vibro viscometer (model SV-10, A&D Company

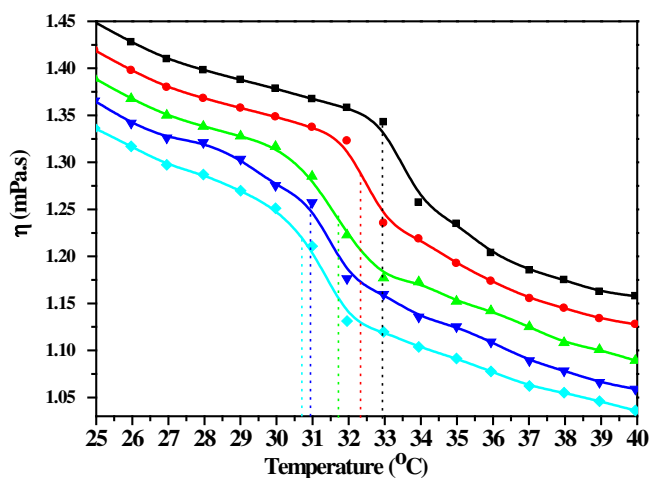
Limited, Japan) with an uncertainty of 1%. The  $\eta$  of sample solutions was collected in the wide temperature range by using a circulating temperature control water bath (LAUDA alpha 6, Japan) with accuracy of temperature of  $\pm 0.02$  K. Field emission scanning electron microscopy measurements (FESEM) studies were carried out using a MIRA3 TESCAN electron microscope operating at 10 kV. All the reported values are an average of three measurements of the sample. The detailed information of instrumentation and measurements used in the present study have been delineated in our earlier papers [36,40].

### 3. Results and discussion

During heating of the PNIPAM aqueous solution without and with a series of ammonium-based ILs, from room temperature to 40  $^{\circ}\text{C}$ , PNIPAM phase transition was examined by thermal fluorescence spectroscopy, DLS and  $\eta$  measurements. Furthermore, steady state fluorescence spectroscopy and FESEM are also performed for the systems at room temperature. The phase transition of PNIPAM in water and in 5, 10, 15, 20 and 25  $\mu\text{L}/\text{mL}$  of each IL is investigated. ANS can be used as the fluorescent probe to examine the characterization of intermediate states and aggregates of PNIPAM induced by the addition of ILs [13, 40]. The concentration of ANS was taken as  $2 \times 10^{-5}$  M to obtain clear spectra. The steady-state emission spectra of ANS in a PNIPAM aqueous solution in the presence and absence of 5  $\mu\text{L}/\text{mL}$  ILs at 25  $^{\circ}\text{C}$  are provided in Fig. 1. ANS is a spatially sensitive molecular fluorescent probe that



**Fig. 3.** Hydrodynamic diameter ( $d_H$ ) of PNIPAM aqueous solution (black line), PNIPAM in (the concentration IL is 5  $\mu\text{L}/\text{mL}$ ) IL-1 (red line), IL-2 (green line), IL-3 (blue line) and IL-4 (cyan line) in the temperature range of 25–40  $^{\circ}\text{C}$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Viscosity ( $\eta$ ) of PNIPAM aqueous solution (black line), PNIPAM in (the concentration IL is 5  $\mu\text{L}/\text{mL}$ ) IL-1 (red line), IL-2 (green line), IL-3 (blue line) and IL-4 (cyan line) in the temperature range of 25–40  $^{\circ}\text{C}$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

exhibits a very low monomeric fluorescence emission intensity peak at 510 nm as shown in the Fig. 1.

As depicted in Fig. 1, ANS in PNIPAM aqueous solution exhibited a relatively low intensity over the range of wavelengths monitored, indicating the absence of a hydrophobic environment for ANS molecules. However, a marked increase in intensity was observed with the addition of the ILs, which implies the formation of a hydrophobic micro-environment from the collapse of the hydrated coil structure of PNIPAM. It is known that an enhancement of the quantum yield of ANS would give an increase in the fluorescence intensity of PNIPAM-IL aggregates. Moreover, the enhancement in the intensity explicitly elucidates that the addition of IL renders the PNIPAM-IL solution more hydrophobic. A similar type of enhancement in the fluorescence intensity of ANS has been observed in the case of PNIPAM in imidazolium-based IL solution [40]. However, in the present study, the extent of the change in emission intensity was strongly influenced by the type of the cation of IL. The maximum intensity was observed for PNIPAM in IL-4 whereas the minimum intensity was observed for PNIPAM in IL-1 (Fig. 1).

It is interesting to note that the fluorescence intensity is increased with increasing the concentration of IL, which is a direct result of the increasing hydrophobic collapse of PNIPAM (Figs. S1, S2, S3 and S4 in ESI†). The observations explicitly uncover the effect of ILs on the emission spectra in PNIPAM aqueous solution, which in turn implies negligible partitioning of ANS onto the PNIPAM surface. From these figures, it has been observed that the bathochromic shift in the emission wavelength is due to the low mobility of molecules. The distinguishable changes in intensities, as shown in Figs. 1, S1, S2, S3 and S4, were expected as a result of the differences in the interactions between the polymer chain and ions of IL and water.

