Thermo-Responsive Poly(N-Isopropylacrylamide) and its Critical Solution Temperature Type Behavior in Presence of Hydrophilic Ionic Liquids

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THERMO-RESPONSIVE POLY(N-ISOPROPYLACRYLAMIDE) AND ITS CRITICAL SOLUTION TEMPERATURE TYPE BEHAVIOR IN PRESENCE OF HYDROPHILIC IONIC LIQUIDS

A Thesis Presented

by

PURNENDU KUMAR NAYAK

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN CHEMICAL ENGINEERING

FEBRUARY 2015

Chemical Engineering
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Approved as to style and content by:

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ACKNOWLEDGEMENTS

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ABSTRACT

THERMO-RESPONSIVE POLY(N-ISOPROPYL ACRYLAMIDE) AND ITS CRITICAL SOLUTION TEMPERATURE TYPE BEHAVIOR IN PRESENCE OF HYDROPHILIC IONIC LIQUIDS

FEBRUARY 2015

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Directed by: Professor Harry Bermudez

Thermo-responsive homopolymer poly(N-isopropylacrylamide), is a widely studied and used polymer. Our recent observations on thermal behavior of aqueous solutions of this polymer requires a short overview of existing results in order to understand the formation of different phases, both stable and unstable with the addition of hydrophilic Ionic liquids (ILs) 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]), 1-Butyl-3-methylimidazolium acetate ([BMIM][OAc]) and 1-Butyl-3-methylimidazolium thiocyanate ([BMIM][SCN]) to the system. PNIPAM is soluble in cold water due to its inter- and intramolecular hydrogen bonding but phase separates upon heating at T > 32°C, which is its lower critical solution temperature (LCST) [1, 2]. PNIPAM exists in an expanded coil like conformation in water below its LCST which gives a transparent homogenous solution but at T > LCST it undergoes hydrophobic collapse marked by cloudiness of solution and conformational change from coil to globule state [3, 4]. All aqueous PNIPAM solutions undergo phase separation or cloud point transition at T > 32 °C, regardless of the molar mass of the polymer [5].
Room temperature Ionic liquids (ILs) are unique designer fluids because of the novel physico-chemical properties arising from their structure, which have tremendous implications in the field of IL as solvents or co-solvents for polymeric solutes [6] [7] [8]. During recent years a number of different imidazolium based ILs have also been tested for solubilization and stabilization of proteins as well as polymers due to hydrogen bond formation of the IL ions [9] [10]. Recent studies have shown that certain imidazolium based ILs can decrease the LCST of PNIPAM aqueous solution by hydrophobic collapse/aggregation of the PNIPAM chains [11], as well as some can induce an upper critical solution temperature (UCST) behavior of PNIPAM in neat IL solution [12]. Even so, experimental studies of such phase transition/instability of thermoresponsive polymer-IL systems has been a challenging task. In this research we have explored the critical solution temperature (CST) type phase behavior of multicomponent systems i.e. PNIPAM in solution media of water, neat IL and aqueous solutions of IL. The overall fundamental challenge is to understand how the interactions among the components control both structure and dynamics of PNIPAM network in solution. For example the disruption of hydrogen bonding or desolvation interactions between blocks of a PNIPAM molecule and solvent molecules in aqueous mixtures that lead to a LCST type transition at higher temperatures.

Interestingly, it was found in our case that PNIPAM shows both LCST and UCST-type phase transition in some aqueous solutions of hydrophilic IL [BMIM][BF₄]. It was found for the first time that this IL can influence the LCST type behavior of PNIPAM in aqueous solutions based on our visual and experimental cloud point (CP) observations. In our experiments the effect of the ILs [BMIM][BF₄] and [BMIM][OAc] is qualitatively similar to influence of Kosmotropic salts on the LCST of aqueous PNIPAM solutions as predicted by the Hofmeister series [13].

**Keywords:** PNIPAM, imidazolium, ionic liquid, LCST, UCST, phase behavior
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>THESIS OUTLINE</td>
<td>1</td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>3</td>
</tr>
<tr>
<td>1.1 Motivations</td>
<td>3</td>
</tr>
<tr>
<td>1.2 Thermoresponsive nature of Poly (N-Isopropylacrylamide)</td>
<td>6</td>
</tr>
<tr>
<td>1.3 PNIPAM and its applications</td>
<td>8</td>
</tr>
<tr>
<td>1.4 The CST transition of PNIPAM</td>
<td>10</td>
</tr>
<tr>
<td>1.5 Hofmeister effect of ionic liquid anions in aqueous solutions</td>
<td>12</td>
</tr>
<tr>
<td>1.6 Ionic liquid as a media for self assembly of polymers</td>
<td>16</td>
</tr>
<tr>
<td>2 CRITICAL SOLUTION BEHAVIOR OF POLY(N-ISOPROPYLACRYLAMIDE) IN PRESENCE OF HYDROPHILIC IONIC LIQUIDS</td>
<td>19</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>19</td>
</tr>
<tr>
<td>2.2 Materials and Methods</td>
<td>21</td>
</tr>
<tr>
<td>2.2.1 Materials</td>
<td>21</td>
</tr>
<tr>
<td>2.2.2 Sample Preparation</td>
<td>22</td>
</tr>
<tr>
<td>2.2.3 Turbidity Measurements</td>
<td>22</td>
</tr>
<tr>
<td>2.3 Cloud point phenomenon in PNIPAM solutions</td>
<td>23</td>
</tr>
<tr>
<td>2.3.1 Phase Behavior of poly(N-isopropylacrylamide) in IL Aqueous Solutions</td>
<td>23</td>
</tr>
<tr>
<td>2.3.2 Critical solution behavior of PNIPAM in IL/water mixtures: Visual cloud point observations and Turbidimetry measurements</td>
<td>26</td>
</tr>
<tr>
<td>2.3.3 Comparison between PNIPAM-32K and PNIPAM-10.3K: LCST systems</td>
<td>28</td>
</tr>
<tr>
<td>2.3.4 Comparison between [BMIM][BF_4] and [BMIM][OAc] ionic liquids</td>
<td>36</td>
</tr>
<tr>
<td>2.3.5 Comparison of Cloud point (CP) Observation trends on the basis of Solvatochromism</td>
<td>45</td>
</tr>
<tr>
<td>2.4 Conclusions</td>
<td>49</td>
</tr>
</tbody>
</table>
3 PNIPAM CHAIN COLLAPSE AND AGGREGATION IN AQUEOUS SOLUTIONS OF AN IONIC LIQUID THROUGH LCST TYPE TRANSITION...53

3.1 Introduction........................................................................................................................................53

3.2 Dynamic Light Scattering Experimental Details ..............................................................................54
  3.2.1 Samples and Materials.................................................................................................................54
  3.2.2 Nano ZS (Technical) experimental details: .................................................................................55

3.3 Results and Discussions ....................................................................................................................55
  3.3.1 CP of PNIPAM Aqueous Solutions by DLS Measurements..........................................................55
  3.3.2 PNIPAM LCST-type chain collapse: single chain to aggregates ..................................................61
  3.3.3 Size Distribution in Aqueous solutions of Ionic liquid [BMIM][BF₄]..............................................75

3.4 Conclusions.......................................................................................................................................79

4 VISCOELASTIC BEHAVIOR OF PNIPAM IN AN IL/WATER MIXTURE
UPON LCST TYPE PHASE TRANSITION .........................................................................................82

4.1 Introduction.......................................................................................................................................82

4.2 Rheology - Materials and Measurement Protocols...........................................................................85

4.3 Results and Discussions ....................................................................................................................87
  4.3.1 Dynamic- Elastic and Viscous Modulus ....................................................................................87
  4.3.2 Dynamic Viscoelastic response....................................................................................................91
  4.3.3 Steady State Shear and Viscosity Responses .............................................................................93
  4.3.4 Influence of Temperature – Viscoelasticity of the Bulk Solution..............................................100

4.4 Conclusions.....................................................................................................................................103

5 CONCLUSION ......................................................................................................................................112

FUTURE PERSPECTIVES .....................................................................................................................116

BIBLIOGRAPHY .....................................................................................................................................119
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Chemical structure of PNIPAM</td>
<td>5</td>
</tr>
<tr>
<td>1.2. Typical Phase diagram of a system exhibiting lower critical solution temperature (LCST)</td>
<td>9</td>
</tr>
<tr>
<td>1.3. Influences of the anion types and concentrations of sodium salts on the LCST of PNIPAM (Adapted from [21, 28])</td>
<td>13</td>
</tr>
<tr>
<td>1.4. (a) Transition of macromolecule chain from freely soluble state to collapsed aggregates in aqueous solution at higher temperatures (b) Interactions between anions, PNIPAM, and hydration waters are involved in the Hofmeister effect: (I) Hydrogen bonding of the amide and its destabilization through polarization by the anion, X\textsuperscript{–}; (II) The hydrophobic hydration of the molecule is associated with surface tension and can be modulated by salt; (III) direct binding of the anion to the amide group of PNIPAM (Adapted from [13])</td>
<td>14</td>
</tr>
<tr>
<td>1.5. Molecular structure of the IL cation and anions studied in this thesis (adapted from [22, 63])</td>
<td>14</td>
</tr>
<tr>
<td>2.1. Comparison between visual and turbidimetry based cloud point measurements for (a) PNIPAM, Mn = 32 kg/mol, Φp = 10 μM in [BMIM][BF4]/H2O mixtures as volume fractions (ΦIL). (b) PNIPAM, Mn = 32 kg/mol, Φp = 40 μM in [BMIM][BF4]/H2O mixtures as volume fractions (ΦIL)</td>
<td>26</td>
</tr>
<tr>
<td>2.2. Absorbance versus temperature measurements of PNIPAM (Mn = 32 kg/mol) in [BMIM][BF4]/H2O mixtures as follows (a) Φp = 40 μM/ 0.13 wt%, ΦIL ranging from 0 to 0.4 (b) Φp = 50 μM/ 0.16 wt%, ΦIL ranging from 0 to 0.3 (c) Φp = 60 μM/ 0.19 wt%, ΦIL ranging from 0 to 0.4</td>
<td>29</td>
</tr>
<tr>
<td>2.3. Absorbance versus temperature measurements of PNIPAM (Mn = 10.3 kg/mol) in [BMIM][BF4]/H2O mixtures, ΦIL ranging from 0 to 0.2 with PNIPAM concentrations as (a) Φp = 50 μM/ 0.05 wt% (b) Φp = 150 μM/ 0.16 wt% (c) Φp = 250 μM/ 0.26 wt%</td>
<td>30</td>
</tr>
<tr>
<td>2.4. Cloud point CP versus polymer concentration phase diagrams for PNIPAM in [BMIM][BF4]/H2O mixtures, at a constant IL volume fraction ΦIL denoted in the inset. (a) Mn = 10.3 kg/mol. (b) Mn = 32 kg/mol. (Adapted from [78]). All Curves are shown with appropriate fits as just guide to the eye using kaleidaGraph software</td>
<td>33</td>
</tr>
<tr>
<td>2.5. Cloud point (CP) versus solvent composition phase diagrams for PNIPAM in IL/ water mixtures, at constant polymer concentration Φp. (a) Mn = 32 kg/mol, Φp = 0.13 wt% (filled circles) in [BMIM][OAc]/H2O. (b) Mn = 10.3 kg/mol, Φp = 0.21 wt% (open circles) and Mn = 32 kg/mol, Φp = 0.13 wt% (filled circles) in [BMIM][BF4]/H2O. (Adapted from [78])</td>
<td>35</td>
</tr>
</tbody>
</table>
2.6. Change in cloud point ΔCP as a function of IL volume fraction for PNIPAM with 
Mn = 32 kg mol⁻¹ at Φp = 0.13 wt% (40 μM). The straight solid line is a guide to the 
cloud point of PNIPAM in water i.e. 33°C. ..................................................40

2.7. Change in (a) HBA basicity β and (b) HBD acidity α with composition for 
[BMM][BF₄]–water (circles), [BMM][SCN]–water (squares), and [BMM][OAc]– 
water (triangles). To the right of each plot, a schematic of possible interactions is shown. 
(Adapted from [78]). ..................................................................................46

3.1. DLS spectra of the intensity distribution graphs of PNIPAM (Mn = 32 kg/mol) polymer 
concentration Φp = 0.16 wt% (50 μM) in [BMM][BF₄]/H₂O mixture at ΦIL = 0.4. 
Shown profile with variation of temperature for (a) low temperature run 30-48°C and (b) 
high temperature run 50-80°C. (Arrow indicates increase/decrease in peak intensity with 
Temp) .............................................................................................................56

3.2. DLS spectra of the intensity distribution graphs of PNIPAM (Mn = 10.3 kg/mol) polymer 
concentration Φp = 0.16 wt% (150 μM) in [BMM][BF₄]/H₂O mixture at ΦIL = 0.2. 
Shown profile with variation of temperature for (a) low temperature run 30-48°C and (b) 
high temperature run 56-80°C. (Arrow indicates increase in peak intensity with Temp)……57

