Unravelling the interactions between biomedical thermoresponsive polymer and biocompatible ionic liquids

Reddicherla Umapathi [a], Imran Khan [a,b,c], João A.P. Coutinho [b,*], Pannuru Venkatesu [a,*]

[a] *Department of Chemistry, University of Delhi, Delhi 110 007, India*
[b] *CICECO - Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal*
[c] *Department of Chemistry, College of Science, Sultan Qaboos University, PO Box 36, PC 123 Muscat, Oman*

ABSTRACT

Studies on the phase behavior of thermoresponsive polymers (TRPs) in the presence of ionic liquids (ILs) are an emerging area for the preparation and design of new polymeric materials. The search to understand the influence of ILs on polymers has come into the limelight as a great challenge. Hitherto, limited work on the phase transition behavior of TRPs in the presence of ILs is available. In this work, we studied the phase behavior of poly-N-isopropylacrylamide (PNIPAM) in presence of cholinium chloride ([Ch][Cl]), cholinium acetate ([Ch][Ac]), cholinium bitartrate ([Ch][Bit]) and cholinium dihydrogen citrate ([Ch][DHCit]) using various techniques such as UV–Visible absorption spectroscopy, steady-state fluorescence spectroscopy, thermal fluorescence spectroscopy, viscosity (η) and dynamic light scattering (DLS). All cholinium-based ILs studied show qualitatively and quantitatively a similar phase behavior, suggesting it to be quite resilient with respect to changes in the anion of the ILs. However, concentration and orientation of ILs have a varied effect on the phase transition temperature and on the aggregation behavior of PNIPAM. Our temperature dependent experimental results explicitly signifies that lower critical solution temperature (LCST) values decrease with increasing the temperature and concentration of studied ILs, which indicates that hydrophobic interactions are dominating. Anions of IL with their charge densities, hydration capacities and hydration energies leads to the hydrophobicity of PNIPAM + IL aqueous solution. High polarity, owing to the charge of the carboxylate groups in [Ch][DHCit] and [Ch][Bit], and hydrogen bond acceptor capability of the Cl⁻ anion causes their affinity for water inducing ability. This is the first report on the influence of cholinium-based ILs on the phase behavior of the PNIPAM. The current research work provides significant information on the phase transition and aggregation behavior of TRPs in ILs, which paves the way for potential applications in various fields.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Poly-N-isopropylacrylamide (PNIPAM) is a well-known and widely studied branched water-soluble thermoresponsive polymer (TRP), with a hydrophobic carbon backbone chain (isopropyl group) and a hydrophilic amide group along the side chain in a single monomer unit [1–5]. Being a TRP, temperature plays a significant role in its solution phase behavior. An increase in temperature decreases its aqueous solubility and results in phase separation above the lower critical solution temperature (LCST). Below the LCST, the hydrogen bonding between the amide group of PNIPAM and water molecules is the key factor which is responsible for PNIPAM coiled state. However, above its LCST, PNIPAM becomes globular due to weak hydrogen bonding, since the kinetic energy of the molecule becomes larger than the energy of

hydrogen bonding between water and the PNIPAM molecules [1–6]. Thus, the hydrophobicity dominates between the isopropyl groups and hydrophobic backbone which in turn causes the PNIPAM transition by collapse from the coil to globule on increasing temperature above the cloud point (phase transition temperature) when dissolved in aqueous solution. Thus, a variation of LCST depends on the interaction behavior of polymer-water and polymer-polymer. Structural factors which increase the polymer-water interactions increase LCST whereas an increase in polymer-polymer interactions leads to a decrease in LCST. Therefore, the interplay of interactions between polymer and water opens a large window of tuning the LCST for various applications [1–11].

Thermoresponsive polymers (TRPs) are being extensively studied in regard to their phase transition behavior [1–11]. Among all the TRPs, PNIPAM, poly(N-vinylcaprolactam) (PVCL), Poly(methyl vinyl ether) (PVME) and Poly(N-vinylpyrrolidone) (PVP) have been widely studied for various purposes in regard to their phase transition behavior which is near to physiological human body temperature. Recently TRPs have drawn great deal of attention in various science and technology

platforms. The extraordinary mechanical, chemical, biophysical, unique optical, and distinctive solution properties of TRPs have made all of these above-mentioned applications possible [1–11].

Ionic liquids (ILs) are attractive alternatives to conventional volatile organic solvents because of their unique physicochemical properties such as low melting point, high thermal and chemical stability, low vapor pressure, non-volatility and non-flammability have a wide electrochemical window and good solvation properties [8,12–15]. Because of these features, ILs are often seen as green solvents. All these characteristics provide unique combinations of properties that enable to design processes with enhanced efficiency or products with better performance. The anions of the ILs have shown a dominating role in tuning the physical properties as well as phase behavior and solubilization of polymers. The understanding of the solubilization behavior and the interaction mechanism can lead to the design of ILs able to solubilize biopolymers and to make novel drug delivery vehicles [8,12–15].

The fundamental understanding of the TRPs self-assembly is of significant importance to the development of drug delivery systems, and for phase transfer of reaction products and reagents. ILs can effectively replace conventional surfactants in protein folding and drug delivery. Several studies have been devoted to illuminating the influence of various additives on the phase transition temperature of TRPs [2,16]. However, studies on the impact of ILs on the phase behavior of TRPs is little explored [4,6,17–19]. The presence of these additives can modulate the solution properties and make it perform better under certain circumstances. One of the issues that are presently attracting attention is the role of biocompatible ILs as co-solvents on solution properties of TRPs. The impact of ILs on the phase behavior of polymers offers fascinating new possibilities for designing new polymeric materials and tuning their behavior [8].

In previous works the influence of imidazolium family ILs on the LCST of TRPs has been explored [4,17,19]. In the last few years cholinium-based ILs have attracted the attention as versatile additives, innovative solvents with enhanced environmental safety, and as stabilizers for proteins. Santos et al. [20] studied a series of cholinium-based ILs aimed at evaluating the influence of the functionalization of the anion and the cation on their ecotoxicity. The effects were evaluated on biological models demonstrating the low toxicity of these family of ILs. Bisht et al. [21] observed improved stability and significantly enhanced activity of cytochrome c against multiple stresses in the presence of cholinium-based ILs. In another study by Bisht et al. [22] found that a series of cholinium-based ILs stabilized the α-chymotrypsin structure against thermal denaturation. Most cholinium-based ILs stabilized proteins and showed good biocompatibility [23].