After completing of steady-state fluorescence of samples, we have performed thermal fluorescence intensity measurements as a function of increasing the temperature. Fig. 2 shows temperature dependence of the fluorescence intensity measurements of ANS in the PNIPAM aqueous solution and in the presence different ILs. As can be seen in Fig. 2, at the temperatures below LCST, the intensity keeps approximately constant until  $\sim 33^\circ\text{C}$  which indicates the existence of a well-coiled conformation of PNIPAM chains. Additionally, below LCST, there are hydrophilic interactions between PNIPAM and water molecules, since water is an extremely good solvent for PNIPAM. Obviously, there is a sudden enhancement in intensity on further heating of the solution which indicates that the PNIPAM starts to collapse, eventually polymer is in a dehydrated state. This sharp and sudden enhancement in intensity at  $\sim 33^\circ\text{C}$  results from the coupling of ANS molecules onto the surface of the hydrophobic portion of PNIPAM chains. As expected, the LCST of polymer was significantly shifted from  $33.0^\circ\text{C}$  (in the absence of IL) to lower temperatures  $32.4, 31.8, 31.0$  and  $30.8^\circ\text{C}$  in the presence of IL-1, IL-2, IL-3 and IL-4, respectively. Moreover, the maximum and minimum shifts in the diminishment in LCST were observed in the presence of IL-4 and IL-1, respectively.

The LCST values further decrease with increasing the concentration of ILs (Figs. S5, S6, S7 and S8 in ESI). The addition of IL leads to the shift to higher intensities towards lower temperatures, which indicates that the aggregation of PNIPAM in the presence of IL is encouraged with the variation in the cation of IL. Moreover, the addition of IL leads to the enhanced surface of hydrophobicity which in turn results in the partitioning of the ANS molecules to the hydrophilic–hydrophobic interfaces, where they would experience reduced mobility. Although the different cations of IL at every concentration reveal the same qualitative behaviour, important quantitative differences arise when they are