3.3. DLS spectra of the intensity distribution graphs of PNIPAM (Mn = 10.3 kg/mol) 
polymer concentration Φp = 0.26 wt% (250 μM) in [BMM][BF₄]/H₂O mixture at 
ΦIL = 0.2. Shown profile with variation of temperature for (a) low temperature run 
30-50°C and (b) high temperature run 50-80°C .............................................58

3.4. Displays the z-average particle size as a function of temperature for PNIPAM of Mn = 32 
kg/mol, Φp = 0.16 wt% (50 μM) in ΦIL ([BMM][BF₄]) = 0.4 obtained from cumulant 
fits. Above the schematic diagram describes steps of the formation of PNIPAM 
aggregates as follows a) free chains b) aggregate c) dense aggregate..................62

3.5. Displays the z-average particle size as a function of temperature for PNIPAM of 
Mn = 10.3 kg/mol, Φp = 0.16 wt% (150 μM) in ΦIL ([BMM][BF₄]) = 0.2 obtained 
from cumulant fits. Above the schematic diagram describes steps of the formation of 
PNIPAM aggregates as follows a) free chains b) aggregate c) dense 
aggregate..................................................................................................62

3.6. Displays the z-average particle size as a function of temperature for PNIPAM of 
Mn = 10.3 kg/mol, Φp = 0.26 wt% (250 μM) in ΦIL ([BMM][BF₄]) = 0.2 obtained 
from cumulant fits. Above the schematic diagram describes steps of the formation of 
PNIPAM aggregates as follows a) free chains b) aggregate c) dense aggregate........63

3.7. DLS spectra of Lag time distribution graphs of PNIPAM (Mn = 32 kg/mol) polymer 
concentration Φp = 0.16 wt% (50 μM) in [BMM][BF₄]/H₂O mixture at ΦIL = 0.4. 
Shown profile with variation of temperature .................................................63

3.8. Peak hydrodynamic size Rₘ as determined by dynamic light scattering for (a) Mn = 32 
kg/mol, Φp = 0.16 wt%, ΦIL = 0.4 in [BMM][BF₄]/water. (b) Mn = 10.3 kg/mol, 
Φp = 0.26 wt%, ΦIL = 0.2 in [BMM][BF₄]/water.............................................65
3.9. Intensity autocorrelation function and distribution of the hydrodynamic dia. (inset),
From the CONTIN analysis of solution of PNIPAM Mn = 32 kg/mol, Φp = 0.16 wt%, ΦIL = 0.4 in [BMIM][BF4]/H2O, as computed at different temperatures: (a) 30°C; (b) 45°C; (c) 56°C; (d) 59°C; (e) 65°C; (f) 80°C………………………………69

3.10. Intensity autocorrelation function and distribution of the hydrodynamic dia. (inset), from the CONTIN analysis of solution of PNIPAM Mn = 10.3 kg/mol, Φp = 0.16 wt%, ΦIL = 0.2 in [BMIM][BF4]/H2O, as computed at different temperatures: (a) 39°C; (b) 45°C; (c) 68°C; (d) 71°C; (e) 80°C………………………………71

3.11. Displays the z-average particle size as a function of temperature for ΦIL ([BMIM][BF4]) = 0.4 obtained from cumulant fits, (a) without PNIPAM. (b) with PNIPAM-32 KDa, Φp = 50 μM (0.16 wt%)…………………………………75

3.12. Displays the z-average particle size as a function of temperature for ΦIL ([BMIM][BF4]) = 0.2 obtained from cumulant fit, (a) without PNIPAM. (b) with PNIPAM-10.3 KDa, Φp = 250 μM (0.26 wt%)……………………………………76

4.1. Storage/elastic (G’, filled symbols) and loss/viscous (G'', hollow symbols) moduli as function of frequency for solutions of PNIPAM with Mn = 32 kg/mol at Φp = 1.5 wt% in [BMIM][BF4]/H2O mixtures at constant IL volume fraction ΦIL = 0.3 (CP ≈ 40°C) at (a) 35°C, (b) 40°C, (c) 50°C…………………………………………………………………………………………87

4.2. Storage/elastic (G’, filled symbols) and loss/viscous (G'', hollow symbols) moduli as a function of frequency for solutions of PNIPAM with Mn = 32 kg/mol at Φp = 1.5 wt% in [BMIM][BF4]/H2O mixtures at constant IL volume fraction ΦIL = 0.4 (CP ≈ 50°C) at (a) 30°C, (b) 50°C…………………………………………………………………………………………88

4.3. Storage/elastic (G’, filled symbols) and loss/viscous (G'', hollow symbols) moduli as a function of frequency for solutions of PNIPAM with Mn = 186.8 kg/mol at Φp = 0.8 wt% in [BMIM][BF4]/H2O mixtures at constant IL volume fraction ΦIL = 0.4 (CP ≈ 37°C) at (a) 30°C, (b) 50°C…………………………………………………………………………………………89

4.4. Comparison of crossover frequencies of G’ and G” (frequency at G’ = G’’), their variation with temperature, ΦIL and ΦP being 1.5 wt% for 32K and 0.8 wt% for 186K……………………………………………………………………………………………91

4.5. Shear rate dependence of steady shear viscosity of PNIPAM solutions with Mn = 32 kg/mol and constant polymer concentration of Φp = 1.5 wt% measured at temperatures of 30°C, 35°C, 40°C, 50°C and 55°C for 2 different IL volume fractions. (a) ΦIL = 0.3 and (b) ΦIL = 0.4………………………………………………………………………………………………………………93

4.6. Shear rate dependence of steady shear viscosity of PNIPAM solutions with Mn = 32 kg/mol and constant polymer concentration of Φp = 0.13 wt% measured at various temperatures of run for different IL volume fractions. (a) ΦIL = 0.1, (b) ΦIL = 0.2, and (c) ΦIL = 0.3…………………………………………………………………………………………………………………94
4.7. Shear rate dependence of steady shear viscosity of PNIPAM solutions with 
Mn = 186 kg/mol and constant polymer concentration of Φp = 0.8 wt% measured at 
temperatures of 30°C, 40°C and 50°C. (a) ΦIL = 0.3, (b) ΦIL = 0.4, and 
(c) ΦIL = 0.6.................................................................................................................................................97

4.8. Variation of viscosity of PNIPAM solutions with temperature for Mn = 32 kg/mol, 
constant polymer concentration of Φp = 0.13 wt% and ΦIL ([BMIM][BF4]) = 0.2 
measured from 25°C to 40°C....................................................................................................................99
THESIS OUTLINE

As one of the widely studied inverse thermoresponsive polymers in aqueous based solutions, PNIPAM polymer exhibits a lower critical solution temperature (LCST) of around 32°C in water [14, 15]. PNIPAM chains are completely hydrated and exist in coil like structure below the LCST. However, above the LCST the same coils undergo rapid dehydration resulting in aggregation. We have tried to tune the critical solution temperature (CST) type behavior of PNIPAM in presence of imidazolium based hydrophilic ionic liquids. Three Chapters comprise the thesis. The overall idea is to illustrate the effect of ILs on the CST of PNIPAM in water as a function of IL volume fraction ($\Phi_{IL}$) and PNIPAM molecular weight ($M_n$) at various temperatures under atmospheric pressure by reliance on visual cloud point observations as well as turbidimetry, dynamic light scattering (DLS) and rheology techniques.

In Chapter-1, we discuss the important physico-chemical properties of PNIPAM and ionic liquids required for assembly of polymers. A special attention is given to PNIPAM phase transition behavior, its hydrogen bonding effects and the Hofmeister effect of IL anions on aqueous solutions of PNIPAM. In Chapter-2, which comprises the main topic of the presented investigation, two imidazolium based ionic liquids i.e. [BMIM][BF$_4$] and [BMIM][OAc] were selected and phase separation temperatures were determined through cloud point measurements. The effect of PNIPAM molecular weight ($M_n$) and IL volume fraction ($\Phi_{IL}$) in aqueous solutions, affecting the LCST type and/or UCST type behavior of PNIPAM is evaluated. The change in hydrogen bonding environment of PNIPAM
with solvent molecules by the addition of IL salts and its influence on the phase transition of the polymers is the main subject of the chapter, and their characterization by turbidimetry technique is described. The aqueous environment of PNIPAM is characterized with respect to its hydration interactions and solvatochromatic nature as favorable or unfavorable for PNIPAM solubilization. In our light scattering experiments (Chapter-3) we have tried to show the transition of PNIPAM chains from solvated state to insoluble aggregate state through the hydrodynamic size measurements, on passing through the CP of solution. The knowledge from hydrodynamic size measurements should support the idea of well known coil-to-globule transition and thus ultimate aggregation of PNIPAM chains in IL/water mixtures as observed in similar polymer systems [16]. The objective being to compare the average hydrodynamic size (\(<d_H>\)) and size distribution before and after the volume CP transition of PNIPAM. We later focused on experimenting with rheology (Chapter-4) to explore the viscoelastic nature of PNIPAM in IL solutions upon CP transition. The temperature dependent volume phase transition of PNIPAM in IL/water mixtures is an interesting phenomenon in polymeric assembly which depends critically on the solution viscoelasticity and polymer network formation [17]. This calls for further interrogation into the steady and dynamic shear stress effects on polymer chain interactions both before and after CP phase transition.
CHAPTER 1

INTRODUCTION

1.1 Motivations

Thermoresponsive Polymers in ILs (Ionic Liquids) is an advancing field of study in polymeric material based self-assembly because of their unique temperature responsiveness [18]. Mostly the IL counter anions play a vital role in determining the phase behavior and solubilization of polymers in them [19]. ILs because of their numerous possible combinations of cations and anions can be used to solubilize biopolymers and to make novel drug delivery vehicles. Certain polymers like PNIPAM show an inverse temperature dependency in aqueous solutions i.e. with increasing temperature the solubility of polymer decreases, and might exhibit a lower critical solution temperature (LCST) phase separation. Whereas from past studies it has been found that PNIPAM in aqueous mixtures of organic solvents like ethanol, 1-propanol and DMF etc. demonstrates both LCST phase behavior due to dominant PNIPAM hydrogen bonding interactions and upper critical solution temperature (UCST) phase separation due to predominant hydrophobic interaction among non-polar groups [20]. The thermo-responsiveness of PNIPAM stems from the presence of a hydrophobic carbon backbone chain, a hydrophobic isopropyl group and a hydrophilic amide group along the side chain in a single monomer unit [21]. Recently it has been found that certain imidazolium based ionic liquids can alter the phase transition of PNIPAM with preferential IL-water interaction over PNIPAM-IL interaction [22]. Reportedly, PNIPAM has been shown to
demonstrate both LCST and UCST phase behavior in certain imidazolium based ILs [12, 23-25].

The imidazolium based Ionic liquids constituting [BMIM]$^+$ cation tested through this work tend to reduce the LCST of PNIPAM indicating a major influence of IL ions. Our experiments illustrate that mostly the IL anions have significant impacts on the LCST/UCST type transition of PNIPAM. In this work we have tried to demonstrate the effect of IL ions ionization capability and loss or gain of hydrogen bonding ability on the LCST of PNIPAM using Cloud point observations, dynamic light scattering and dynamic rheological measurements. Ions play a major role in influencing protein solubility, protein and polymer folding, denaturation and aggregation [21]. Nevertheless the unique structure forming ability of ionic liquids with proteins due to hydrogen bonding calls for detailed understanding of ion-protein interactions. One of the methods of understanding the influence of salt ions on protein physical properties and aggregation is based on the Hofmeister series [26]. The structural changes of proteins can be appropriately modeled through phase transition of PNIPAM in salt solutions. Furthermore, solvatochromatic and calorimetric studies have shown that ions play a major role in affecting the solvation/hydration layer of macromolecules [27]. There have been past studies to correlate the Hofmeister series with the solvation entropy of the ions and other physical and thermodynamic properties of the ions [21]. Nevertheless, the entire series and the inverse Hofmeister series have failed to explain the details of aggregation sometimes. Since the monomer unit in PNIPAM contains an amide group along the side chain, it
often serves as structurally similar to proteins for investigating ionic effect of protein (also ELPs) stability/unstability. Cremer and coworkers have suggested that IL anion plays a critical role in the phase transition of PNIPAM [21, 28, 29]. The presence of different anionic species can affect the hydrogen bonding ability between the amide group in PNIPAM and water through polarization effects. Thus, giving IL anions unique strength to raise or suppress the LCST of PNIPAM.

In this thesis we have tried to explain the phenomenon of observed CST (critical solution temperature) type transition on the basis of visual observations and turbidity measurements of CPs (cloud points), chain collapse-aggregation through light scattering and dynamic viscoelastic changes with temperature of the bulk PNIPAM in IL/H₂O mixtures. We report the investigation of the phase behavior of linear PNIPAM in several water–[BMIM]⁺ based IL systems. ILs with chaotropic and kosmotropic anions were selected so as to determine the effects of individual chemical groups on the aspects of temperature–composition plots. The effects produced by the different solvents tested were qualitatively compared, considering aspects related to their particular molecular structures, such as the potential to form hydrogen bonds and the implications of the size and shape of non-polar groups for hydrophobic hydration. ILs for this study were selected according to the similarity of their molecular structures and their miscibility with water in the whole range of compositions. By using [BF₄]⁻, [OAc]⁻ and [SCN]⁻ anions it was possible to evaluate the influence of hydrophilic and hydrophobic groups on phase separation temperatures. We believe similar to conventional molecular solvents,
PNIPAM in IL/H\textsubscript{2}O mixture holds potential for smart material variable self-assemblies in solution.