PNIPAM a widely used and well know biomedical thermoresponsive polymer is a chemical isomer of polyleucine, it is often used as a simple model of proteins owing to the presence of amide groups in the side chains, instead of the backbone. Thermodynamically the coil for collapse transition of PNIPAM with increasing temperature corresponds to the transition from the unfolded protein structure to the folded protein structure, namely a model of the inverse process of cold denaturation across the cold renaturation temperature [2,16,24–26]. As mentioned PNIPAM has been used as a biomedical thermoresponsive polymer, and is also used as a model for protein studies. However, studies on the effect of cholinium-based ILs on the phase behavior of PNIPAM are completely absent in the open literature.

It is therefore desirable to understand the effect of cholinium-based ILs on the phase behavior of PNIPAM. In the current work it is done a comprehensive examination of the influence of various ILs such as cholinium chloride ([Ch] Cl; $C_5H_{14}ClNO$), cholinium acetate ([Ch][Ac]; $C_7H_{17}NO_3$), cholinium bitartrate ([Ch][Bit]; $C_9H_{19}NO_7$), cholinium dihydrogen citrate ([Ch][DHCit]; $C_{11}H_{21}NO_8$) on the LCST behavior of PNIPAM in aqueous solution. The chemical structures of ILs used in the present study were schematically illustrated in Fig. 1. These investigations have been carried out using a set of state of the art bio physicochemical techniques, viz., UV–visible absorption spectroscopy, steady-

state fluorescence spectroscopy, thermal fluorescence spectroscopy, viscosity (η) and dynamic light scattering (DLS). The results obtained are presented and discussed below.

2. Experimental section

2.1. Materials

8-Anilinonaphthalene-1-sulfonic acid (ANS), PNIPAM (Mn = 20,000–25,000), cholinium chloride (purity >98%) ([Ch]Cl), cholinium acetate (purity≥95%) ([Ch][Ac]), cholinium bitartrate (purity >98%) ([Ch][Bit]) and cholinium dihydrogen citrate (purity >98%) ([Ch][DHCit]) were procured from Sigma-Aldrich and used as received. Nano pure water with a resistivity of 18.3 MΩ cm was used for the preparation of polymer and IL solutions.

2.2. Sample preparation

A Mettler Toledo weighing balance with a precision of ±0.0001 g, was used for the preparation of sample solutions. The required amount of PNIPAM and ANS was weighed for stock solutions. Aliquots of these solutions were mixed to prepare the sample solutions at a desired concentration of polymer. The final copolymer concentration for all measurements was 6 mg/mL. The weighed amounts of ILs at various concentrations (5, 10 and 15 mg/mL) were added directly to the aqueous polymer solution. 2×10^{-5} M concentration of ANS probe was used for the UV–Visible absorption spectroscopy and fluorescence spectroscopy measurements. UV–Visible absorption spectroscopy and steady-state fluorescence spectroscopy measurements were performed at 25 °C. Temperature dependent fluorescence spectroscopy, dynamic light scattering, and viscosity (η) measurements were performed as a function of temperature (25–40 °C). All resulting sample solutions were equilibrated at room temperature prior to the measurements. The polymer samples were stored in a cool place and kept the container tightly to prevent water absorption. Before performing the measurements, all the sample solutions were filtered with 0.45 μm disposable filters (Millipore, Millex-GS) through a medical syringe. For temperature dependent analysis, the polymer + IL aqueous solutions were equilibrated for at least 10–15 min at each temperature before measurement to attain equilibrium. The polymer + ionic liquid aqueous sample solutions were measured 3 times and the average of the measurements was taken. The variations in LCST of polymer + ionic liquid aqueous solutions were ± 0.3 °C.

3. Instrumentation and measurements

UV–Visible absorption spectroscopy measurements were performed with UV-1800, Shimadzu Co., Japan. Cary Eclipse fluorescence spectrophotometer (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia) with an intense Xenon flash lamp as the light source was used for fluorescence spectroscopy measurements. Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK), equipped with He-Ne (4 mW, 632.8 nm) was used for particle size measurements. Sine-wave vibro viscometer (model SV-10, A&D Company Limited, Japan) with an uncertainty of 1% was used for measuring viscosities. The comprehensive information on experimental methods used in the present study has been exemplified in the supporting information.

4. Results and discussion

4.1. Influence of ILs on the phase behavior of PNIPAM in an aqueous solution by UV–visible absorption spectroscopy measurements

The UV–vis absorption spectra are quite sensitive to the variations in absorption and emission bands of the molecule's conformation which can identify the tiny structural vibrations of biological macromolecules.
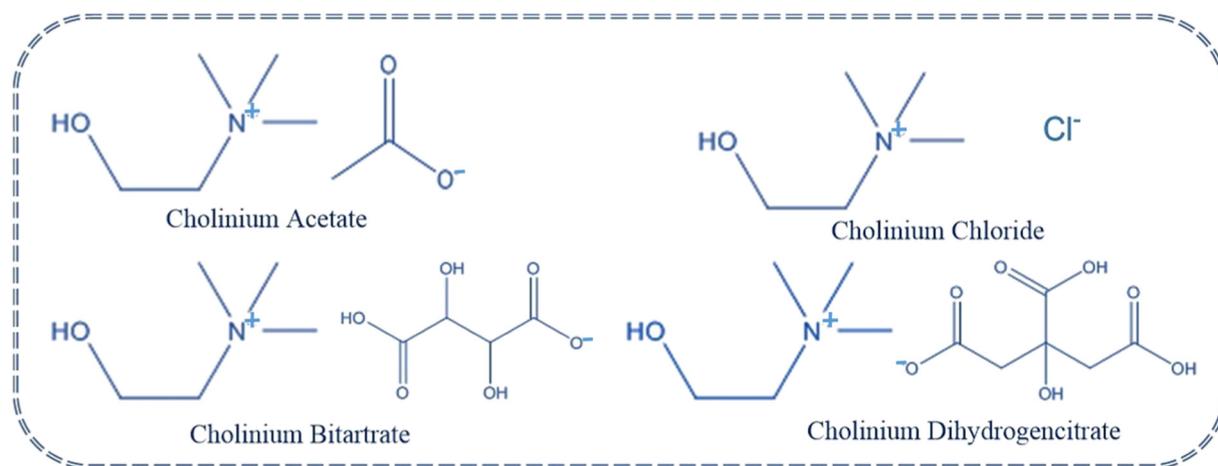
Fig. 1. Chemical structures of the cholinium ionic liquids used in the study.