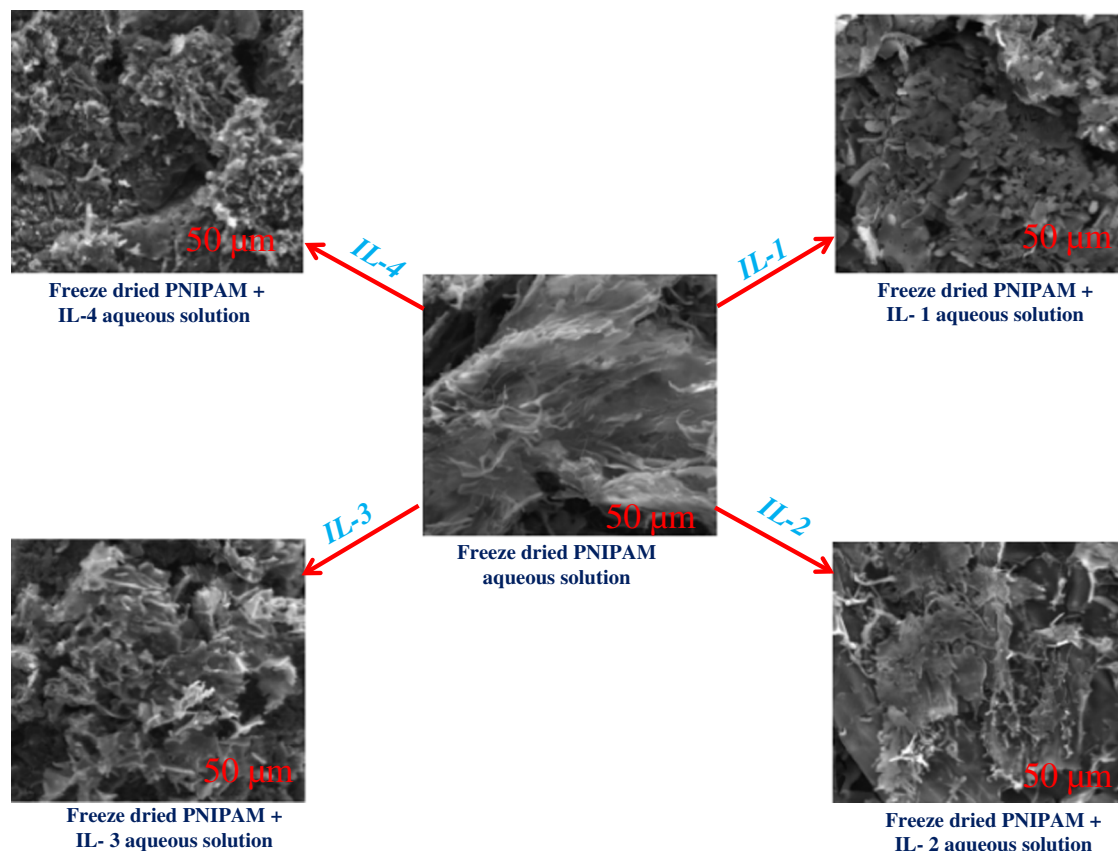
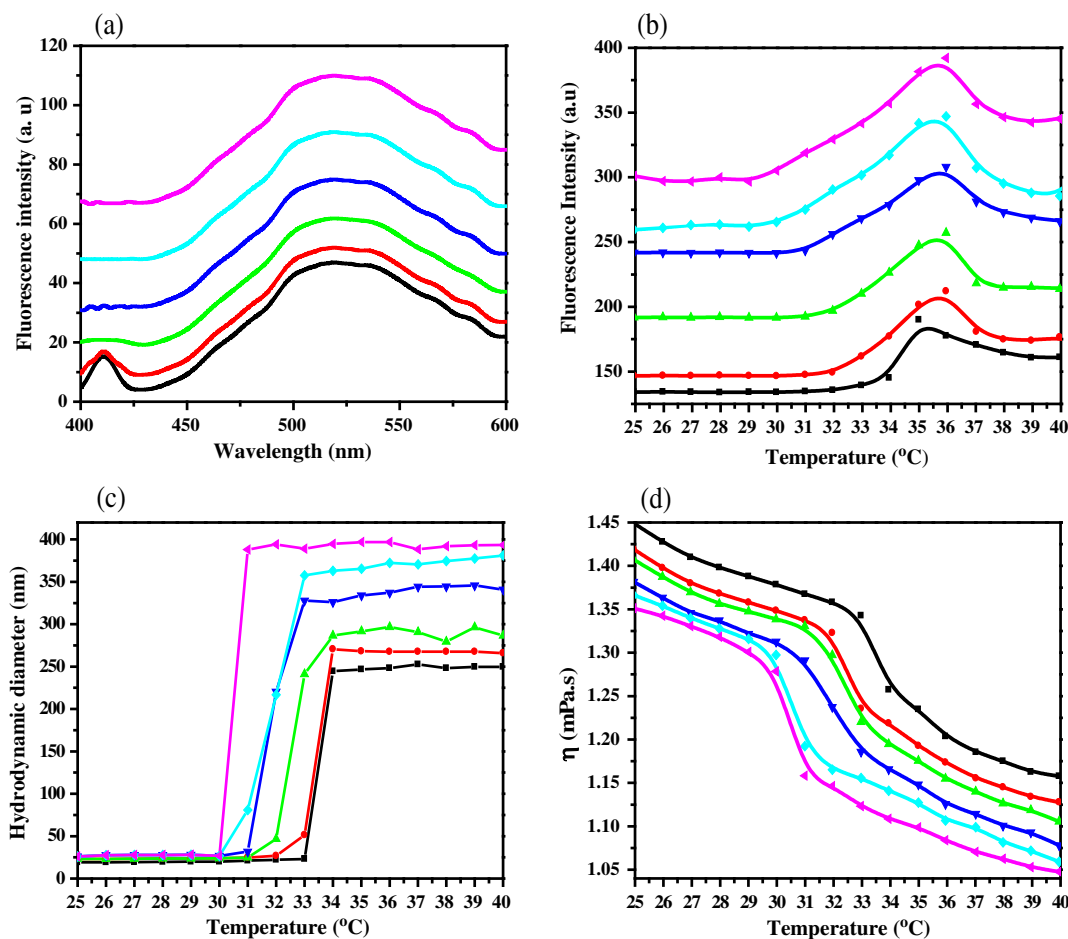


Fig. 5. FESEM micrographs of freeze dried PNIPAM aqueous solution in the absence and presence of different ionic liquids at a concentration of  $5\ \mu\text{L}/\text{mL}$ .



**Fig. 6.** The influence of IL-1, 0  $\mu\text{L}/\text{mL}$  (black line), 5  $\mu\text{L}/\text{mL}$  (red line), 10  $\mu\text{L}/\text{mL}$  (green line), 15  $\mu\text{L}/\text{mL}$  (blue line), 20  $\mu\text{L}/\text{mL}$  (cyan line) and 25  $\mu\text{L}/\text{mL}$  (pink line) on PNIPAM aqueous solution. (a) steady state fluorescence spectroscopy, (b) temperature dependent fluorescence spectroscopy, (c) DLS and (d) viscosity measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compared individually. In PNIPAM-IL aqueous solution, the cations of the IL interact with water molecules to form a symmetric complex via hydrogen bonding. At this condition, there is molecular bonding interaction between ions of the IL because the cations of the IL preferentially interact with water molecule to form hydrogen bonding. The probable molecular interaction between the polymer and ILs at the phase transition state has been schematically shown in Scheme 1.