1.2 Thermoresponsive nature of Poly (N-Isopropylacrylamide)

Stimulus-responsive or intelligent polymers, are polymeric materials that exhibit reversible property changes in response to a small external stimulus such as temperature, pH, ionic strength, mechanic constraints, electric field or light (UV exposure) [30-32]. All the stimulus driven transitions are completely reversible giving rise to switch on/off structural changes [14]. The application of stimulus-responsive smart polymers cover a range of domains like immunoassays, biomedical implants, protein-polymer conjugates and drug-delivery systems based on their physical forms like molecularly dispersed chains in solutions, cross-linked hydrogels, micelles, grafted or adsorbed on surfaces [33-36].

Among different classes of smart polymers thermo-responsive polymers are of special interest because of their biomedical and pharmaceutical applications [18, 37]. Thermoresponsive polymers exhibit a miscibility gap in their temperature-composition phase diagrams known as critical solution temperature. If a miscibility gap is found at a lower temperature and the polymer phase-separates above the critical temperature, the critical temperature is called a lower critical solution temperature (LCST) or cloud point (CP) of the polymer. On the other hand, if the solution is miscible above the critical temperature and the solution is phase-separated below the critical temperature, it is called an upper critical solution temperature (UCST).
The most studied thermo-responsive polymer is poly (N-isopropylacrylamide) (PNIPAM), which undergoes a coil-globule phase transition above its Lower critical solution temperature (LCST) [16]. PNIPAM contains a hydrophobic as well as a hydrophilic moiety. The isopropyl moiety along with the carbon chain backbone is hydrophobic whereas the amide moiety is hydrophilic. In its thermo responsiveness, solutions of PNIPAM exhibit fast, reversible phase transition/phase separation phenomena. At temperatures below LCST, PNIPAM exhibits a coil conformation due to hydrogen bonding between the hydrophilic group and water [1]. When temperature increases, hydrogen bonding weakens, the hydrophobic interaction between the adjacent groups dominate and PNIPAM takes a globular conformation [4]. The structure of PNIPAM is shown in Figure 1.1.

![Chemical structure of PNIPAM](image)

**Figure 1.1.** Chemical structure of PNIPAM.

Current research has shed light on polymers with double thermo sensitivity in water and other organic solvent media. In ILs, block copolymers based on PNIPAM have been found to show double thermo-sensitivity i.e. LCST and UCST behavior [23]. The hydrophilic to hydrophobic phase transition is based on the entropy effects in solution [38-40]. Although it remains the subject of further study, the hydrogen bond accepting
and donating ability between IL ions and a polymer plays an important role in determining its solubility behavior [41]. LCST type behavior of PNIPAM is driven by favorable enthalpic contribution due to hydrogen-bonding interactions between the amide group on PNIPAM and water so that PNIPAM possesses an ordered solvation shell. On the other hand, UCST type behavior is driven by dominant entropic effect of hydrophobic isopropyl groups and the carbon backbone and PNIPAM solution shows the lack of an ordered solvation shell [19].

1.3 PNIPAM and its applications

The first appearance of PNIPAM in literature traces back to 1956 dealing with the synthesis and polymerization of its monomer N-isopropylacrylamide, NIPA [42]. After that PNIPAM has been studied in different forms such as single chains [3, 4], grafted or adsorbed on surfaces [34], microgels [3, 43], macroscopic gels [44], coatings [37] and protein-polymer conjugates [36], which has brought applications in a wide range of disciplines including biology, biochemistry, separation science, biotechnology, and medicine. The novel thermo-responsiveness of PNIPAM and its copolymers has stemmed vital research in the fields of drug delivery systems, tissue engineering and as surface modifiers in green technology [18, 45, 46]. The protein structure mimicking characteristic of PNIPAM due to its conformation change from a hydrophilic coil to a hydrophobic globule around its LCST has interested particular applications as temperature sensitive drug delivery carrier in various forms such as micelle, oligonucleotide-polymer conjugate and hydrogels [34, 36, 47].
Protein mimicking copolymers

A recent emphasis to mimic the behavior of globular proteins in physical systems has motivated scientists to study protein-like copolymer systems. Upon heating the aqueous solution, PNIPAM undergoes a coil-to-globule transition at its LCST, a behavior very similar to globular proteins [3, 4, 16]. The LCST behavior in aqueous PNIPAM solutions is based on the hydrophobic interactions (intra- and interchain interactions) and solubilising effect of the hydrophilic shell like in protein solutions. Similar to globular proteins there is an ordering of the surrounding solute molecules around PNIPAM driven by hydrogen bonding. This ordering can be tailored in presence of anions or cations like IL ions. The matrix of PNIPAM can be strengthened by cross-linking, grafting and conjugation etc. Conventional cross-linked PNIPAM gels usually undergo a sharp phase transition from swollen to shrunken structures around the LCST of PNIPAM [14]. This methodology is the principle behind using PNIPAM hydrogels (grafted/crosslinked) as drug carrier in targeted drug delivery. The gels release drug through a core–shell structure when the temperature reaches below its LCST [35]. When temperature increases above its LCST, PNIPAM chains collapse to develop a dense layer like a shell of the cross-linked hydrogel, thus preventing water and drug permeation from core to outside.

Polymeric self-assemblies like micelles

Aqueous solution of amphiphilic AB type block copolymers self-assemble to form spherical, lamellar, vesicle, cylindrical and core-corona structures like micelles. Recently Lodge et.al reported that in a single ionic liquid solvent, at low and high temperatures, the block copolymer of PEO-PNIPAM self-assembles into PNIPAM-core and PEO-core
micelles, respectively [24]. Also, the core and corona of the micelles are reversibly switchable in response to the stimulus of temperature giving double thermo-responsivity. This depends on polymer concentration and/or solvent composition owing to competing interactions between the polymer segments. In dilute aqueous solutions, the copolymers having hydrophobic and hydrophilic parts form spontaneously polymeric micelles resulting in spherical core-shell structures [42, 48]. The colloidal stability of such micelles, as well as other particles may be achieved sterically and/or electrostatically by using IL salts [49].

1.4 The CST transition of PNIPAM

Most studied temperature-responsive polymers exhibit a coil-to-globule or globule-to-coil phase transition by surpassing a certain temperature known as critical solution temperature (CST). In all reported studies such phase transition is reversible; at lower temperature they are soluble (coil state) due to hydrogen bonding with water and become insoluble (globule) above lower critical solution temperature (LCST) [50] or vice-versa in case of upper critical solution temperature (UCST) behavior. The liquid-liquid phase separation curve is determined by plotting the temperature of phase separation as a function of the overall polymer concentration. The solution is initially homogeneous at low temperature, but with increase in temperature above CST a macroscopic phase separation appears known as LCST [51]. The LCST is the minimum in the phase separation diagram, frequently reported in literature as cloud point (CP) [52]. A typical curve of cloud point temperature versus polymer composition for a thermo-responsive system is shown in Figure 1.2. The x-axis of the phase diagram is representative of the
polymer composition undergoing temperature inverse solubility at CP. The lower the CP, the more hydrophobic is the polymeric system. On the other hand, UCST phase behavior originates from the aggregation of long alkyl side chains at low temperatures. In this case, hydrophobic interactions between the long polymer alkyl side chains play a major role in polymer aggregation [20].

![Figure 1.2. Typical Phase diagram of a system exhibiting lower critical solution temperature (LCST).](image)

The major cause of coil-to-globule transition in case of PNIPAM, determined by hydrophobic effects and hydrogen bonding effects still needs further clarification [53]. Especially, the effect of additives on such structural transition has made this phenomenon interesting. As the temperature rises, the strong hydrogen bonds formed around the polymer coil between water molecules and the amide group of PNIPAM breaks, followed by increased hydrophobic interaction in between adjacent groups. Consequently,
polymer-polymer interactions drive self-aggregation and subsequent precipitation, ruled by decrease in entropy [52, 54]. However, the information about the phase diagram for the aqueous solution of PNIPAM is still confusing. While some authors reported that the LCST of high molecular weight PNIPAM is independent of molecular weight and end group functionality [55]. Whereas, the LCST of low molecular weight PNIPAM is depended on molecular weight and terminal hydrophobic groups. Others like, Schild and Tirrell [56] argued that the cloud point for PNIPAM in water depends on polymer molecular weight and concentration. There are questions which still need to be answered regarding the effect of polymer molecular weight, polydispersity and side chain functionality on the LCST of PNIPAM.

1.5 Hofmeister effect of ionic liquid anions in aqueous solutions

Ionic liquids are often called as designer solvents because their physical and chemical properties like polarity, low volatility, hydrophobicity, viscosity, thermal conductivity and solubility, can be fine tuned by appropriate combination of cations and anions. This is an important advantage, as it allows an IL to be designed for a specific purpose, such as to solubilize a macromolecule, to increase a substrate solubility, to modify a protein selectivity, to enhance the macromolecule stability, or to control the reaction rate. However, this advantage can only be utilized based on a thorough understanding about how the macromolecule’s performance is affected by changing the structure and composition of the IL.
So far different types of macromolecules have been demonstrated to show solubility in ILs. It is vital to discuss this from the aspect of the constituting IL salt ions and their effect on phase transition. Earlier experiments by Cremer et al. found that salt anions have significant impacts on the LCST of PNIPAM [21, 28]. It appears that the anion concentration, valence and size play important roles in influencing the LCST of PNIPAM. However, the effects of the cations on LCST are not obvious. Taking the above into consideration, one can place the PNIPAM in aqueous solution with addition of different ILs and their associated ions. This can provide valuable information on elucidating the importance of the IL's ionic nature in affecting PNIPAM’s LCST type behavior. Similar effect of inorganic salt ions in protein precipitation has already been reported by Hofmeister in 1888. Where the ions are categorized in regards to their “salting out” and “salting in” potential in aqueous solution of proteins, following a sequence now known as the Hofmeister series [57]. In this thesis, the effect of anions of the water soluble ILs [BMIM][BF₄] and [BMIM][OAc] on the LCST of PNIPAM is first examined. This leads to our next consideration whether the PNIPAM phase behavior in aqueous IL mixtures also follows a characteristic of the Hofmeister series. Numerous studies [58] have revealed that the effect of ions on protein stability usually follows the Hofmeister series, and that a kosmotropic anion strengthens the water structure and promotes protein stabilization/precipitation, where as a chaotropic anion breaks the water structure and aids in protein solubilization/unfolding (Figure 1.3- for kosmotropes and chaotropes). The behavior of Kosmotropes and Chaotropes has been explained on the basis of certain mechanisms. According to Zhang and Cremer [28], the stabilization
effect of kosmotropic anions can be well illustrated by preferential hydration and promotion of hydrogen bonding network in water [28]. Because of its strong interaction with water, a kosmotropic anion like [OAc]$^-$ (Figure 1.4) polarizes the adjacent water molecules originally associated with the macromolecule, thereby interfering with the hydrophobic hydration of the macromolecule. This increases the surface tension surrounding the backbone chain and the isopropyl group. Thus favoring the acquirement of its native compact state and stability due to the salting out of the macromolecule [59]. According to the same Researchers, unlike its kosmotropic partner, however, a chaotropic anion like [SCN]$^-$ has a low water affinity, thereby preferring to bind to the macromolecule–water interface directly and hence tends to destabilize the macromolecule.

As per the Hofmeister series, cations on the other hand have a less dominant effect relative to anions of the same ionic radius, because anions are more polarizable [60] and hydrate more strongly. Additionally, cations seem to present their impact indirectly via interaction with anions via ion-pairing effects, and the kosmotropic behavior of anions could be lessened in the presence of kosmotropic cations [61]. All such effect of ions in presence of a macromolecule somehow depends on the bulk water structure in the water rich regimes. In presence of too little ions the bulk water structure may be unaffected to reflect any change, and in presence of too much ions the specific water effects are suppressed. On the basis of the “law of matching water affinity” it is predicted that oppositely charged ions possessing equal affinity for water tend to form contact ion-pairs in solution but ions with different water affinity dissociate easily [62]. All
macromolecules are stable in solution when they are surrounded by fully hydrogen bonded water molecules and any disturbance in water network results in increase in entropy and denaturation of macromolecules. We have made an attempt to explain the Hofmeister effect of IL salt anions in PNIPAM aqueous solution based on ion concentration and CP temperature.

Figure 1.3. Influences of the anion types and concentrations of sodium salts on the LCST of PNIPAM (Adapted from [21, 28]).
Figure 1.4. (a) Transition of macromolecule chain from freely soluble state to collapsed aggregates in aqueous solution at higher temperatures (b) Interactions between anions, PNIPAM, and hydration waters are involved in the Hofmeister effect: (I) Hydrogen bonding of the amide and its destabilization through polarization by the anion, $X^-$; (II) The hydrophobic hydration of the molecule is associated with surface tension and can be modulated by salt; (III) direct binding of the anion to the amide group of PNIPAM (Adapted from [13]).