The change in the conformational states of PNIPAM can be analyzed by the shift in the wavelength in the near UV region [4]. The UV–vis absorption spectrum of 8-Anilinonaphthalene-1-sulfonic acid (ANS) - PNIPAM aqueous solutions in absence and in presence of various cholinium-based ILs was measured at 25 °C and the obtained spectra are displayed in Fig. 2. Changes in the absorption spectra specify changes in the ANS environment subsidized by the variations in conformational states of PNIPAM. ANS in aqueous solution displays two core absorption peaks at $\lambda_{max} \sim 270$ or 370 nm [4]. However, in the present study, we have considered the changes in the peak at 270 nm. The absorption spectra were primarily caused by the electronic excitation of the ANS microenvironment. The absorption spectra of ANS microenvironment varies with the variation in the type and condition of the solution. The absorption peak at 270 nm is caused by the transition affinity of hydrophobic ANS.

Fig. 2. clearly shows that the absorption of ANS-PNIPAM aqueous solution in the absence of ILs is minimum (i.e. 0.24). Further with the addition of 5 mg/mL of ILs the absorbance has been increased to 0.28, 0.30, 0.31 and 0.33 in the presence of [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit], respectively. This minimal absorption signifies the well-hydrated coil conformation of polymer presenting less affinity for hydrophobic probes such as ANS. Absorption spectra of PNIPAM varied with the addition of 5 mg/mL of [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit] ILs,
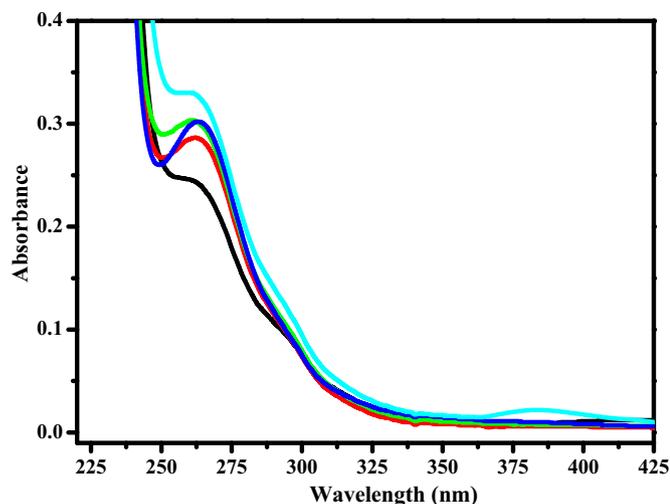
supplemented by a bathochromic shift in the absorption maxima wavelength, which signifies the hydrophobic collapse of the polymer. Noticeable changes have not been observed with the further addition of ILs at higher concentrations. The minimum and maximum degree of capability to perturb the hydration layer around the PNIPAM molecule was observed in the presence of [Ch][Ac] and [Ch][Bit], respectively. The increase in the ultraviolet absorption spectral intensity and shift in the absorption maximum may be attributed to the perturbation of the ANS-PNIPAM aqueous solution microenvironment in the presence of ILs, which further leads to the variations in structural and conformational states of PNIPAM.

4.2. Influence of ILs on the phase behavior of PNIPAM in an aqueous solution by fluorescence spectroscopic measurements

To understand the impact of ILs on the phase transition behavior of PNIPAM, we also employed steady-state fluorescence spectroscopy measurements, which is a significant tool to probe the solvation and aggregation properties of TRPs in aqueous media [4,27,28]. For these measurements we have used ANS as an extrinsic fluorescent probe in very low concentrations to assess how the PNIPAM microenvironment changes with the addition of 5 mg/mL of [Ch]cl, [Ch][Ac], [Ch][Bit] and [Ch][DHCit] ILs. ANS exhibits a characteristic peak in the wavelength range of around 510 nm, which is sensitive to variations in the microenvironment [4,27]. The steady-state emission spectra of ANS-PNIPAM aqueous solutions in the presence of various cholinium-based ILs at room temperature are measured and the spectra obtained are displayed in Fig. 3(a). From these results, it is evident that ANS-PNIPAM aqueous solution displays a relatively low intensity in the whole wavelength range signifying the absence of hydrophobic environment for ANS molecules. Further the ANS fluorescence emission intensity keeps increasing with the addition of [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit]. This increase in fluorescence emission intensity is an indication of the significant role played by the ILs, inducing nanoscale aggregation and the collapse of the hydrophobic environment for PNIPAM in an aqueous medium. Among the various ILs [Ch][Ac] has shown minimum significant enhancement in fluorescence emission intensity, whereas [Ch][Bit] has shown maximum significant enhancement in fluorescence emission intensity.