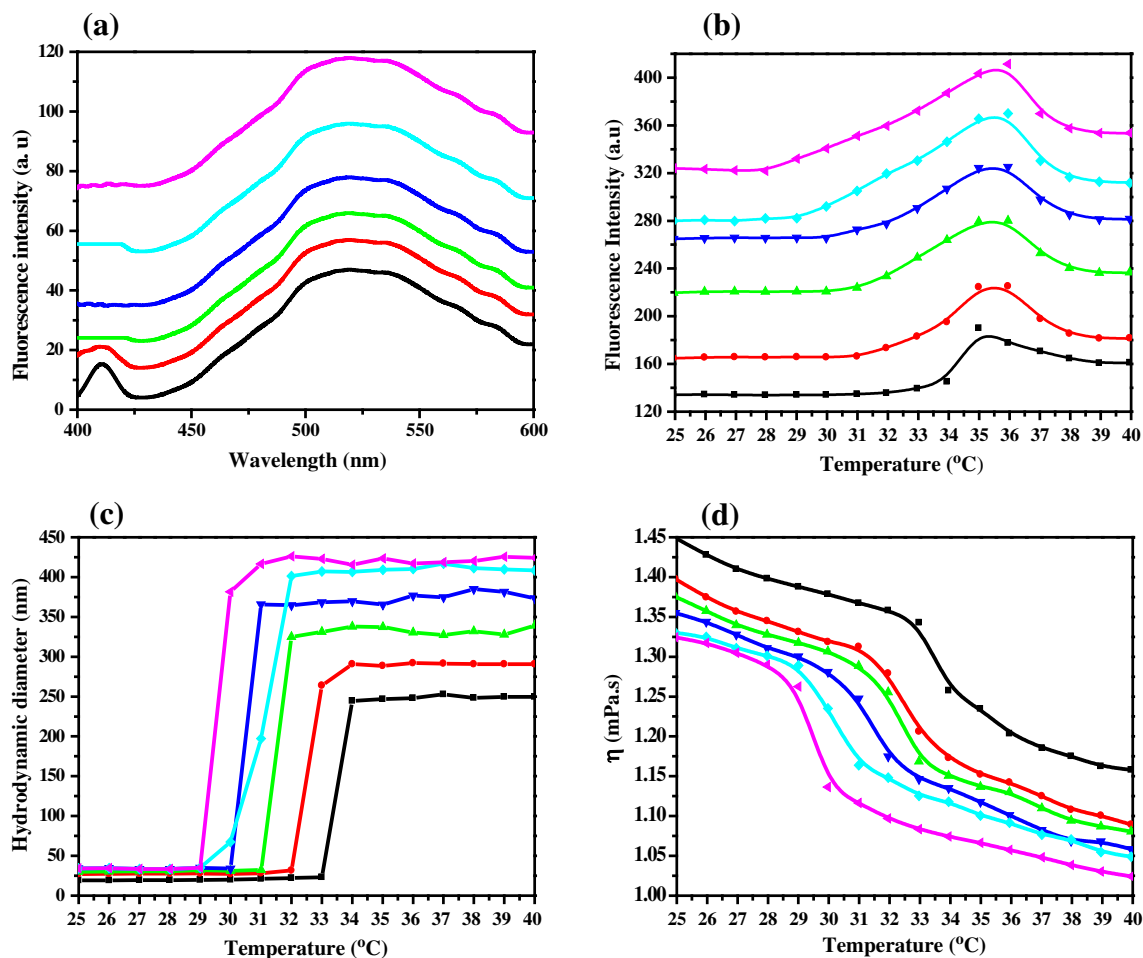
More detailed information relating to the aggregation behaviour of the polymer-IL can be obtained from the DLS measurements through the hydrodynamic diameter ( $d_H$ ) changes during the phase transition of the polymer. The  $d_H$  values of the samples with different ammonium-based ILs were carried out in the temperature range 25–40  $^{\circ}\text{C}$ , and the results are illustrated in Fig. 3. All the polymer-IL solutions exhibit a clear phase transition range between 30.8 and 32.4  $^{\circ}\text{C}$ . The PNIPAM sample without IL shows a phase transition temperature (LCST) at 33.0  $^{\circ}\text{C}$  during heating. Furthermore, with the addition of ILs to PNIPAM aqueous solution, the LCST values decrease gradually towards lower temperatures from 33.0 (without IL) to 32.4, 31.8, 31.0 and 30.8  $^{\circ}\text{C}$  in the presence of IL-1, IL-2, IL-3 and IL-4, respectively. As shown in Fig. 3, IL-4 shows maximum ability to induce the hydrophobic collapse of PNIPAM, whereas IL-1 shows minimum ability to induce the hydrophobic collapse of PNIPAM. The LCST values of PNIPAM solution systematically decrease as the IL concentration increases (Figs. S9, S10, S11 and S12) which indicate the strong interactions are occurred between ions of IL and polymer segments.

In the absence of IL, the size of the PNIPAM in aqueous solution at its LCST is 63 nm, furthermore, with the addition of 5  $\mu\text{L}/\text{mL}$  ILs the

evolution of  $d_H$  has been raised to 66, 74, 81 and 101 nm in the presence of IL-1, IL-2, IL-3 and IL-4, respectively. Later, after approaching the LCST, the evolution in  $d_H$  has been raised enormously from 242 nm in PNIPAM aqueous solution to 271, 291, 317 and 365 nm with the addition IL-1, IL-2, IL-3 and IL-4, respectively. Apparently, the PNIPAM chain becomes longer size at the LCST, due to more hydrophobicity increased by the IL, ultimately water molecules are being squeezed out away from the PNIPAM and we obtained collapsed monomers of polymer aggregate state. Later, there is no significant change in the  $d_H$  values of aggregates above the LCST values of each IL systems (Fig. 3).