1.6 Ionic liquid as a media for self assembly of polymers

We present an outlook on possible explanations of the solubility of a temperature-responsive polymer in presence of ILs. The assembly of water soluble thermo-responsive polymer systems for biotechnology and therapeutics has interested researchers, where CST type phase separations are exploited to trigger changes in their structural properties and functional parameters.

Figure 1.5. Molecular structure of the IL cation and anions studied in this thesis (adapted from [22, 63]).
Ionic liquids (ILs) provide a unique platform for material assembly where the behavior depends on the structural parameters of the ions and the experimental conditions. Structure and properties of ionic liquids play a major role as a solvent media for polymers. These properties include thermal and electrochemical stability, low vapor pressure, high ionic conductivity, wide liquidus range, high dissolution capability, etc. [19, 64, 65]. ILs are generally regarded as “designer solvents” because the properties of ILs can be controlled by numerous possible combination of cations and anions [19]. Recent emphasis on the phase behavior and colloidal stability of particles in ionic liquid has given rise to examination of hydrogen bonding ability, aggregation and structure formation in ILs [66]. The phase transition and separation of polymers in IL may generate novel smart materials with reversible property changes in response to temperature and other physical properties. Specifically these include amphiphilic block copolymers with an associated stimulus responsive block/ monomer unit. These polymers self assemble in IL like surfactant micelles depending on their structural parameters and experimental conditions [47]. In addition, if the stimulus is thermo-sensitivity, it can show specific volume change from swollen to collapse aggregation in ILs [23]. Thermo-responsive polymers in IL can be used as soft materials with reversible structural changes at the macroscopic and molecular level [10, 12]. Such materials are based on NIPAM as one of the monomer unit or PNIPAM as a block, which undergo rapid coil-to-globule transition above the LCST. In addition the solubility of such smart polymers can also be controlled by the hydrophobicity/hydrophilicity of the IL ions. Recent studies on protein solubility and denaturation in ILs [67] are also being used to
study the copolymers with functionality similar to globular proteins, which show protein
like conformational effects. From this perspective in order to understand the solubility
and structuring of polymers in IL, forces inherent in the IL like electrostatic, steric and
hydrogen bonding need to be understood first. Certain combinations of polymers in ILs
exhibit lower and/or upper critical solution temperature type phase separation by
changing the temperature. We have identified PNIPAM as such a polymer in select
imidazolium based ionic liquids towards studying its critical solution behavior and
solubility. Also, the phase separation temperature can be easily altered by the change in
IL functionalities, in our case the emphasis has been laid upon the IL anion
CHAPTER 2

CRITICAL SOLUTION BEHAVIOR OF POLY(N-ISOPROPYLACRYLAMIDE) IN PRESENCE OF HYDROPHILIC IONIC LIQUIDS ¹

2.1 Introduction

The wide diversity of room-temperature ionic liquids (ILs) presents opportunities for studying, and controlling, polymer phase behavior. We have examined the phase behavior of poly(N-isopropyl acrylamide) (PNIPAM) in imidazolium based ILs and their mixtures with water. We find there is a strong influence of the IL anion; specifically, the tetrafluoroborate anion ([BF₄⁻]) yields a complex phase diagram with both LCST and UCST type regimes. PNIPAM is generally miscible at intermediate IL/water compositions, although this range depends on the polymer molecular weight. Solvatochromatic characterization of both neat and mixed solvents reveals a key role for the interplay between PNIPAM-IL hydrogen-bonding and ion-pairing within the IL. These results demonstrate that appropriate selection of ILs should allow for increased control over polymer phase behavior.

PNIPAM exhibits a well-known lower critical solution temperature (LCST) of ≈ 32 °C in water [68], i.e. phase separation occurs on increasing temperature. We have used this property to study the phase behavior of linear PNIPAM in IL/water mixtures. Two

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¹ The work from this chapter has been published as the following paper:

imidazolium based ionic liquids i.e. [BMIM][BF₄] and [BMIM][OAc] were selected and phase separation temperatures were determined through cloud point (CP) measurements. The diverse and tunable solvent properties of ILs have recently motivated re-examination of PNIPAM phase behavior. Ueki and Watanabe [12] first reported that PNIPAM showed upper critical solution temperature (UCST) behavior when dissolved in the hydrophobic IL 1-ethyl-3-methylimidazolium bis(trifluoromethyl) sulfonylamide ([EMIM][TFSA]). Such results immediately suggested to us the possibility of complex phase behavior for PNIPAM at intermediate compositions of IL–water mixtures. Because [EMIM][TFSA] is immiscible with water, we instead selected anions (acetate [OAc]⁻, thiocyanate [SCN]⁻, and tetrafluoroborate [BF₄]⁻) that result in hydrophilic ILs which are fully miscible with water. The cation was chosen to be the relatively common 1-butyl-3-methylimidazolium [BMIM]⁺.

We discover that PNIPAM solubility is reduced within a range of intermediate solvent concentrations in binary IL–aqueous solutions, originating a curious phenomenon of critical solution type phase transitions. All the studied systems with ILs exhibited the LCST type behavior, i.e. lower PNIPAM solubility within definite ranges of composition in water–IL mixtures. However, we have detected that there exists coexistence of phase separation temperatures in a particular IL. Both lower critical solution temperature (LCST) type with an upper critical solution temperature (UCST) type phase separation based on solvent concentrations existed in [BMIM][BF₄] based systems. This depended on the hydrophobic nature of the solvent. The change from a LCST to an UCST was correlated with the competition between IL–water, polymer–water and polymer–IL
interactions controlled by their compositions. A variety of additives and cosolutes have been reported to affect significantly the phase transition of PNIPAM/water systems [69]. Recently, the addition of IL salts has been shown to promote an increase/decrease in the LCST, which has been demonstrated by swelling experiments with PNIPAM gels [70, 71] and cloud point or calorimetric measurements of linear PNIPAM solutions [56, 72]. Contrary to what might be expected, we found that the addition of small amounts of [BMIM]+ based IL to PNIPAM–water solutions initially decreases the CP temperature, and only a further addition of IL promotes an increase in it, similar to a phenomena reported by Schild and coworkers for alcohols [56]. Thus, indicating the major influence of local interactions between PNIPAM and solvent in both IL and organic solvent based systems, dictating the cononsolvency phenomenon. The LCST type behavior suggests that water-IL interactions might be preferable over PNIPAM-water hydrogen bonding with in definite concentration ranges in water rich mixtures.

2.2 Materials and Methods

2.2.1 Materials

Poly(N-isopropylacrylamide) (PNIPAM) was obtained from Polymer Source, Inc. with number-average molecular weights $M_n = 10.3$ kg/mol and $32$ kg/mol having polydispersity indices of 1.12 and 1.5 respectively. Ionic liquids 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF$_4$]) ($\geq 98\%$ purity, $\leq 0.5\%$ water) and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]) ($\geq 95\%$ purity, $\leq 2.0\%$ water) were obtained from Sigma-Aldrich and used after drying under reduced pressure for 72 h at 65 °C. The dried IL samples were analysed for water content by Karl Fischer titration.
and was found to contain < 0.2% water for [BMIM][BF₄] and < 0.1% water for [BMIM][OAc]. X-ray photoelectron spectroscopy (XPS) was used to confirm the elemental composition and purity [73]; no trace metals or otherwise unexpected atomic peaks were found. Reverse-osmosis purified water was used for all sample preparations.

2.2.2 Sample Preparation

Samples for cloud-point, plate reader absorbance and DLS measurements were prepared by pre calculating the weight of PNIPAM required on milligrams basis and the total volume of neat system (i.e. either IL or water) or mixed systems consisting of IL and water to get the required micromolar concentration needed. All mixture samples were prepared gravimetrically using a Mettler Toledo, Inc. balance with a precision of 0.0001 g. Calculated amount of PNIPAM (molar concentration basis) was measured into a 5ml Glass Vial followed by addition of required volume of RO water and vortexed for 15 seconds. The sample was then allowed to stand for 15 minutes followed by addition of calculated volume of IL and further subjected to vortexing. The sample was let to stand for atleast 3 hrs for complete mixing of IL-water-PNIPAM before measurements were done. For some studies, smaller quantities of polymer stock solutions were diluted to a required total volume with different compositions of IL and water or neat solution.

2.2.3 Turbidity Measurements

Independent confirmation of the visually determined cloud point (CP) data was obtained for selected samples by turbidimetry. Briefly, absorbance measurements were obtained on a SpectraMax® M5 Multi-Mode Microplate reader. For this purpose the optical
density of the aqueous oligomer solution was monitored as a function of the temperature at 500 nm. The absorbance values of light through the PNIPAM samples in heating process are plotted against temperature in the Figure 2.2. All sample solutions consisted of 200 μL of aliquots in a 96-well at-bottom polystyrene microplates. The microplate lids were sealed with parafilm to minimize loss of water. Absorbance measurements were recorded at a wavelength of 500 nm starting from room temperature to 60 °C. Temperature was manually ramped with a step increase of 2.5°C. At each temperature the sample was equilibrated for 5 min and subjected to shaking for 15 s before measurement runs were performed. All data were normalized by the maximum and minimum values, and cloud points are determined from the initial break points in the resulting absorbance versus temperature curves.

2.3 Cloud point phenomenon in PNIPAM solutions

2.3.1 Phase Behavior of poly(N-isopropylacrylamide) in IL Aqueous Solutions

Our focus is to evaluate the reversible thermoresponsive property of PNIPAM and to investigate how the IL ions interact with PNIPAM over different concentration and temperature. The ability of PNIPAM to exhibit thermoresponsive behavior in aqueous solutions has led to investigation of its critical solution temperature (CST) behavior in presence of cosolutes like ILs [25]. This rapid reversible phase transition of PNIPAM has been attributed to the coil-to-globule transition of PNIPAM chains [74]. Therefore, the addition of any cosolute to PNIPAM/water system is expected to affect its CST behavior. In general the cosolute competes for/with the solvation water of the PNIPAM chains affecting its hydrogen bonding with water and hydrophobic effect [56]. ILs are a class of
functional salts whose properties can be desirably tuned by changing the chemical structure of the ions. Like simple salts, ILs can also be imagined to influence the water structure through salting in/salting out effect [8]. In this chapter the influence of imidazolium based IL salts on the CST type phase transition of aqueous PNIPAM solutions are investigated. We have specially focused our discussions in the water rich solutions on the basis of Hofmeister effect of IL anions [13, 28, 57] and in case of IL rich solutions on the basis of dominant PNIPAM-IL interactions and hydrophobic hydration effects.

Here we have specially highlighted the LCST type phase transition of polymer solution system comprising PNIPAM, hydrophilic Ionic liquid [BMIM][BF₄] or [BMIM][OAc] in water. PNIPAM has been shown to demonstrate UCST phase behavior in certain imidazolium IL [75], and LCST phase behavior in water [2, 63, 68, 76, 77]. At the same time amphiphilic copolymers engineered with PNIPAM have been shown to demonstrate both LCST and UCST type behavior in ILs [24]. Among the cited authors, some have argued that understanding of the molecular mechanism of both, polymer–solvent interactions and bonding/ structure formation among solvent molecules would be quite important to elucidate the observed phenomena. Most recently, we reported that PNIPAM is soluble in neat [BMIM][OAc] over a wide range of temperature but exhibits an LCST-type phase behavior in some aqueous solutions of the same IL [78]. Similarly, PNIPAM exhibits both LCST and UCST type phase transition in aqueous solutions of IL [BMIM][BF₄]. Temperature-composition phase diagrams in these PNIPAM/[BMIM][BF₄] systems with cloud point curves are largely asymmetric, with the
critical composition strongly dependent on the volume fraction of Ionic liquid. Thus PNIPAM/IL systems with tunable phase boundaries either upon heating or cooling can be used to design novel materials for controlled release of protein based drugs. However, modifying the phase behavior of PNIPAM in pure water is challenging due to physico-chemical limitations of water like high vapor pressure and viscosity [79-81]. In addition, for large macromolecules other effects like hydrogen bonding and hydrophobic interactions need to be addressed as well [20, 56]. The effect of the IL anions on the CP is very similar to Hofmeister effect of inorganic salt anions on aqueous PNIPAM solutions as reported by Cremer et al. [28]. Proper selection of the IL ions, specifically IL anion is essential for desired UCST/LCST type solvation property of a macromolecule [82]. Watanabe and coworkers [19] studied the phase behavior of PNIPAM in different neat ILs and water at a constant temperature and determined that the addition of a more hydrophobic IL promotes PNIPAM chain collapse at a lower IL concentration. Similar effect of alcohols on the LCST of PNIPAM have been evaluated for a more hydrophobic (1-propanol) and a relatively less hydrophobic alcohol (ethanol) by Costa et al. [20]. This effect was explained on the basis of a highly co-operative dehydration of solvent molecules from polymer chain network. The same authors also report, addition of an organic solvent like methanol and acetone decreases the LCST to some extent and then increases it. We intend to use such concepts to study the hydrophobic effects of LCST type phase behavior of PNIPAM solutions in imidazolium based ILs with different anions.
2.3.2 Critical solution behavior of PNIPAM in IL/water mixtures: Visual cloud point observations and Turbidimetry measurements

The LCST type phase behavior of PNIPAM in ILs was investigated using visual cloud point (CP) observations as well as turbidimetry measurements using a spectrophotometer. The absorbance based CP measurement is the simplest technique that can provide reliable indication of phase separation in a polymer solution. The absorbance of light is expected to increase steeply with increasing temperature at a threshold temperature i.e., the cloud point. This is the indication of macroscopic phase separation, if the solution under consideration really shows the LCST type miscibility behavior. This phenomenon of CP is completely reversible and the phase separated aggregates disassemble slowly to form a homogenous solution upon cooling.