The fluorescence emission intensity was further increased to greater extents with the addition of 10 and 15 mg/mL of ILs as presented in Fig. S1(a and b). This increase in intensity is due to the increasing hydrophobic collapse of the polymer at the higher concentrations of ILs. The changes in intensities with the addition of cholinium-based ILs were expected to result from the interactions between the IL and polymer segments as well as water molecules and ions of IL. The order of ILs in
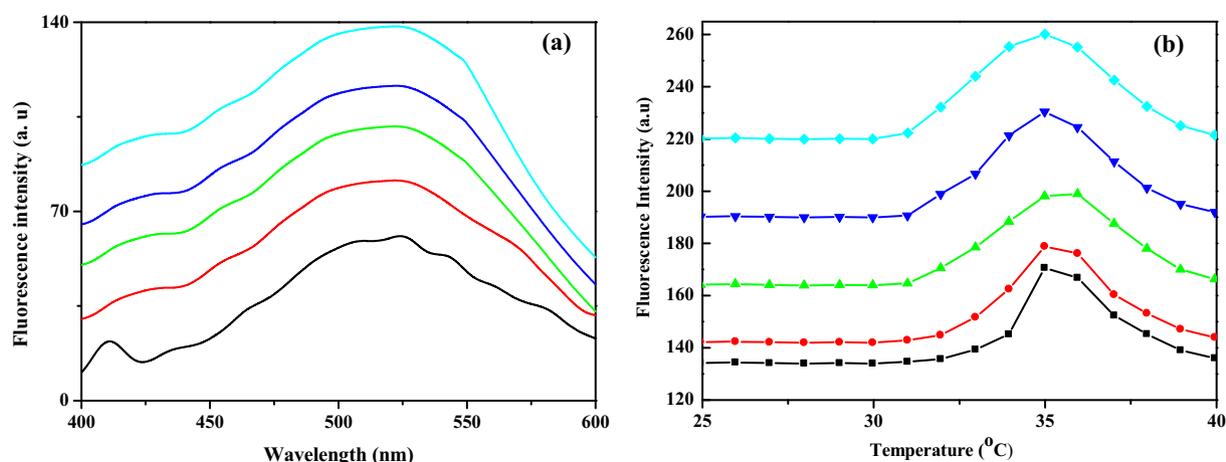

Fig. 2. UV–Visible absorption spectroscopy of ANS in PNIPAM aqueous with and without ILs at 25 °C. ILs: IL free (black line), [Ch][Ac] (red line), [Ch]cl (green line), [Ch][DHCit] (blue line) and [Ch][Bit] (cyan line). Concentration of materials; ANS = 2×10^{-5} M; PNIPAM = 6 mg/mL; ILs = 5 mg/mL.

Fig. 3. (a) Fluorescence emission spectra of ANS in PNIPAM aqueous solution without IL (black line) and in presence of ILs at 25 °C. (b) Temperature-dependent fluorescence emission spectra of ANS in PNIPAM aqueous solution without IL (black line) and in presence of ILs in the temperature range of 25–40 °C; excitation wavelength 360 nm. ILs: IL free (black line), [Ch][Ac] (red line), [Ch]cl (green line), [Ch][DHCit] (blue line) and [Ch][Bit] (cyan line). Concentration of materials; ANS = 2×10^{-5} M; PNIPAM = 6 mg/mL; ILs = 5 mg/mL.

increasing fluorescence emission intensity of PNIPAM aqueous solution is [Ch][Ac] < [Ch]cl < [Ch][DHCit] < [Ch][Bit]. The increase in intensity is pronounced in the ILs with increasing kosmotropicity. A similar type of enhancement in fluorescence emission intensity was observed during the hydrophobic collapse of PNIPAM with the addition of imidazolium-based ILs with fixed cation and different anions [4]. The intensity trend obtained for the studied cholinium-based ILs in aqueous PNIPAM solution agrees with the results reported for poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (PEG– PPG–PEG) [29]. The increase in intensity may be ascribed to the additional dehydration of the polymer leading to the hydrophobic collapse of the polymer. Also, additional dehydration of the polymer may result from a balance between the interactions of IL-polymer, water-polymer, and water-IL as observed for polyethylene glycol (PEG) + IL aqueous solutions [30].

Further temperature dependent fluorescence spectroscopic analysis was carried out to evaluate the influence of cholinium-based ILs on the phase transition behavior of PNIPAM in an aqueous medium, in the presence and in absence of ILs, and the obtained spectra are illustrated in Fig. 3(b). They show that there is no increase in fluorescence intensity until ~33.0 °C, which results from the coiled conformation of the polymer chains. Further increase in temperature (heating) of the PNIPAM aqueous solution leads to a sudden raise in intensity, representing the hydrophobic collapse of polymer chains, which results in a reduction of the mobility of their hydrophobic environment for ANS molecules, eventually leading to an enhancement of the fluorescence intensity. The point at which a sudden increase in intensity observed is determined as the LCST [4,27,28,31]. As there is no significant variation in fluorescence intensity until the phase transition temperature of the polymer, this suggests that the polymer remains in a well-hydrated structure.

Further, addition of 5 mg/mL of IL to the aqueous polymer solution showed a substantial decrease in LCST with [Ch][Ac], ~32.6 ± 0.3 °C; [Ch]cl, ~32.0 ± 0.3 °C; [Ch][DHCit] 31.6 ± 0.3 °C and [Ch][Bit] 31.0 ± 0.3 °C. The variation in LCST with the addition of ILs is driven by the anion as the cation is the same, these results are in agreement with our previous studies on the micellization behavior of triblock copolymer [29]. Further, the sharp increment in fluorescence intensity at the phase transition temperature derives from the coupling of probe molecules onto the surface of the hydrophobic portion of polymer. From these findings, we can predict that the hydrophobic collapse in PNIPAM-ILs solution is induced by the type of anion and thermal effect. This variance in intensity might be due to changes in the performance of ILs on collapsing the hydrated polymer. Further we can assume that these changes might be due to the hydration energies of the studied ILs.

The spectra obtained suggest that although the ILs decrease the LCST of the polymer, their mechanisms for decreasing the phase transition temperature are different. In general, in aqueous IL solution, the water molecules interact more strongly with the anions than with the cations [4,32,33]. Moreover, the maximum and minimum shifts in decreasing the LCST were observed in the presence of [Ch][Bit] and [Ch][Ac], respectively. The addition of the ILs leads to the hydrophobic collapse of the PNIPAM chains and thereby decreases the LCST value. Further, the LCST values of polymer were significantly decreased to lower temperatures with the addition of 10 and 15 mg/mL of ILs as depicted in Fig. S2(a and b). The shift of fluorescence emission intensities towards lower wavelengths with increasing temperature and concentration specifies the onset of interactions between ILs and polymer, which results in more aggregation. Although the ILs belongs to the same family with common cation "choline" with different anions the phase transition behavior of the ILs in the presence of PNIPAM is different which is based on the nature of the anions, their charge densities, hydration capacities and hydration energies. High polarity, owing to the charge of the carboxylate groups in [Ch][DHCit] and [Ch][Bit], and hydrogen bond acceptor capability of the Cl^- anion causes their affinity for water inducing ability.