The shrinkage of the polymer chain at higher size of IL-PNIPAM is generally seen in Fig. 3. On the other hand, the sudden increase in  $d_H$  around the phase transition point indicates, highly aggregation of cations and the hydrophobic interaction of polymer chains and ILs. Increase of  $d_H$  is accompanied by an increase in the intensity of the scattered light that implies the aggregation of PNIPAM chains solution. As the aggregates become bigger, the solution changes visually turbid in ILs. The LCST values obtained from steady state fluorescence, thermal fluorescence and DLS measurements are consistent with each other.

Furthermore, to explore the phenomena of polymer – IL aggregates, we measured  $\eta$  values for PNIPAM in the absence and presence of ammonium-based ILs. Fig. 4 displays the  $\eta$  values of aqueous PNIPAM solution and in the presence of different types of ILs at the concentration of 5  $\mu\text{L}/\text{mL}$  as function of temperature. From Fig. 4, the  $\eta$  value is 1.43 mPa.s for PNIPAM in aqueous solution below the polymer's LCST in which the solution has a larger  $\eta$  value due to the hydrated coil conformation. Later, the  $\eta$  values decrease with increasing temperature



**Fig. 7.** The influence of IL-2, 0  $\mu\text{L}/\text{mL}$  (black line), 5  $\mu\text{L}/\text{mL}$  (red line), 10  $\mu\text{L}/\text{mL}$  (green line), 15  $\mu\text{L}/\text{mL}$  (blue line), 20  $\mu\text{L}/\text{mL}$  (cyan line) and 25  $\mu\text{L}/\text{mL}$  (pink line) on PNIPAM aqueous solution. (a) steady state fluorescence spectroscopy, (b) temperature dependent fluorescence spectroscopy, (c) DLS and (d) viscosity measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

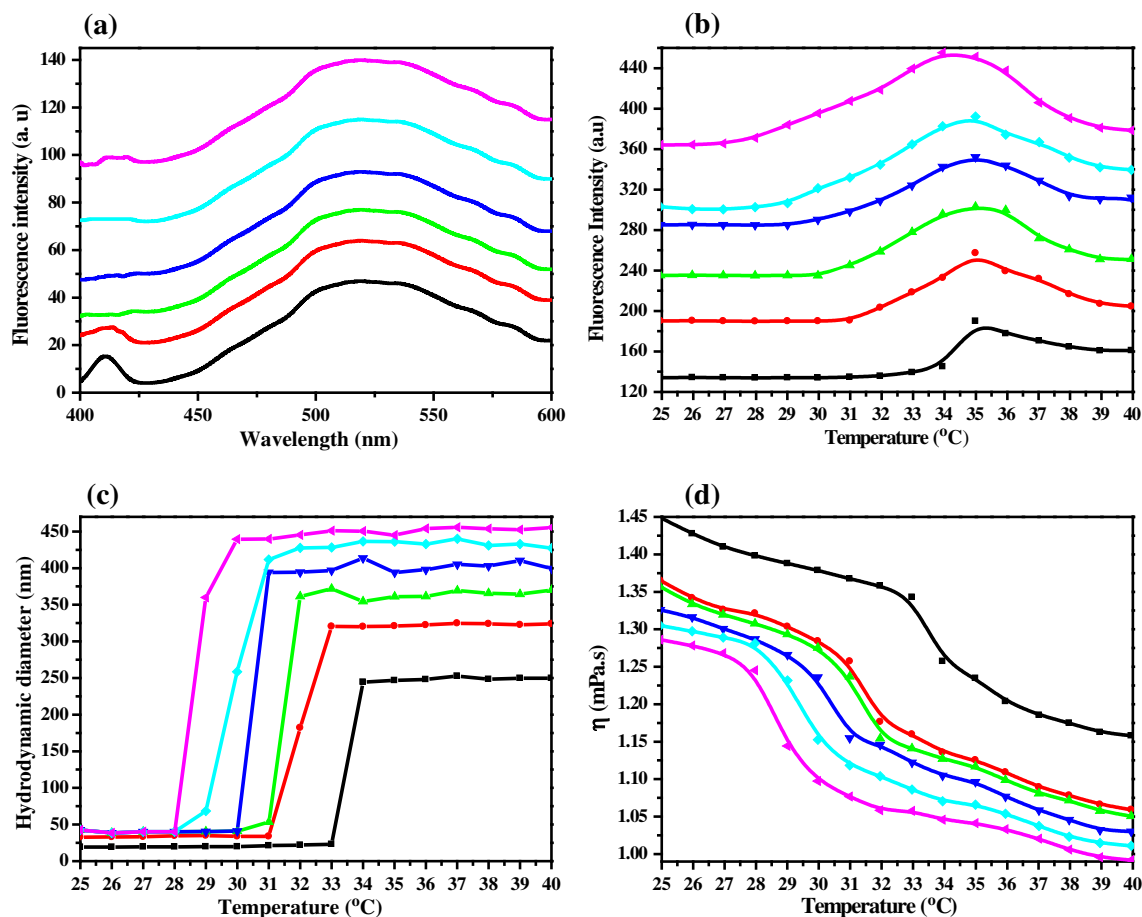
which leads to the hydrophobic isopropyl group drive to collapse the chain to minimise contact with the aqueous phase, ultimately, we obtained the dehydration of the polymer. Apparently, above its LCST, the polymer becomes dehydrated and it would collapse and takes to the compact globule structure eventually, the solution has a lower  $\eta$ .