We now consider various factors that might affect the CP of PNIPAM. The differences in cloud point between solutions is influenced by the PNIPAM molecular weight ($M_n$) and concentration used and/or the conditions (i.e. heating rate, experimental method) used in the measurements of the CP [83, 84], which has a significant influence on the LCST especially when it is measured using optical and spectrophotometer based turbidimetry techniques. Idziak [83] and Boutris [84] also investigated the effect of PNIPAM concentrations and the heating rates on the optical measurement of their LCST, and found that LCST is influenced by above factors. An increase in heating rate may result in shift of the CP to a higher temperature due to insufficient time for nucleation. At low concentrations of our PNIPAM solutions (below 0.5 wt %) the polymer chains fail or are very slow to aggregate to a size that can be detected by the spectrophotometer. This
leads to an understanding that cloud point techniques are sensitive only to the macroscopic phase separation phenomena (formation of polymer aggregates), and at low polymer concentrations this process might be very slow [56]. The sample solution concentrations used here are dilute and substantially below the overlap concentrations ($\Phi_p^*$) of the polymer i.e. for 32 kg/mol, $\Phi_p^* \approx 1.1$ wt% and for 10.3 kg/mol PNIPAM, $\Phi_p^* \approx 2.0$ wt%. Such effect is particularly pronounced for a high heating rate of a dilute solution. If the solution temperature is increased too quickly it seldom results in a less sharp transition and over prediction of cloud point temperature. Therefore, there is a minimum threshold limit of detection and interpretation of the spectrophotometer based absorbance results, such that at a particular wavelength setting the polymer particles should be large enough to be detected. Lack of or delayed aggregation of the particles may result to inappropriate high CP reading. The temperature effect on turbidity and CP in our systems can thus be determined by selecting an appropriate scanning wavelength. Since both of these factors were kept constant during our investigation, ramping rate (2.5 °C) and polymer concentrations, (all < 0.5 wt %) they are well suited for measuring the phase transition of polymers using turbidimetry. Figure 2.1 shows the relative variation and similarity in visual CP and turbidimetry measurements.
Figure 2.1. Comparison between visual and turbidimetry based cloud point measurements for (a) PNIPAM, $M_n = 32$ kg/mol, $\Phi_p = 10$ μM in [BMIM][BF$_4$]/H$_2$O mixtures as volume fractions ($\Phi_{IL}$). (b) PNIPAM, $M_n = 32$ kg/mol, $\Phi_p = 40$ μM in [BMIM][BF$_4$]/H$_2$O mixtures as volume fractions ($\Phi_{IL}$).

2.3.3 Comparison between PNIPAM-32K and PNIPAM-10.3K: LCST systems

Figure 2.2 shows the temperature dependence of the absorbance at 500 nm for PNIPAM-32K ($M_n = 32$ kg/mol) in [BMIM][BF$_4$]/H$_2$O solutions with different PNIPAM and IL concentrations. The absorbance of 0% indicates a single-phase solution and an increase in absorbance indicates that the solution undergoes clouding. At low temperatures,
PNIPAM-32K in [BMIM][BF₄]/H₂O with Φ_{IL} (volume fraction of Ionic Liquid) < 0.45 is completely miscible; a clear solution is observed. As the sample approaches its CP, the bulk solution becomes hazy and shows an increase in absorbance. The CPs of 40, 50 and 60 μM of PNIPAM-32K in [BMIM][BF₄]/H₂O solutions were determined at different volume fractions of the IL. Similar LCST-type phase behavior was also observed for 50, 150 and 250 μM of PNIPAM-10.3K (Mₙ = 10.3 kg/mol) in [BMIM][BF₄] aqueous solutions as shown in Figure 2.3. According to the figures, on increasing the temperature of the solutions from 25°C, the absorbance of solution is nearly constant, but at a particular temperature (CP), a sharp increase is observed which is due to appearance of preliminary particles from formation of a new phase. However from the LCST measurements it can be seen that the shape of the absorbance curve depends on Mₙ and its increase is rather gentle except for some samples with lower volume fractions of [BMIM][BF₄]. There may be some doubt depending upon the shape of the absorbance curve, whether the increase in absorbance really corresponds to the macroscopic phase separation in all cases. We can’t ignore the fact that there is some ambiguity in the determination of the cloud point with absorbance, so we adopt the temperature at which the absorbance starts to rise steeply (from the base value) or becomes 80% as the cloud point, for convenience.

In both the types of PNIPAM/IL systems, the phase transition becomes broader and tends to plateau as the concentration moves away from the critical composition (see Figure 2.4). The change in solubility with temperature in both systems is completely reversible. Figures 2.4 shows the temperature-composition diagrams for PNIPAM-10.3K and
PNIPAM-32K in [BMIM][BF₄]/H₂O mixtures. The diagrams were constructed by plotting the CPs determined by visual cloud point observations and turbidity measurements illustrated in Figure 2.1. The minimum in CP (CPₘᵢₙ) are estimated for each cloud point curves as: for example, for Mₙ = 10.3 kg/mol at Φₗₑ = 0.2, the CPₘᵢₙ ≈ 35°C and for Mₙ = 32 kg/mol at Φₗₑ = 0.4, the CPₘᵢₙ ≈ 42°C with critical compositions (Wc) of roughly 1.5 and 1.8 wt% for PNIPAM-32 and PNIPAM-10.3, respectively. LCST decreases by atleast 10°C and Wc shifts to lower concentration as the molecular weight increases from 10.3 kg/mol to 32 kg/mol for all the volume fractions of IL considered in our studies. Such a trend is commonly observed in other polymer solutions and blends that exhibit LCST-type phase behavior. The results in above figures therefore indicate that the value of CP can be easily controlled over a wide range by appropriately adjusting the volume fraction of [BMIM][BF₄] and [BMIM][OAc] in the solutions. Since PNIPAM exhibits hydrophobic-hydrophilic phase transition at its CP and the presence of IL anions presents a significant impact on the CP value. It would be necessary to understand the fundamental mechanisms for the effects of IL salts on CP due to many potential applications of PNIPAM in biotechnology and targeted drug delivery.
Figure 2.2. Absorbance versus temperature measurements of PNIPAM (Mₙ = 32 kg/mol) in [BMIM][BF₄]/H₂O mixtures as follows (a) Φᵥ = 40 μM/ 0.13 wt%, ΦᵢL ranging from 0 to 0.4 (b) Φᵥ = 50 μM/ 0.16 wt%, ΦᵢL ranging from 0 to 0.3 (c) Φᵥ = 60 μM/ 0.19 wt%, ΦᵢL ranging from 0 to 0.4.
Figure 2.3. Absorbance versus temperature measurements of PNIPAM (Mₙ = 10.3 kg/mol) in [BMIM][BF₄]/H₂O mixtures, Φᵢ ranging from 0 to 0.2 with PNIPAM concentrations as (a) Φₚ = 50 μM/ 0.05 wt% (b) Φₚ = 150 μM/ 0.16 wt% (c) Φₚ = 250 μM/ 0.26 wt%.
Figures 2.4(a) and (b) also indicate that the length of the PNIPAM chain (molecular weight) has a significant impact on the solubility of PNIPAM in the IL solutions; an increase in chain length results in appreciable decrease in CP. This result is consistent with the decrease in CP with chain length increase [85]. Watanabe and co-workers also provided strong experimental evidence to show that the length of the side chain in the imidazolium ring has a significant impact on the solubility of PNIPAM in ILs; a small increase in alkyl side chain length (C2, C4, C6) results in huge increase in the CP [8]. However we are not interested about the group substitution effect in the cation of IL, rather chose to vary the anion of [BMIM]+ based IL to observe if there is any Hofmeister type effect of IL anion on the CP of PNIPAM in the low IL conc. regime. All the polymer concentrations studied in Figure 2.4 are substantially below their overlap concentration, with each solid curve connecting smoothly the data point against Φp for the same sample. This can be compared with the cloud point observations of various aqueous PNIPAM samples reported in the literature. Wherein, the effect of molecular weight is to decrease CP [55, 85], which agrees with the trend for the critical temperature Tc (as a matter of simplicity, Tc is referred to in this thesis as either ‘LCST type’ or ‘UCST type’ to indicate the sense of the phase separation temperature) as predicted from a modified Flory–Huggins solution theory where the interaction parameter χ increases with temperature [86].

The observed trend in CP over the concentration range studied also agrees with a Flory–Huggins-type prediction of an initial decrease of LCST with polymer concentration. There is some controversy regarding the relationship between M_n and T_c with some
papers [55, 85] reporting an inverse correlation (as herein) and others [87, 88] reporting a direct correlation. These discrepancies can be attributed to factors including, but not limited to, end-group hydrophobicity, stereochemistry, and polydispersity. The data of Figure 2.4 is consistent with a relatively moderate hydrophobicity of polymer chain ends, and should become less pronounced as $M_n$ increases. We emphasize that the “critical solution temperature” (e.g., LCST or UCST) can only be identified from the binodal line of the entire phase diagram, [86, 89] which is here approximated by each of the cloud point curves in Figure 2.4. This approximation is validated by reversible behavior upon heating/cooling near the phase boundary (Figures S1 and S2 supporting-image), as well as selected turbidimetry measurements. We also note that all polymer concentrations examined are substantially below their overlap values $\Phi_p^* \approx N^{-4/5}$, if we conservatively assume good solvent conditions [86]. Therefore, the effect of increasing $\Phi_p$ is to decrease the cloud point till $\Phi_p$ nears the overlap concentration region.
Figure 2.4. Cloud point CP versus polymer concentration phase diagrams for PNIPAM in [BMIM][BF₄]/H₂O mixtures, at a constant IL volume fraction Φᵢₗ denoted in the inset. (a) Mₙ = 10.3 kg/mol. (b) Mₙ = 32 kg/mol. (Adapted from [78]). All Curves are shown with appropriate fits as just guide to the eye using kaleidaGraph software.
2.3.4 Comparison between [BMIM][BF₄] and [BMIM][OAc] ionic liquids

The CP temperatures obtained for PNIPAM–water systems containing [BMIM][OAc] and [BMIM][BF₄] are shown in Figure 2.5(a) and (b). It can be observed that the small addition of IL promotes a continuous decrease in CP from 33°C for PNIPAM in pure water to around 26°C for PNIPAM in the IL aqueous solution with a volume fraction of [BMIM][OAc] (Φ_I) ≅ 0.1 (Figure 2.5 (a)). Likewise it can be observed in Figure 2.5(b) that PNIPAM–water–[BMIM][BF₄] system exhibits a minimum CP at 29°C for Φ_I ≅ 0.1 of [BMIM][BF₄]. In both cases, additional amounts of IL increases CP, so that the LCST disappears at higher concentrations (Φ_{[BMIM][OAc]} > 0.3 and Φ_{[BMIM][BF₄]} > 0.45). Despite the similarities between both systems, the CP promoted by [BMIM][OAc] ceases at a lower volume fraction and it is less pronounced than in the case of [BMIM][BF₄]. For all the IL–water solvent mixtures that we examined, PNIPAM displayed LCST-type behavior in the water-rich regime (Figure 2.5). Such effects have been seen with mixtures of molecular solvents and water [56, 90] and also with inorganic salts in water [28]. In the water rich regimes our understanding is qualitatively similar to the Hoffmeister mechanism of anions. Nonetheless there needs to be further understanding related to the molecular effects of hydrogen bond formation and hydrophobic hydration of various groups.
The influence of cosolute addition on phase transition of PNIPAM can be explained due to the entropy loss (dehydration) of bound water surrounding polymer chains. Which might be caused by the presence of solvent molecules with groups capable of hydrogen bonding with water/polymer like IL ions [56]. This effect is majorly influenced by the structure of water produced by an extensive network of hydrogen-bonded molecules, in presence of ionic or non-ionic solutes [91]. When a small amount of a low polarity IL like [BMIM][OAc] or [BMIM][BF₄] is added to water, these solute molecules are cocooned/shelled by water molecules, this phenomenon being known as hydrophobic hydration. Such a hydration shell is stabilized by the hydrogen bonds formed among water molecules in a configuration different from that in pure water and the shape of the shell depends on the IL ions. In similar line of analogy, organic solvents with lower polarity like alcohols and ketones interact poorly with water and hence don’t destroy the
water structure. The low polarity molecules are also called ‘structure makers’ due to their ability to increase the stability of water structure, while polar molecules like DMSO and amides are called as “structure breakers” due to their interaction with water through hydrogen bonding. However we should also remember the fact that such effects are influenced by extrinsic factors like temperature and pressure changes.