4.3. Influence of ILs on the phase behavior of PNIPAM in an aqueous solution by dynamic light scattering measurements

To further ascertain our results, dynamic light scattering (DLS) measurements as a function of temperature were performed to assess the hydrodynamic diameter (d_H) of polymer in the presence of cholinium-based ILs. DLS is one of the most powerful methods to acquire detailed information regarding the nano-size distribution of macromolecular assemblies and to determine the size of the particles [2]. The d_H is influenced by the shape of the ions of ILs and chains of the polymer. The temperature dependent d_H values of polymer in the presence and absence of ILs are presented in Fig. 4. Evidently, the d_H value of aqueous polymer solution in the absence of IL was below ~100 nm at lower temperatures (<LCST) and remained comparatively constant up to 33 °C signifying that the PNIPAM chains were solubilized in the water below the phase transition temperature. Further, there is an intense increase in the size of particles above this temperature which can be viewed as the LCST. The obtained d_H values are displayed in Fig. 4. With the further increment in the temperature, a sudden enhancement in d_H was noticed above the LCST.

The d_H values of polymer increased from ~22–25 nm in pure water at their respective LCST to 31.0, 34.1, 44.0 and 46.0 nm in the presence of 5 mg/mL of [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit], respectively (Fig. 4). This indicates that the process of breaking hydrogen bonds
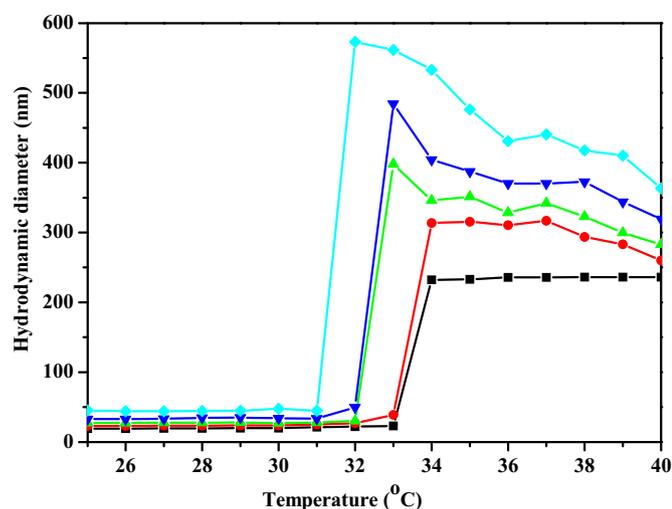
Fig. 4. Hydrodynamic diameter, of PNIPAM aqueous solution without IL (black colour line) and with the presence of ILs in the temperature range of 25–40 °C. ILs: [IL free (black line), Ch][Ac] (red line), [Ch]cl (green line), [Ch][DHCit] (blue line) and [Ch][Bit] (cyan line). Concentration of materials; PNIPAM = 6 mg/mL; ILs = 5 mg/mL.

Fig. 5. Temperature-dependent viscosity of PNIPAM aqueous solution without (black colour line) and with the presence of ILs in the temperature range of 25–40 °C. ILs: IL free (black line), [Ch][Ac] (red line), [Ch]cl (green line), [Ch][DHCit] (blue line) and [Ch][Bit] (cyan line). Concentration of materials; PNIPAM = 6 mg/mL; ILs = 5 mg/mL.

between the water molecules and amide group of the PNIPAM can be enhanced by the anions of IL. The d_H data illustrated that when the solution temperature reaches the LCST of the polymer, the polymer chains aggregate which results in dehydration upon heating. With the addition of 5 mg/mL of IL to aqueous polymer solution, the LCST was decreased to lower temperatures from 33.0 °C (IL free) to ~32.6 ± 0.3 °C [Ch][Ac], ~32.0 ± 0.3 °C [Ch]cl, 31.6 ± 0.3 °C [Ch][DHCit] and 31.0 ± 0.3 °C [Ch][Bit]. These results are in agreement with the previously studied thermal fluorescence spectroscopy analysis. This decrease of LCST results from the preferential interactions of the IL and consequent dehydration of the polymer which affects the size of the aggregates as shown in Fig. 4. Further, the phase transition temperature of polymer decreased further with increasing concentration of ILs (Fig. S3).

4.4. Influence of ILs on the phase behavior of PNIPAM in an aqueous solution by viscosity measurements

In order to appraise the influence of ILs on the phase transition temperature of the polymer in aqueous solution, we have studied the viscosity (η) as a function of temperature in the presence and absence of ILs and the obtained results are shown in Fig. 5. They show that below the phase transition the PNIPAM aqueous solution has higher η values owing to hydrated coil conformation. Above the phase transition temperature, the PNIPAM becomes dehydrated and it collapses into a compact globular form; as a result, the solution has a lower η. From Fig. 5 it is evident that IL free PNIPAM is showing 1.45 mPa·s η value at 25 °C, which is in good agreement with the existing literature η value of 1.43 mPa·s [4]. However the η values gradually decreases from 1.45 in the presence of PNIPAM aqueous solution to 1.42, 1.39, 1.35 and 1.31 mPa·s in the presence of [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit] ILs, respectively. The decrease in the η values with the addition of ILs, suggests that the IL induces the hydrogen bonds destruction between water molecules and PNIPAM that eventually leads to the hydrophobic collapse of the polymer.