As can be seen in Fig. 4, the  $\eta$  values gradually decrease from 1.43 mPa·s (PNIPAM aqueous solution) to 1.41, 1.38, 1.36 and 1.33 mPa·s in the presence of IL-1, IL-2, IL-3 and IL-4, respectively. Indeed, the addition of the IL significantly affected the LCST values of PNIPAM solution. This may be due to the stronger interactions of monomer-IL than that of monomer-water. We observed a noticeable change in decreasing the  $\eta$  values towards lower temperature with the addition of ILs. In addition, we have observed a similar trend in decreasing the LCST values towards lower temperatures with increasing the concentration of ILs (10, 15, 20 & 25  $\mu\text{L}/\text{mL}$ ) as shown in the Figs. S13, S14, S15 and S16 in ESI. This can be described that ions of IL are getting associated with PNIPAM with increasing the concentration of IL, eventually, PNIPAM-IL interaction are progressively replaced by the interactions of PNIPAM-water, which indicates hydrophobicity increased by the IL.

To explore the effect of the ammonium-based IL on the phase transition of the PNIPAM aqueous solution, furthermore, FESEM technique was carried out at room temperature. FESEM can provide consequent information to the methods discussed above, regarding the morphological behaviour of PNIPAM in the presence of ILs. The obtained FESEM micrographs of pure PNIPAM aqueous solution and PNIPAM in the presence of ILs were measured at 50  $\mu\text{m}$  and the images are portrayed in Fig. 5. As can be seen in Fig. 5, well-defined hydrated structure of

PNIPAM aqueous solution, which indicates, less affinity of hydrophobic environment. However, upon the addition of small amount (5  $\mu\text{L}/\text{mL}$ ) of IL to the PNIPAM aqueous solution, the hydrogen bonds between PNIPAM and water molecules are ruptured by the ions of IL and a clear change is observed in the non-hydrated structure of PNIPAM in the presence of IL-1, IL-2, IL-3 and IL-4. This indicates that the morphology of PNIPAM structure is changing to dehydrated state with the addition of ILs. Further, with increasing the concentration of ILs (10, 15, 20 & 25  $\mu\text{L}/\text{mL}$ ), the molecular interactions between the PNIPAM and ions of IL become entangled undergoes hydrophobic collapse and accounts for the molecular interactions and ultimately leads to the nano-scale aggregation of PNIPAM monomers as shown in Figs. S17, S18, S19 and S20 in ESI. A large amount of aggregates are formed and connect with each other to form huge and narrow structures. This deviation in the associating structures is due to the formation of dehydrated state of polymer chains by the addition of IL and eventually we observed that the polymer is in the collapsed state. This observation is consistent with the DLS results discussed in the earlier section. The results reveal that strong charge shielding effect between the polymer and IL associating structures. The associating structures became larger (denser), and block-like aggregates are formed because of the strengthened intermolecular associations among hydrophobic groups, thereby, the polymer undergoes hydrophobic collapse and ultimately contributes to the nano-scale range aggregation of PNIPAM molecules.

Finally, for the sake of clarity and presentation of the role of the concentration of individual IL on hydrophobic collapse of PNIPAM, we made an attempt to plot all of the steady state fluorescence spectroscopy,



**Fig. 8.** The influence of IL-3, 0  $\mu\text{L}/\text{mL}$  (black line), 5  $\mu\text{L}/\text{mL}$  (red line), 10  $\mu\text{L}/\text{mL}$  (green line), 15  $\mu\text{L}/\text{mL}$  (blue line), 20  $\mu\text{L}/\text{mL}$  (cyan line) and 25  $\mu\text{L}/\text{mL}$  (pink line) on PNIPAM aqueous solution. (a) steady state fluorescence spectroscopy, (b) temperature dependent fluorescence spectroscopy, (c) DLS and (d) viscosity measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