In the water rich region, we observed a decrease in CP upon initial addition of [BMIM][BF$_4$] or [BMIM][OAc] (till $\Phi_{\text{IL}} \approx 0.1$) shown in Figure 2.5, which might result from the loss of water molecules surrounding the PNIPAM chains to participate in hydration of IL ions [20]. According to Costa et al. apart from the existence of hydrogen bonds between water molecules and PNIPAM amide groups, a large amount of water molecules are also involved in hydration structures of PNIPAM isopropyl group and main backbone. Therefore, according to their hypothesis in terms of entropic effect it is reasonable to assume that IL solvent molecules with only small methyl or butyl groups is preferable for hydrophobic hydration over the larger PNIPAM segments. Furthermore, Schild and coworkers [56] showed that the enthalpy of the endothermic LCST transition decreases with increasing cosolute concentration, probably due to the reduction of either the number or the strength of PNIPAM–water contacts driven by PNIPAM–water hydrogen-bonding breakage [68]. In case of their systems, they found similar decrease in LCST of aqueous PNIPAM solutions with the addition of organic solvents like methanol, tetrahydrofuran and dioxane. Thus leading to a lower enthalpy of mixing and decrease in entropy term $T\Delta S$ favoring LCST type behavior of PNIPAM in water–IL mixtures.
In further experimentation with [BMIM][BF$_4$] and [BMIM][OAc] we observed an increase in CP and extent of PNIPAM solubility with higher amounts of ILs ($\Phi_{IL} \geq 0.1$). Such phenomenon can be explained in terms of dominant hydrophobic interaction at higher IL concentrations. At higher IL concentration, some of the IL ions are able to overcome their own hydration shell, avoid the hydrophobic hydration and favor interaction with PNIPAM chains. Based on the observations one could confirm the influence of chemistry of PNIPAM, as well as the shape and property of IL ions on hydration shell affecting the CP behavior. Both [BMIM][OAc] and [BMIM][BF$_4$] are kosmotropic salts for PNIPAM and, apart from hydrophobic hydration, their addition would be expected to decrease PNIPAM solubility or, in other words, to depress the LCST. It is also consistent with the fact that [OAc]$^-$ system exhibits a lower range of CP at a volume fraction ($0 \leq \Phi_{IL} \leq 0.3$) than [BF$_4$]$^-$ system ($0 \leq \Phi_{IL} \leq 0.45$) because [BMIM][BF$_4$] (weaker kosmotrope) behaves as a more hydrophobic solvent than [BMIM][OAc] (stronger kosmotrope) and is probably able to overcome its hydration shell and interact more with PNIPAM. Similar effect of organic solvents like methanol and acetone on increasing PNIPAM LCST in water has been shown due to increased solvent-PNIPAM interactions, in case of good solvents for PNIPAM [20].

A comparison between [BMIM][BF$_4$] and [BMIM][OAc] ILs draws us to discuss their anion nature and hydration effects. The former produces an extensive LCST type region till $\Phi_{IL} = 0.45$. The difference between these two ILs resides only in the molecular shape of their anion, which leads to an interesting discussion, again involving hydration shells. Although both [BMIM][BF$_4$] and [BMIM][OAc] are miscible with water in any
proportion, each IL demands a different hydration structure. Because the [OAc]− anion has a stronger dipole moment, its hydration process probably leads to a less significant disturbance in water structure and promotes a less extensive dehydration of PNIPAM segments. On the other hand [BF₄]− anion has a weaker dipole moment. This means that the hydration shell involving [BMIM][BF₄] is less stable and it is more likely to create a larger disturbance in the structure of water molecules when compared with [BMIM][OAc], thus promoting an extensive LCST type behavior. While compounds like [BMIM][BF₄] are considered to be weaker kosmotropes because their favorable interactions with water limits stability of the hydrogen-bonded water structure [21]. Nevertheless, the referred structure making by an IL is limited to positive hydration of the PNIPAM chains and the strengthening of water structure. Overcoming this, hydrophobic interactions are promoted within PNIPAM chains leading to subsequent chain collapse and phase separation. In contrast to the above works that were restricted to the water rich regime, the phase behavior in the IL-rich regime depends much more strongly on the nature of the IL anion. In neat [BMIM][OAc] we find that PNIPAM is completely soluble in the temperature range studied (Figure 2.5(a)). Similar behavior is observed for neat [BMIM][SCN] (data not shown). On the other hand, in neat [BMIM][BF₄], PNIPAM is only soluble above 85°C. At lower temperatures in neat [BMIM][BF₄], PNIPAM appears as an insoluble dispersed phase (Figure 2.5 (b)). Such appearance of UCST type regions have been predicted for PNIPAM-water-ethanol system, which is driven by dominant effect of non-specific dipole-dipole interactions over hydrogen bonding in PNIPAM at higher co-solvent concentrations. However we are
unable to explain any specific reasons for this behavior, but it might be driven by change in \([\text{BF}_4^-]\)–PNIPAM interactions. Thus in [BMIM][BF_4]–water mixtures, the phase behavior of PNIPAM depends in a complex manner on the combination of temperature and IL volume fraction \(\Phi_{\text{IL}}\).

An interesting fact observed is that [BMIM][OAc] system does not exhibit a UCST type behavior at higher mole fractions unlike [BMIM][BF_4]. The only LCST type behavior in [BMIM][OAc] system indicates the relatively strong hydrogen bonds around the polymer chains between N–H groups of PNIPAM segments and C=O groups of [OAc]⁻. On the other hand, the UCST type behavior in [BMIM][BF_4] system indicates that upto some extent, hydrophobic interactions among the backbone chain and isopropyl group predominates thermodynamically over hydrogen bonds between C=O of PNIPAM and H⁺ of imidazolium. We do not present any specific quantitative thermodynamic data involving PNIPAM in these ternary mixtures, rather qualitatively discuss the interactions between PNIPAM, water and IL molecules. [BMIM][BF_4] and [BMIM][OAc] are both miscible with water in all proportions and both compounds are considered to be structure-makers [28], while experimentation reveals that an [OAc]⁻ anion is less hydrophobic than a [BF_4]⁻ anion [57, 92]. According to this explanation, it is possible to imagine that the more hydrophobic [BMIM][BF_4] non-polar groups would contribute more significantly to the formation of non-specific interactions with PNIPAM segments, thus showing a UCST type behavior more likely than [BMIM][OAc], as it is actually observed. The observed behavior arises due to a delicate balance between the polymer-water interactions mediated by hydrogen bonding and hydrophobic interactions. Along with
this the structure of IL ions, more specifically the relative positions of their hydrophobic and hydrophilic groups, and their ability to interact with either a N-H or a C=O group in PNIPAM segments plays an important role too.

![Figure 2.6](image-url)

**Figure 2.6.** Change in cloud point $\Delta CP$ as a function of IL volume fraction for PNIPAM with $M_n = 32$ kg mol$^{-1}$ at $\Phi_p = 0.13$ wt% (40 μM). The straight solid line is a guide to the cloud point of PNIPAM in water i.e. 33°C.

For all the ionic liquid systems that we examined, there exists a LCST type phase transition of PNIPAM in the same concentration range. This suggested us to compare the cloud point changes ($\Delta CP$) when the three Ionic liquids are added to the PNIPAM solution. In the presence of $[\text{OAc}]^-$ and $[\text{BF}_4]^-$ anions the cloud point temperature of the solutions is lowered by approximately 2-6°C for $\Phi_{IL} < 0.2$ (Figure 2.6), and
[BMIM][OAc] has obviously the strongest “salting out” effect among the investigated IL salts; at $\Phi_{IL} = 0.1$, [BMIM][OAc] solution reduces the CP to around 25°C (Figure 6). It is followed by [BMIM][BF$_4$]; which shows a maximum depression of the CP to around 29°C at $\Phi_{IL} = 0.1$ (Figure 2.6). For [BF$_4$]$^-\text{anion the effect is less pronounced, since [BF$_4$]$^-\text{being a weak kosmotrope where as [OAc]$^-\text{being a strong kosmotrope favors more intermolecular hydrogen bonding of water molecules to bring down the CP. A strong effect of the increase in CP could also be observed for [BMIM][SCN] (chaotropic anion). The CP of a solution of [BMIM][SCN] at $\Phi_{IL} = 0.2$ is increased to around 43°C in 0.13 wt% PNIPAM solution (Figure 2.6). For [BF$_4$]$^-\text{anion the effect is less pronounced and, as the volume fraction of the IL increases this anion tends to slightly elevate rather than depress the cloud point (“salting in” effect). At higher volume fractions of [BMIM][OAc], however, the cloud point temperature with increasing salt concentration remains steady at 27°C, albeit to the lowest degree of depression of all investigated salt species. Moreover, the observed relative changes in the CP correspond in quality to those previously observed by Freitag and Garret-Flaudy [93, 94] for PNIPAM. Similarly Reddy et al. [11] also reported a decreasing effect on the LCST of PNIPAM solutions with the addition of hydrophilic ionic liquid [BzMIM][BF$_4$]. “Salting-in” anions (chaotropes) or anions with a rather large volume like [SCN]$^-\text{break the structure of the water (“structure breaker”) and as a result stabilize e.g. hydrophobic hydration; the coil-like structure of the thermo-responsive polymers is favored in these solutions [13]. In the case of our investigated PNIPAM samples, this would mean that dissolution is aided and that precipitation occurs at higher temperature than in pure water. “Salting-out” anions or
anions with a smaller volume or more surface charge density like \(\text{[OAc]}^{-}\) and \(\text{[BF}_{4}^{-}\), on the contrary, function as “structure makers” for water and as a result, they strengthen hydrophobic interactions among the side chains and promote the formation of the globular structures (collapse-state) of the polymers.

Previous studies have already shown that for any thermoresponsive polymer, its initial thermal response is the individual chain collapse in a coil-to-globule transition [95]. Based on this we intend to shed light on such phenomenon and its occurrence in our systems, the details of which are discussed in the next chapter. The well established coil-to-globule transition is driven by two factors which include the breakdown of polymer-water hydrogen bonding in controlling the macromolecular contraction followed by chain collapse due to changes in the “hydrophobic effect” [96, 97]. Furthermore there is self-association of the amphiphilic polymers which can be explained as follows. On one hand, the addition of a kosmotropic salt \([\text{BMIM}][\text{BF}_{4}]\) increases the hydrogen bonding among water molecules, therefore decreases that between water and PNIPAM hydrophilic chains. Subsequently the hydrogen bonding among the hydrophilic portion of the polymer chain increases causing the chain association and decrease in CP [98, 99]. Simultaneously, even though \([\text{BMIM}][\text{BF}_{4}]\) being less polar than \([\text{BMIM}][\text{OAc}]\) increases the polarity of water slightly leading to increased hydrophobic-hydrophobic interaction. Thus stronger tendency for the PNIPAM chains to self-aggregate; a feature that will result in lower solubility of the polymer in water, hence a decrease in CP [93]. Therefore, both mechanisms reason for the decrease in the CP of PNIPAM.
2.3.5 Comparison of Cloud point (CP) Observation trends on the basis of Solvatochromism

Similar relationship as discussed previously for preferable IL or PNIPAM hydration can also be derived from the values of hydrogen-bond donor (HBD) acidity $\alpha$ and hydrogen-bond acceptor (HBA) basicity $\beta$ of the IL solvents and water without PNIPAM [78]. Solvatochromatic characterization of ILs has revealed that the IL anion is primarily responsible for the HBA basicity $\beta$ [41], and therefore we sought to use this approach to better understand the phase behavior of PNIPAM in IL–water mixtures. It has been shown in the study [78] that neat [BMIM][OAc] has the highest $\beta$ value of the ILs studied, indicating that [OAc]$^-$ has the strongest interactions with PNIPAM. According to the study, HBD acidity $\alpha$ will reflect interactions of the IL cation, primarily the $C^2$ proton of the imidazolium ring, with either the IL anion or the amide oxygens of PNIPAM, and the magnitude of $\alpha$ provides a relative measure of the strength of ion-pairing [100]. Water was reported to have the lowest $\beta$ value and the highest $\alpha$ value. Such a combination suggests that water’s primary interaction with PNIPAM is as a hydrogen-bond donor. However, LCST-type behavior requires not only attractive interactions ($\Delta H_m < 0$) but also the formation of an ordered solvation shell ($\Delta S_m < 0$) [19, 89]. Indeed, the phase diagram of PNIPAM in water can be accurately discussed by invoking cooperative hydrogen-bonding [101].