The LCST for the aqueous solution in presence of cholinium ILs, obtained from the η measurements, are 33.0 °C (IL free), ~32.6 ± 0.3 °C [Ch][Ac], ~32.0 ± 0.3 °C [Ch]cl, 31.6 ± 0.3 °C [Ch][DHCit] and 31.0 ± 0.3 °C [Ch][Bit], respectively at 5 mg/mL concentration of IL in aqueous polymer solution. The LCST values from the η measurements are in good agreement with the thermal fluorescence spectroscopy and DLS measurements. Further increase in the concentration of ILs, the polymer LCST has shifted towards lower temperatures as illustrated in Fig. S4.
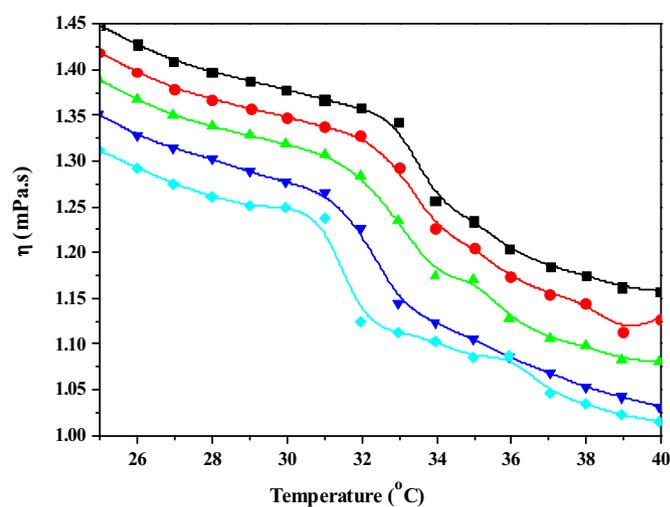
The influence of concentration of ILs on the polymer aqueous solution was more pronounced at the higher concentration of IL which may be ascribed to the increasing tendency in rupturing of the hydrogen bonds between the polymer and water and thus the dehydration of the polymer. As the [Ch][Ac] containing PNIPAM aqueous solution possesses a small tendency of decreasing the LCST value (~32.6 °C), this suggests that it has a little tendency in rupturing the hydrogen bonds, whereas, [Ch][Bit] containing polymer aqueous solution possess highest ability to decrease the LCST value (31.1 °C) suggests that it has the greatest capability to dehydrate the PNIPAM and thereby, it decreases the η values to a larger extent. This is in agreement with the results obtained by the other techniques discussed above and suggest that the mechanism of LCST lowering by the cholinium based ionic liquids is similar to that described by Pereira et al. for polyethylene glycol (PEG) + IL aqueous solutions [30].

The LCST values between PNIPAM and cholinium-based ILs drawn from different experimental methods are depicted in Fig. 6. Schematic representations illustrating the interactions between the biomedical thermoresponsive polymer and biocompatible ILs are illustrated in Scheme 1. The ILs [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit] perturb the hydration layer around the PNIPAM molecule in aqueous solution
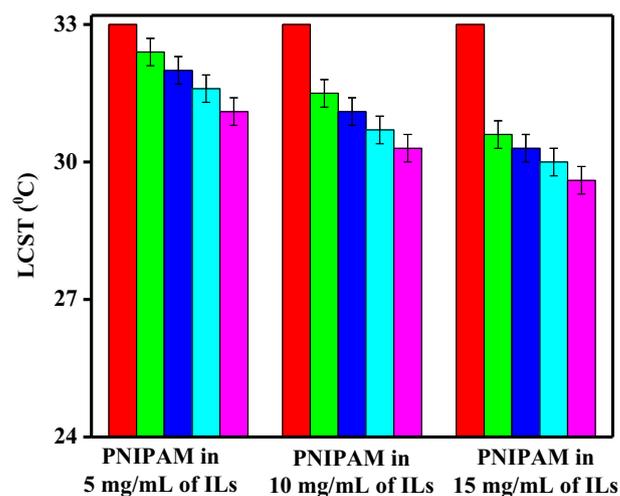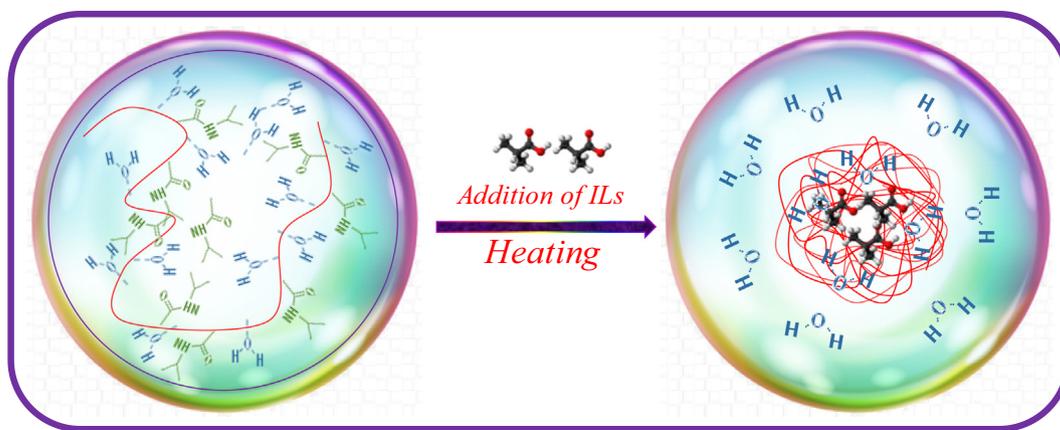

Fig. 6. LCST values of aqueous PNIPAM solution as a function of anion and concentration of ILs; {IL free (red colour), ([Ch][Ac] (green colour), [Ch]cl (blue colour), [Ch][DHCit] (cyan colour) and [Ch][Bit] (pink colour)}.

Scheme 1. Schematic representations illustrating the interactions between the biomedical thermoresponsive polymer and biocompatible ILs.

at all concentrations, which ultimately leads to the improvement of hydrophobic collapse process of PNIPAM in the solution. This phenomenon can be visualized from Fig. 6 and Scheme 1. As depicted in Fig. 6, in the presence of [Ch][Ac] the PNIPAM in aqueous solution, exhibits shrunken behavior as the LCST decreased. The shrunken behavior of PNIPAM further intensifies in the presence of [Ch][Ac], [Ch]cl, [Ch][DHCit] and [Ch][Bit] as the decrease in LCST intensified more towards lower temperatures. The increased hydrophobic interactions among PNIPAM chains and ions of IL drive the water out and cause the chains to collapse and entangles in the presence of ILs. The impact of the ILs on polymer has been systematized by the nature of the structural, molecular interaction, ILs concentration and on the chemical environment. The phase transition trend of polymer in the presence of the ILs exhibited IL induced aggregation. The changes observed in the PNIPAM hydration layer with the addition of ILs resulted from the preferential interactions between the polymer-water molecules and ions of IL. $[Bit]^-$ anion is significantly strong enough to perturb the hydrogen bonds between polymer and water molecules, compared with that of the cl^-, $[DHCit]^-$ and $[Ac]^-$ anions. Hydrogen-bonding interactions happening between the anions ($[Bit]^-$ cl^-, $[DHCit]^-$ and $[Ac]^-$) of IL and the terminal –OH groups of the polymer are more predominant, and further by varying the hydrogen-bond ability of the respective anion. The decrease in the LCST of PNIPAM depends on the strength of the interaction between the anions ($[Bit]^-$ cl^-, $[DHCit]^-$ and $[Ac]^-$) of IL and water molecules. Generally, in aqueous IL solutions water molecules interacts more strongly with the anions than with cation [32,33].