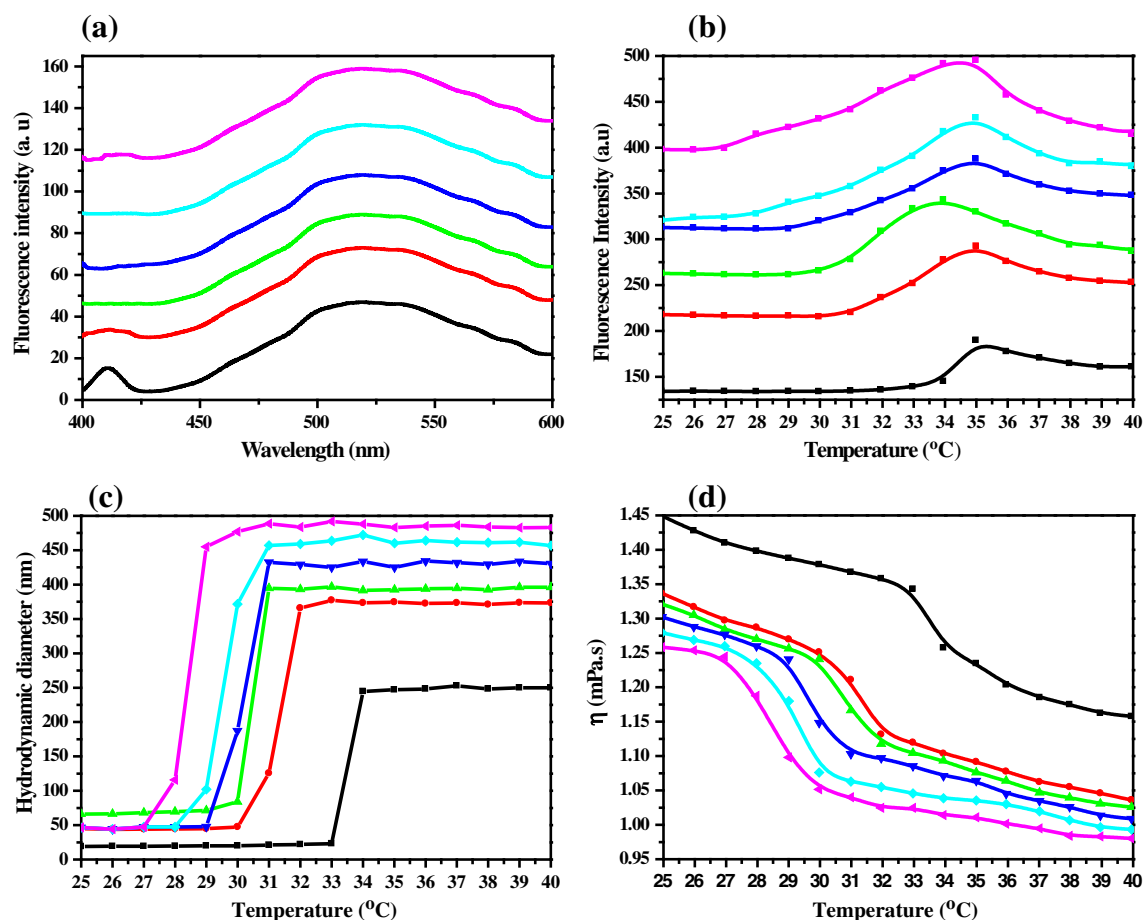
thermal fluorescence spectroscopy, dynamic light scattering and viscosity experimental results in Figs 6–9 as a function of IL concentration. Noticeable qualitative differences have been arisen when they are compared with the individual IL. As can be seen in the Figs 6–9, there is a linear modulation in phase transition of PNIPAM have been observed with the increase in the concentrations of IL from 5 to 25  $\mu\text{L}/\text{mL}$ . The fluorescence intensity of PNIPAM in all studied ILs is increased with increasing the concentration of ILs (Figs 6–9 (a) and (b)). The LCST values of PNIPAM in ILs further decrease with increasing the concentration of ILs. The evolution of  $d_H$  and decrease in LCST of the PNIPAM is more pronounced with increasing ILs concentration as shown in Figs 6–9 (c). Based on our studies, we concluded that the IL-4 is strongest inducer for hydrophobic collapse of PNIPAM whereas IL-1 is weakest inducer for hydrophobic collapse of PNIPAM. From these results, one can clearly understand that concentration and type of cation of IL plays a substantial role in promoting the PNIPAM towards dehydrated state. With the addition of IL to PNIPAM aqueous solution, the ions of the ILs destroy the hydrogen bond network between polymer and water molecules during heating process and the ions of the ILs also form symmetric complex via hydrogen bonding. These interactions were observed because of considerable change in the PNIPAM chain conformation around the phase transition state and gradual dehydration process in the aggregation of the polymer.

### 3.1. Molecular mechanism of the alkylammonium-based ILs effect on the phase behaviour of PNIPAM aqueous solution

Finally, to authenticate the experimental results and LCST values between PNIPAM and alkylammonium-based ILs that were drawn from

various techniques such as steady state fluorescence spectroscopy, thermal fluorescence spectroscopy, DLS,  $\eta$  and FESEM were graphically represented in Fig. 10 as a function of the concentration of ILs. The results from various biophysical techniques have potentially proved that the LCST of PNIPAM in aqueous solution gradually decreased with increasing the concentration of ILs. The reason behind this decrease in LCST values would be the various structural alterations and molecular mechanism brought by these ILs at different concentration ranges. However, the extent of decrease in LCST of PNIPAM varies from cation to cation depending upon its water structure making and breaking tendency. By taking all the present results into account, the order of the diminishment of LCST of PNIPAM in aqueous solution towards lower temperatures of the investigated cations is IL-4 < IL-3 < IL-2 < IL-1. This behaviour suggests that the variation in the carbon chain length of cation of IL is having intense influence on the LCST of PNIPAM in aqueous solution.