Based on such experimentation, interplay of hydrogen-bonding can possibly explain the trends in $\alpha$ and $\beta$ values. Based on the fact that $\beta$ never increased with the addition of water (Figure 2.7(a)), water is a weak base and not readily accept protons from PNIPAM.
As water is introduced into [BMIM][OAc] (Φ_{IL} > 0.6), the dominant effect is [OAc]^− accepting protons from water. This interaction largely explains why α remains nearly constant and why β decreases (Figure 2.7). At higher water contents (Φ_{IL} < 0.6), water donates protons to PNIPAM, causing α to increase (Figure 2.7(b)). By contrast, as water is introduced into [BMIM][BF_4]^- (Φ_{IL} > 0.6), it acts as an acid, donating protons to PNIPAM and increasing α values. Because both water and [BF_4]^- are weak bases, β stays relatively constant. At intermediate water content (Φ_{IL} = 0.2–0.6), [BF_4]^- begins to accept protons from water, which causes α to plateau and β to decrease. In this case, the donation of protons from water to PNIPAM is only clear at the highest water contents (Φ_{IL} < 0.2). Curiously, the LCST-type phase boundaries for both [BMIM][OAc] and [BMIM][BF_4]^- (Figure 5) correspond to β ≤ 0.35 (Figure 2.7(a)), suggesting a critical role for IL anion basicity. Unfortunately, no such correlation is obvious for the UCST-type boundaries.

In the water-rich regimes, Φ_{IL} < 0.3, there was almost no difference in either α or β values among the various ILs (Figure 2.7). Since water is in abundance, LCST-type behavior for PNIPAM is expected and observed (Figure 2.5). Differences of CP in the water-rich regime are explained by the Hofmeister effect that is well known for inorganic salts in water. Work by Cremer et al. [13] has established that specific interactions of inorganic anions with PNIPAM determine the polymer’s solubility, with [SCN]^− known to be a strong chaotrope. It is natural from such findings that [OAc]^− is a strong kosmotrope while [BF_4]^- is a weak kosmotrope [13]. Indeed, the changes in cloud point ΔCP (Figure 2.6) agree quite well with the Hofmeister series. As further support of the
above, Reddy et al. [11] showed that when small amounts of 1-benzyl-3-methylimidazolium tetrafluoroborate ([BzMIM][BF$_4$]) are added to water, a depression of PNIPAM’s LCST type behavior occurs.
Figure 2.7. Change in (a) HBA basicity $\beta$ and (b) HBD acidity $\alpha$ with composition for [BMIM][BF$_4$]–water (circles), [BMIM][SCN]–water (squares), and [BMIM][OAc]–water (triangles). To the right of each plot, a schematic of possible interactions is shown. (Adapted from [78]).
2.4 Conclusions

The effect of the molecular weight in our experiments is to decrease the CP, which agrees with the trend for the “critical point” LCST as predicted from a modified Flory-Huggins solution theory. This theory also predicts an initial decrease of LCST with polymer concentration, which agrees well with the observed trend in CP over the concentration range studied. It is apparent from Figure 2.4 that further increasing IL content increases CP. However, this trend does not continue indefinitely. The effect of IL content on PNIPAM phase behavior is more clearly depicted in Figure 2.5. From this perspective, it is seen that PNIPAM is generally soluble at intermediate IL–water compositions. Interestingly, for [BMIM][BF₄]/H₂O mixtures, the width of this solubility “window” decreases with increasing molecular weight (Figure 2.5(b)), suggesting an eventual convergence of the two insoluble regimes. And we should mention here that others have observed multi-phase behavior in imidazolium IL–alcohol mixtures [102, 103]. In contrast to PNIPAM phase behavior here, Smith et al. found that silica microparticles remain colloidally stable in both neat ethylammonium nitrate (EAN) and water, but not for their mixtures [40].

In the study of [BMIM][BF₄] systems with PNIPAM of different Mₙ, the coexistence of a LCST and a UCST type behavior at different [BMIM][BF₄] volume fraction ranges was disclosed. The LCST type phenomenon was qualitatively explained on the basis of weakening of PNIPAM–water interactions due to the preferential hydrophobic hydration of IL molecules in water-rich region. The rupture of hydration shells with increasing IL concentration allows IL molecules to interact with PNIPAM segments. Thus, the
incidence of a UCST type behavior results from the predominance of non-specific PNIPAM–IL interactions. The magnitude of the effects caused by the increasing IL concentration was correlated with factors such as: ionic structure of IL, potential to form hydrogen bonding, according to the size and shape of its hydrophobic groups and the potential to hinder hydrophobic hydration. While [BMIM][OAc] either promoted LCST type behavior or complete solubility, the [BMIM][BF₄] system showed LCST type behavior in the water-rich region and a UCST type behavior in IL-rich region. At this moment, we are unable to offer a solid explanation for the occurrence of this narrow solubility window in case of [BMIM][BF₄]. However the observed phenomena in ILs and their mixtures with water should provide many future opportunities for unique manipulation of such phase behavior and structural assembly.
Supporting information:

Confirmation of reversibility of the observed phase behaviors:

Fig. S1. LCST type behavior: Photograph of 40 μM PNIPAM (M_n =32 kg/mol) in [BMIM][BF_4]/water mixture of φ_IL = 0.2 showing the thermoreversibility of LCST type transition behavior. From left to right: (t<0) sample before LCST type transition at T=30 °C during heating; (t=0) sample upon LCST type phase transition at T= 35 °C; (t=45 secs) sample on cooling at RT for 45 seconds after LCST type phase transition; (t=150 secs) on cooling at RT for 150 seconds after LCST type transition, similar to previous condition at t<0. The sample in a 5ml glass vial was heated on an analog dry block heater and temperature was read by a thermometer inserted into one of the block heater apertures. The temperature was increased manually in approximately 5 °C intervals starting from RT and allowed to equilibrate for atleast 5 min.
Fig S2. UCST type behavior: Opposite thermoreversibility phenomenon as above.
3.1 Introduction

After completing the Turbidimetry experiments of PNIPAM in aqueous solutions of Ionic liquid [BMIM][BF₄] the mechanism for the interactions between IL ions, water and PNIPAM are elucidated from a structural point of view. To detect the formation of polymer aggregates upon phase transition or clouding, we have recourse to dynamic light scattering (DLS) as described in the following sections. DLS was used to measure the hydrodynamic diameter of the polymer particles below the CP, and to confirm the onset of aggregation at the CP. Dynamic light scattering analysis was performed using both cumulants and CONTIN algorithms. PNIPAM chains can undergo a reversible coil-globule-aggregation behavior hydro-dynamically, when it shows clouding or LCST phase separation [16]. Such reversible transition is an interplay of chain demixing and the hydrogen bonded structure formation between polymer and solvent molecules [104]. The origin of this transition of PNIPAM has long been discussed through DLS techniques. First we investigate the coil-to-globule transition with temperature rise for PNIPAM-32K (Mₙ = 32 kg/mol) with [BMIM][BF₄]/H₂O mixtures and varying concentration followed by similar experiments with PNIPAM-10.3K (Mₙ = 10.3 kg/mol).

PNIPAM was studied at different concentrations and they had varying chain length (Mₙ) and polydispersity (same parent chain). We believe the trend in hydrodynamic size with
temperature holds valuable information regarding PNIPAM-IL/H$_2$O interactions. In order to further investigate the effects of Ionic liquid volume fractions in water and polymer molecular weight on the CP of PNIPAM in the same solution, light scattering of PNIPAM in aqueous solutions of Ionic liquid [BMIM][BF$_4$] were carried out above and below its estimated CP. We experimented with aqueous IL solutions of PNIPAM-32K (M$_n$ = 32 kg/mol) for $\Phi_p = 0.16$ wt% (50 μM), $\Phi_{IL} = 0.4$ and PNIPAM-10.3K (M$_n$ = 10.3 kg/mol) for $\Phi_p = 0.16$ wt% (150 μM), $\Phi_{IL} = 0.2$; $\Phi_p = 0.26$ wt% (250 μM), $\Phi_{IL} = 0.2$. The above concentrations of IL and PNIPAM were chosen to be the same as studied in our cloud point experiments.

3.2 Dynamic Light Scattering Experimental Details

3.2.1 Samples and Materials

Samples for DLS measurements were prepared by pre calculating the weight of PNIPAM required on milligrams basis and the total volume of neat system (i.e. either IL or water) or mixed systems consisting of IL and water to get the required micromolar concentration needed. All mixture samples were prepared gravimetrically using a Mettler Toledo, Inc. balance with a precision of 0.0001g. Calculated amount of PNIPAM (molar concentration basis) was measured into a 5ml glass vial followed by addition of required volume of RO water and vortexed for 15 secs. The sample was then allowed to stand for 15 mins followed by addition of calculated volume of IL and further subjected to vortexing. The sample was let to stand for at least 3 hrs for complete mixing of IL-water-PNIPAM before measurements were done. For some studies, smaller quantities of polymer stock solutions were diluted to a required total volume with different
compositions of IL and water or neat solution. Prior to dynamic light scattering (DLS) experiments, the prepared sample of a given concentration of PNIPAM and volume fraction of IL was filtered using a 0.22 μm Millex® syringe filter unit with Millipore Express® polyethersulfone (PES) membrane into a low volume cuvette. Sample volume was 450 μL.

3.2.2 Nano ZS (Technical) experimental details:

Particle size distributions at temperatures between 25°C and 80°C were determined with a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) operating with a 4 mW He-Ne laser (633 nm), a detector positioned at the scattering angle of 173°C and a temperature-control jacket for the cuvette. The cuvette cell with sample was placed in the sample chamber and a cell cap was placed at the mouth of the cell to secure it from dust. The cuvette was left for 10 mins to allow temperature equilibration before each measurement. Five measurements consisting of up to 12 consecutive runs of duration of 20s were performed for each sample at a particular temperature. Dynamic correlation functions were fitted by a second-order cumulant and CONTIN method to obtain the size distributions. Hydrodynamic sizes (d_H) were calculated using the Stokes-Einstein equation with the temperature-dependent viscosity measurements of Zhou et al [105].

3.3 Results and Discussions

3.3.1 CP of PNIPAM Aqueous Solutions by DLS Measurements

To determine the contribution of IL on the phase transition of PNIPAM aqueous solution, we have further exploited DLS as a function of IL concentration at various temperatures.
We reveal the intensity distribution graphs (Figures 3.1, 3.2 and 3.3) for different PNIPAM concentration and various concentrations of IL (volume fractions) solutions, at different temperatures starting from far below their CP to their respective CP temperature and higher. Near the phase transition region, we observed a significant increase in the intensity of scattering from larger peaks. One of these peaks (Figure 3.1 (a)) was obtained from a population (majorly ≈ 5-50 nm) of comparably lower intensity. This peak shows that the PNIPAM molecules are aggregated with a probability of higher inter-chain interaction in the solution. The other population (the second peak, ≈ 1-3 nm) corresponds to a higher percentage of intensity. This peak might correspond to free polymeric chains in collapsed/folded (referred to dehydration) form. Such a result leads to the hypothesis that inter-chain interactions are just starting to propagate or seed. For the sake of clarity, we do not present all the distribution graphs at each temperature. It is also noted that, in the intensity distribution graph, the peak area for high aggregation will appear at least $10^6$ times larger than that of the first peak for smaller particles (Rayleigh approximation). The change in the hydrodynamic diameter with temperature clearly exhibited the existence of LCST type phase transition. In this work we present our recent light-scattering studies on the stages of PNIPAM single chain collapse and aggregation in IL/H$_2$O solution. Hence, relate with the LCST-type (CP) phase transition.