Anions of IL with their charge densities, hydration capacities and hydration energies leads to the hydrophobicity of PNIPAM + IL aqueous solution. High polarity, owing to the charge of the carboxylate groups in [Ch][DHCit] and [Ch][Bit], and hydrogen bond acceptor capability of the Cl^- anion causes their affinity for water inducing ability. The occurrence of molecular interactions between anions and water molecules are significant concerns to understand these consequences and their corresponding effects in the dehydration of the PNIPAM. At lower temperatures the hydrogen bonding between the water-ions of IL-polymer has minimal effects on the water–water hydrogen bonded networks. However, with further increase in the temperature and the increase in the concentration of ILs reduces the strength of hydrogen bonding, which results in the destruction of the hydrogen bonds between water molecules and PNIPAM that eventually leads to the hydrophobic collapse of the polymer. Although the ILs have the same cation with different anions, the phase transition forming ability of these ILs must be determined by the nature of the anions. The ability to induce phase transition behavior of ILs is determined by the capacity of the anion to form hydration complexes. This specifies that the process of breaking hydrogen bonds between the water molecules and amide group of the

PNIPAM can be enhanced by the anions of IL. Also, additional dehydration of the polymer may result from a balance between the interactions of IL-polymer, water-polymer, and water-IL as observed for polyethylene glycol (PEG) + IL aqueous solutions [30].

5. Conclusions

We demonstrated the effect of a series of ILs having different anions ($[Ac]^-$, cl^-, $[DhCit]^-$ and $[Bit]^-$) with the fixed cation (cholinium) on the phase transition behavior of aqueous PNIPAM solutions by employing UV–Visible absorption spectroscopy, steady-state fluorescence spectroscopy, thermal fluorescence spectroscopy, viscosity (η) and dynamic light scattering (DLS) techniques. The results obtained from a set of techniques are in good agreement with each other and have shown that the addition of the ILs decreased the LCST of the aqueous PNIPAM solution. The added ILs decreased the LCST of PNIPAM aqueous solution, by causing the well-swollen PNIPAM to collapse into a shrunken structure. The influence of the ILs at lower concentration was varied from their behavior at higher concentration showing various conformational changes among the components. The results explicitly elucidated that the decrease in LCST of aqueous PNIPAM solution in presence of ILs is mainly due to the self-assembly of PNIPAM into nano-scale range particles, which is ascribed to the nature of the structural, molecular interaction, ILs concentration and on the chemical environment. The added ILs decreased the LCST of PNIPAM, but the extent to which the LCST is changed is significantly more for [Ch][Bit] than for [Ch] cl, [Ch] $[DHCit]^-$ and [Ch][Ac]. Although the ILs have the same cation with different anions, the phase transition forming affinity of these ILs must be determined by the nature of the anions. The ability to induce phase transition behavior of ILs is determined by the capacity of the anion to form hydration complexes. This specifies that the process of breaking hydrogen bonds between the water molecules and amide group of the PNIPAM can be enhanced by the anions of IL. Overall, the results obtained in this work lead to the conclusion that stimuli-responsive systems may be tuned by using biocompatible cholinium-based ILs, and this may be useful for the design of novel materials and new self-assembled compositions of polymers.

CRediT authorship contribution statement

Reddicherla Umapathi: Writing - original draft, Writing - review & editing, Investigation, Validation. **Imran Khan:** Writing - original draft, Investigation. **João A.P. Coutinho:** Conceptualization, Funding acquisition. **Pannuru Venkatesu:** Conceptualization, Funding acquisition, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2019.112362.

References

[1] D. Roy, W.L.A. Brooks, B.S. Sumerlin, New directions in thermoresponsive polymers, Chem. Soc. Rev. 42 (2013) 7214–7243.

[2] R. Umapathi, P.M. Reddy, A. Rani, P. Venkatesu, Influence of additives on thermoresponsive polymers in aqueous media: a case study of poly(N-isopropylacrylamide), Phys. Chem. Chem. Phys. 20 (2018) 9717–9744.

[3] C. Zhao, Z. Ma, X.X. Zhu, Rational design of thermoresponsive polymers in aqueous solutions: a thermodynamics map, Prog. Polym. Sci. 90 (2019) 269–291.

[4] P.M. Reddy, R. Umapathi, P. Venkatesu, Interactions of ionic liquids with hydration layer of poly(N-isopropylacrylamide): comprehensive analysis of biophysical techniques results, Phys. Chem. Chem. Phys. 16 (2014) 10708–10718.

[5] A. Halperin, M. Kröger, F.M. Winnik, Poly(N-isopropylacrylamide) phase diagrams: fifty years of research, Angew. Chem. Int. Ed. 54 (2015) 15342–15367.

[6] P.M. Reddy, C.J. Chang, S.R. Hsieh, H.C. Huang, M.C. Lee, Overview of the effect of monomers and green solvents on thermoresponsive copolymers: phase transition temperature and surface properties, RSC Adv. 5 (2015) 86901–86909.

[7] M.A. Ward, T.K. Georgiou, Thermoresponsive polymers for biomedical applications, Polymers 3 (2011) 1215–1242.

[8] J. Lu, F. Yan, J. Texter, Advanced applications of ionic liquids in polymer science, Prog. Polym. Sci. 34 (2009) 431–448.