The phase transition trend of PNIPAM in ILs showed that IL induced transition in the structure of PNIPAM do not occur simultaneously and therefore, suggests that ILs have slowly led to aggregation changes in PNIPAM depending on the structural and molecular attributes with increasing the temperature as shown in Scheme 1. Furthermore, the ion-ion pair interactions of ILs played a major role in the hydrophobic collapse of PNIPAM chains. Presumably, when PNIPAM in IL solutions are warmed above the phase transition temperature, intrachain contraction may be occurred between the monomers of the polymer, which leading to collapse of individual chains from water-swollen coils to compact globule structures [1]. On the other hand, interchain association with IL also occurred between polymer-IL, which indicates aggregation of the polymer.



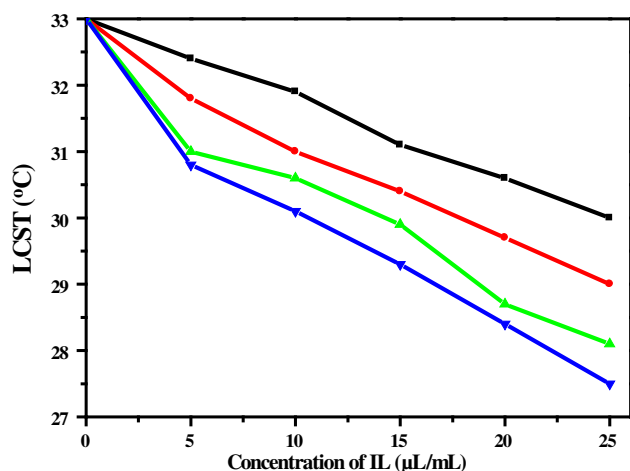
**Fig. 9.** The influence of IL-4, 0  $\mu\text{L/mL}$  (black line), 5  $\mu\text{L/mL}$  (red line), 10  $\mu\text{L/mL}$  (green line), 15  $\mu\text{L/mL}$  (blue line), 20  $\mu\text{L/mL}$  (cyan line) and 25  $\mu\text{L/mL}$  (pink line) on PNIPAM aqueous solution. (a) steady state fluorescence spectroscopy, (b) temperature dependent fluorescence spectroscopy, (c) DLS and (d) viscosity measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Therefore, the effect of these ILs on PNIPAM in IL solutions has been systemized by the nature of the molecular and structural interaction and on the alkyl chain length and the concentration of the ILs. The model for envisaging the phase behaviour of polymer and IL induced aggregates in water can be understood from ion-ion pair interactions to account for the conformational changes. Further, based on the FESEM

surface morphologies, the interaction of alkylammonium cation with polymer hydrophobic group is maximum for IL-4 and minimum for IL-1 among all the studied ILs, confirming the fact the dehydrating tendency of the polymer increased with the variation in alkyl chain length of the cation.

#### 4. Conclusions

In this context, to extend the knowledge about aggregation of polymer in the presence of ILs, in the present work we have explored the phase transition behaviour of PNIPAM in the presence of ILs containing various cations and the same anion. Further, an attempt was made to rank the cations of these ILs series according to their ability to solubilize polymer in water. In this study we combined the methods of fluorescence spectroscopy, DLS and  $\eta$  measurements as a function of temperature as well as FESEM at room temperature to explore the role of ammonium-based ILs on the phase transition temperature of PNIPAM aqueous solution. Apparently, the LCST of the PNIPAM is affected by the size of the cation of IL as well as the concentration of IL. Moreover, the maximum and minimum shifts in the diminishment in LCST were observed in the presence of IL-4 and IL-1, respectively. The decrease in the LCST towards lower temperatures is due to strong hydrogen bond formation between the polar amide units in the macromolecule and ions of IL. As the  $d_H$  values for PNIPAM-ILs solution approach higher on warming, obviously, the solvent (water) quality decreases and ultimately the polymer chain shrink towards hydrophobic moieties. The morphologies explicitly elucidate that the hydrated polymer state becomes dehydrated and a denser polymer-IL associated structures are



**Fig. 10.** LCST values of PNIPAM aqueous solution as a function of cation and concentration of IL (IL-1) (black line), (IL-2) (red line), (IL-3) (green line), and (IL-4) (blue line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observed after addition of the IL. As expected a more dramatic decrease in LCST with increasing the concentration of ILs occurs around the phase transition temperature because chain collapse results in a more compact particle. The knowledge of how the phase transition depends on the nature, size and composition can be useful in the design of new responsive materials and possible application of these studies in drug-delivery systems. Presumably, the thermo-responsive polymer studies with ILs may lead to the materials with improved compatibility required in the pharmaceutical and biomedical fields.

## Acknowledgements

We acknowledge the financial support from the Department of Science and Technology (DST), New Delhi, India (Grant No. SB/SI/PC-109/2012). We are grateful to the Department of Physics, University of Delhi, Delhi for providing FESEM.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.molliq.2016.11.060>.

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