[9] S. Fujishige, K. Kubota, I. Ando, Phase transition of aqueous solutions of poly(N-isopropylacrylamide) and poly(N-isopropylmethacrylamide), J. Phys. Chem. 93 (1989) 3311–3313.

[10] K. Nagase, T. Okano, H. Kanazawa, Poly(N-isopropylacrylamide) based thermoresponsive polymer brushes for bioseparation, cellular tissue fabrication, and nano actuators, Nano-Structures & Nano-Objects 16 (2018) 9–23.

[11] J.F. Mano, Stimuli-responsive polymeric systems for biomedical applications, Adv. Eng. Mater. 10 (2008) 515–527.

[12] N.V. Plechkova, K.R. Seddon, Applications of ionic liquids in the chemical industry, Chem. Soc. Rev. 37 (2008) 123–150.

[13] T. Welton, Room-temperature ionic liquids. Solvents for synthesis and catalysis, Chem. Rev. 99 (1999) 2071–2084.

[14] K.S. Egorova, E.G. Gordeev, V.P. Ananikov, Biological activity of ionic liquids and their application in pharmaceutics and medicine, Chem. Rev. 117 (2017) 7132–7189.

[15] H. Weingartner, C. Cabrele, C. Herrmann, How ionic liquids can help to stabilize native proteins, Phys. Chem. Chem. Phys. 14 (2012) 415–426.

[16] L.B. Sagle, Y. Zhang, V.A. Litosh, X. Chen, Y. Cho, P.S. Cremer, Investigating the hydrogen-bonding model of urea denaturation, J. Am. Chem. Soc. 131 (2009) 9304–9310.

[17] R. Umapathi, P. Venkatesu, Assessing the efficiency of imidazolium-based ionic liquids on the phase behavior of a synthetic biomedical thermoresponsive polymer, J. Colloid Interface Sci. 511 (2018) 174–183.

[18] Z. Wang, P. Wu, The influence of ionic liquid on phase separation of poly(N-isopropylacrylamide) aqueous solution, RSC Adv. 2 (2012) 7099–7108.

[19] R. Umapathi, P. Venkatesu, Solution behavior of triblock copolymer in the presence of ionic liquids: a comparative study of two ionic liquids possessing different cations with same anion, ACS Sustain. Chem. Eng. 4 (2016) 2412–2421.

[20] J.I. Santos, A.M.M. Gonçalves, J.L. Pereira, B.F.H.T. Figueiredo, F.A. e Silva, J.A.P. Coutinho, S.P.M. Ventura, F. Gonçalves, Environmental safety of cholinium-based ionic liquids: assessing structure-ecotoxicity relationships, Green Chem. 17 (2015) 4657–4668.

[21] M. Bisht, D. Mondal, M.M. Pereira, M.G. Freire, P. Venkatesu, J.A.P. Coutinho, Long-term protein packaging in cholinium-based ionic liquids: improved catalytic activity and enhanced stability of cytochrome c against multiple stresses, Green Chem. 19 (2017) 4900–4911.

[22] M. Bisht, P. Venkatesu, Influence of cholinium-based ionic liquids on the structural stability and activity of α-chymotrypsin, New J. Chem. 41 (2017) 13902–13911.

[23] M. Reslan, V. Kayser, Ionic liquids as biocompatible stabilizers of proteins, Biophys. Rev. 10 (2018) 781–793.

[24] P. Bruscolini, C. Buzano, A. Pelizzola, M. Pretti, Lattice model for polymer hydration: collapse of poly(N-isopropylacrylamide), Macromol. Symp. 181 (2002) 261–273.

[25] H.G. Schiid, D.A. Tirrell, Microcalorimetric detection of lower critical solution temperature in aqueous polymer solutions, J. Phys. Chem. 94 (1990) 4352–4356.

[26] S. Kunugi, K. Kameyama, T. Tada, N. Tanaka, M. Shibayama, M. Akashi, Differences in pressure and temperature transitions of proteins and polymer gels, Braz. J. Med. Biol. Res. 38 (2005) 1233–1238.

[27] R. Umapathi, S.B. Vepuri, P. Venkatesu, M.E. Soliman, Comprehensive computational and experimental analysis of biomaterial toward the behavior of imidazolium-based ionic liquids: an interplay between hydrophilic and hydrophobic interactions, J. Phys. Chem. B 121 (2017) 4909–4922.

[28] R. Umapathi, K. Kumar, G.M. Rani, P. Venkatesu, Influence of biological stimuli on the phase behaviour of a biomedical thermoresponsive polymer: a comparative investigation of hemeproteins, J. Colloid Interface Sci. 541 (2019) 1–11.

[29] I. Khan, R. Umapathi, M.C. Neves, J.A.P. Coutinho, P. Venkatesu, Structural insights into the effect of cholinium-based ionic liquids on the critical micellization temperature of aqueous triblock copolymers, Phys. Chem. Chem. Phys. 18 (2016) 8342–8351.

[30] J.F. Pereira, K.A. Kurnia, O.A. Cojocaru, G. Gurau, L.P. Rebelo, R.D. Rogers, M.G. Freire, J.A.P. Coutinho, Molecular interactions in aqueous biphasic systems composed of polyethylene glycol and crystalline vs. liquid cholinium-based salts, Phys. Chem. Chem. Phys. 16 (2014) 5723–5731.

[31] R. Umapathi, K. Kumar, G.M. Rani, P. Venkatesu, Influence of biological stimuli on the phase behaviour of a biomedical thermoresponsive polymer: a comparative investigation of hemeproteins, J. Colloid Interface Sci. 541 (2019) 1–11.

[32] I. Khan, K.A. Kurnia, F. Mutelet, S.P. Pinho, J.A. Coutinho, Probing the interactions between ionic liquids and water: experimental and quantum chemical approach, J. Phys. Chem. B 118 (2014) 1848–1860.

[33] I. Khan, K.A. Kurnia, T.E. Sintra, J.A. Saraiva, S.P. Pinho, J.A.P. Coutinho, Assessing the activity coefficients of water in cholinium-based ionic liquids: experimental measurements and COSMO-RS modeling, Fluid Phase Equilib. 361 (2014) 16–22.