The most important factors that affect the PNIPAM solution aggregation size and size distribution are polymer concentration, IL concentration, and temperature. Figure 3.1 shows the PNIPAM size distribution at different temperatures. Here polymer concentration and [BMIM][BF$_4$] volume fraction ($\Phi_{IL}$) are kept at 0.16 wt% and 0.4
respectively. The CP of this solution is around 57°C. The temperatures are selected from a range of values starting from T lower than the CP of the PNIPAM solution to T higher than the CP. Figure 3.1, shows the main \( d_H \) (hydrodynamic dia.) peak positions and in Figure 3.1(a) we observe two distinct peaks for each temperature i.e. i) \( d_H \approx 0.5-5 \) nm ii) \( d_H \approx 5-70 \) nm. As shown in Figure 3.1(a), increase in temperature leads to peak narrowing for the peak at 5-70 nm \( d_H \). We, assume this as an indication of the chains becoming more compact. Also, the intensity of scattering for this peak increases with increase in temperature till \( T = \text{CP} \). This suggests that the PNIPAM chains are aggregating and the population of larger sized aggregates is growing. Thus, an important characteristic of a polymeric solution undergoing volume phase transition at CP [16, 74, 106]. Scattering at temperatures of 50-80°C (Figure 3.1(b)) shows significant shift in peak positions and three distinct peaks, with third peak around 300 nm. Thus, at temperatures higher than 50°C (CP \( \approx 57°C \)), the solution shows significant amount of aggregation as suggested by \( <d_H> \) (average hydrodynamic dia.) \( \leq 300 \) nm. The shape of the intensity size distribution plots show significant polydispersity of \( <d_H> \), with the larger size end of the distribution curve being stretched out. The experimental observation could imply that, in the CP regime aggregation of polymer chains due to intermolecular interactions occur. Thus, yielding apparent larger values of \( <d_H> \) as given by coil-to-globule type volume phase transition in dilute polymer solutions [4, 106].
Figure 3.1. DLS spectra of the intensity distribution graphs of PNIPAM ($M_n = 32$ kg/mol) polymer concentration $\Phi_p = 0.16$ wt% (50 μM) in [BMIM][BF4]/H2O mixture at $\Phi_{IL} = 0.4$. Shown profile with variation of temperature for (a) low temperature run 30-48°C and (b) high temperature run 50-80°C. (Arrow indicates increase/decrease in peak intensity with Temp)
Figure 3.2. DLS spectra of the intensity distribution graphs of PNIPAM \( (M_n = 10.3 \text{ kg/mol}) \) polymer concentration \( \Phi_p = 0.16 \text{ wt\%} \) (150 μM) in [BMIM][BF₄]/H₂O mixture at \( \Phi_{IL} = 0.2 \). Shown profile with variation of temperature for (a) low temperature run 30-48°C and (b) high temperature run 56-80°C. (Arrow indicates increase in peak intensity with Temp)
Figure 3.3. DLS spectra of the intensity distribution graphs of PNIPAM ($M_n = 10.3$ kg/mol) polymer concentration $\Phi_p = 0.26$ wt\% (250 μM) in [BMIM][BF$_4$/H$_2$O mixture at $\Phi_{IL} = 0.2$. Shown profile with variation of temperature for (a) low temperature run 30-50°C and (b) high temperature run 50-80°C.
3.3.2 PNIPAM LCST-type chain collapse: single chain to aggregates

The programmed temperature scan of the sample compartment during DLS allows computation of both the size and scattering intensity at different temperatures. The marked point where both the size and intensity start to increase significantly is near the CP region. The \( <d_{H}> \) of PNIPAM solutions plotted against temperature at various [BMIM][BF_4] volume fractions (\( \Phi_{IL} \)) and polymer concentrations (\( \Phi_{p} \)) are shown in Figures 3.4, 3.5 and 3.6. It can be seen that, for each solution, the \( <d_{H}> \) of the polymer chains increases sharply at a certain temperature, near the CP (30-40 °C) of the corresponding PNIPAM solution as determined visually and by turbidimetry technique. At temperatures below the CP of the mixture, the observed \( <d_{H}> \) was small (5-10 nm, which can be attributed to the polymer chains in random coil conformation. It is observable that \( <d_{H}> \) (55-350 nm) rapidly increases around 56-60 °C. This increase in hydrodynamic size of PNIPAM depends on the heating rate of sample and the equilibration time. Some of the papers have reported prolonged equilibration times of hours before taking scattering measurements [107, 108]. For experiments with larger equilibration times a CP transition (increase in size) might take place even at lower temperatures. Moreover, the increase of the average hydrodynamic diameter occurs in a quite narrow temperature range (2-5 °C) due to the well-known polymeric coil to globule conformation change, revealing that phase transition of PNIPAM occurs in this region. Figures 3.4, 3.5 and 3.6 also show that the value of \( <d_{H}> \) gradually decreased or ultimately did not change at temperatures above 65°C (higher than respective CP).
Figure 3.4 shows the $<d_H>$ of chains increases with increase in temperature as the solution approaches its CP and the $<d_H>$ versus temperature curve shows a peak at around 65°C with $<d_H>$ max = 250 nm, indicating maximum aggregation at around that temperature. As seen from Figure 3.4, the $<d_H>$ value shows a steep increase from 50 nm at 57°C resulting a peak max at 65°C with further increase in temperature. This further confirms the onset of aggregation and volume phase transition at 57°C, which is exactly the CP temperature for this particular solution of PNIPAM as observed visually. This suggests a common mechanism for volume change in both IL/water mixture and just water. Similarly, for a low $M_n$ PNIPAM we can see that the solutions have approximately the same CPs i.e. 45°C (Figure 3.5) and 41 ~ 42°C (Figure 3.6) as determined from visual cloud point observations. The comparison of their $<d_H>$ below and above the CP shows that the average size has increased at least 36 times. The $<d_H>$ keeps on increasing continuously till 70-72°C due to rapid aggregation between collapsing PNIPAM chains aided by slow heating. Firstly, since the solutions being dilute it should favor the intramolecular interactions between the collapsing PNIPAM chains. Secondly, increasing the temperature of the solution should have an increased desolvation effect on the collapsing PNIPAM chains and it also improves the steric stabilization of the forming aggregates due to intermolecular associations.

Figures 3.5 and 3.6 explicitly demonstrate that the onset of CP driven chain aggregation of the PNIPAM aqueous solution is altered as a function of PNIPAM concentration. It has been stated in many literatures that sometimes the coil-to-globule transition can occur in a single phase dilute solution without any phase separation [106]; i.e., in very dilute
conditions, the distance between the single-chain globules is so large that the inter-chain interaction is minimum. However the time scales of phase separation process and aggregation are larger, so can be practically distinguished from collapse of individual coils through DLS[16]. The intra-molecular chain collapse often occurs first, followed by the inter-molecular chain aggregation, which can be characterized using DLS because the aggregates are much bigger in size than the globules. However, all such chain collapse and aggregation phenomena are influenced by polymer concentration and temperature of the solution. We however, do not attempt to quantify and differentiate the size ranges of chain collapse, globule and aggregate formation, rather qualitatively use the DLS data for justification of CP phenomenon and phase separation. The lack of quantitative agreement is due to limitations of maximum attainable temperature for DLS, sample heating rate and needed time for equilibration.
Figure 3.4. Displays the z-average particle size as a function of temperature for PNIPAM of $M_n = 32$ kg/mol, $\Phi_p = 0.16$ wt% (50 $\mu$M) in $\Phi_IL([\text{BMIM}][\text{BF}_4]) = 0.4$ obtained from cumulant fits. Above the schematic diagram describes steps of the formation of PNIPAM aggregates as follows a) free chains b) aggregate c) dense aggregate.

Figure 3.5. Displays the z-average particle size as a function of temperature for PNIPAM of $M_n = 10.3$ kg/mol, $\Phi_p = 0.16$ wt% (150 $\mu$M) in $\Phi_IL([\text{BMIM}][\text{BF}_4]) = 0.2$ obtained from cumulant fits. Above the schematic diagram describes steps of the formation of PNIPAM aggregates as follows a) free chains b) aggregate c) dense aggregate.
Figure 3.6. Displays the z-average particle size as a function of temperature for PNIPAM of $M_n = 10.3$ kg/mol, $\Phi_p = 0.26$ wt\% (250 μM) in $\Phi_{IL}$ ([BMIM][BF$_4$]) = 0.2 obtained from cumulant fits. Above the schematic diagram describes steps of the formation of PNIPAM aggregates as follows a) free chains b) aggregate c) dense aggregate.

Figure 3.7. DLS spectra of Lag time distribution graphs of PNIPAM ($M_n = 32$ kg/mol) polymer concentration $\Phi_p = 0.16$ wt\% (50 μM) in [BMIM][BF$_4$/H$_2$O mixture at $\Phi_{IL} = 0.4$. Shown profile with variation of temperature.
Temperature driven aggregation is often a random process and thus aggregate nucleation rates and lag times can vary from experiment to experiment. This intrinsic variation makes it difficult to compare results between different aggregation experiments. So, we have tried to interpret the Lag time distribution function (Figure 3.7) on course to CP transition for one particular sample of 0.16 wt% PNIPAM of $M_n = 32$ kg/mol. The particles observed by DLS during the lag phase of this solution before the CP tend to have $<d_H>$ in the range of 30-60 nm. The Lag time distribution suggests that the particle size is small initially at a lower temperature, below the CP of sample. But, as the solution approaches its CP and at $T > CP$, dominant effect of phase separation and chain aggregation is reflected from the shorter lag time and higher intensity due to larger size of particles.

Following the approach of Watanabe et al., we have shown the peak hydrodynamic sizes (Rh) for $M_n = 32$ kg/mol, $\Phi_p = 0.16$ wt% and $M_n = 10.3$ kg/mol, $\Phi_p = 0.26$ wt% in Figures 3.8 (a) and (b). The polymer concentration is substantially below the overlap concentration of $\Phi_p^* \approx 1.1$ wt% (32 kg/mol) and $\Phi_p^* \approx 2.2$ wt% (10.3 kg/mol), and therefore the hydrodynamic size of individual coils should decrease with temperature, as observed. For the 32 kg/mol sample, a larger size peak appears at $T \approx 50^\circ$C, and continues to increase with temperature. Similar phenomenon is observed in case of 10.3 kg/mol sample, at $T \approx 50^\circ$C. This result is in qualitative agreement with the cloud point data (Figure 2.4 of Ch-2) supporting PNIPAM chain collapse and aggregation near CP. However further quantitation through viscosity and detailed size measurement would be a plus.
Figure 3.8. Peak hydrodynamic size $R_h$ as determined by dynamic light scattering for (a) $M_n = 32$ kg/mol, $\Phi_p = 0.16$ wt%, $\Phi_{IL} = 0.4$ in [BMIM][BF$_4$]/water. (b) $M_n = 10.3$ kg/mol, $\Phi_p = 0.26$ wt%, $\Phi_{IL} = 0.2$ in [BMIM][BF$_4$]/water.
We measure the intensity correlation functions $g_2(t)$ with the lag time $t$. This indicates the decay character of these functions, as shown in Figures 3.9 and 3.10. The temperature dependence of the normalized intensity autocorrelation function, determined at different stages of polymer volume transition is shown in Figures 3.9-10, along with the changes in the corresponding hydrodynamic diameter $d_H$ distribution shown in the insets. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size ranges and is therefore known as an intensity size distribution. Each scattering center in our study refers to either a free polymer chain or an aggregated complex. Our experimental results (Figures 3.9 and 3.10) are completely independent of any theoretical models based on the coil-globule type CP transition.

The $d_H$ distribution curves plotted show quite symmetrical distribution, as shown in parts (a) and (b) of Figure 3.9, which was measured at 30 °C and 45 °C, respectively. The average measured size remained relatively constant with $<d_H> \approx 4$-6 nm (individual chains), but the intensity of second (II) peak (5-70 nm) (aggregates + single chains), increased from 2 (Figure 3.9a) to a value of 8 (Figure 3.9b), before passing through the CP. The $<d_H>$ value in case of first peak doesn’t change by much, which might be because of coil contraction of single chains when compared with the size of a coil in a good solvent. As the solution approaches its CP temperature, the intensity of second peak starts decreasing and a third peak (III) appears between 30-300 nm. The value of $<d_H>$ increased from 7 nm to 77 nm at 56-60°C. The sudden increase in $<d_H>$ could be due to the presence of a very small amount of PNIPAM aggregates which are growing in size with temperature. The CONTIN method of analysis however could resolve the bimodal
distribution to a trimodal distribution thus. This appearance of a third peak in size distribution, also suggests the possibility of small amount of aggregates. In addition to the \( \langle d_H \rangle \) value increasing constantly, the decrease in intensity of peak II, could imply that the size of single coils is shrinking slowly. The rise and fall of \( \langle d_H \rangle \) value (Figure 3.4) corresponds well with the increase (Figures 3.9c-e) and decrease (Figure 3.9f) in intensity of peak III. In addition, the \( d_H \) distribution after the phase transition (Figure 3.9e) represents an upper limit (\( I_{\text{max}} = 13 \)) since it includes maximum contribution from aggregates. Viewing the correlogram from a measurement can also give a lot of information about the quality of sample. The time at which the correlation starts to decay significantly is an indication of the average size (\( \langle d_H \rangle \)) of the sample. If the decay curve is steeper, the sample is more monodisperse. Conversely, if the decay curve becomes more extended, the greater is the sample polydispersity. Consequently, the sample solution at 30°C and 45°C (Figures 3.9a and b) show a steeper decay compared to 56°C and 65°C (Figures 3.9c and e), hence suggest an increase in polydispersity of PNIPAM particle size due to phase change (insolubility) with increase in temperature. If the particles are large the signal changes slowly and the correlation will persist for a long time (Figures 3.9c, d and e). If the particles are small and moving rapidly then correlation reduces more quickly (Figures 3.9a and b). The time taken by the correlation function to decay completely at 30°C and 45°C was estimated to be 0.17 and 2 ms respectively, whereas that at 56°C and 65°C was estimated to be 1 and 100 ms respectively. The solution at 30°C (Figure 3.9a) had the minimum correlation decay time (0.17 ms) and also smallest \( \langle d_H \rangle \) value of 3.8 nm. Thus, similar to a non-aggregated single PNIPAM
chain in a good solvent. On increasing the solution temperature through CP, at 65°C the decay time was maximum (100ms) and correspondingly largest $<d_H>$ value of 250 nm was observed. At 56°C, closer to the phase transition temperature (Figure 3.9c), the single chain collapse process almost reached a compact form like globules of chains. The intensity of the small globules, with $d_H = 10$-$12$ nm, attained a minimum value of around 4, as shown in Figure 3.9c, and the size of the large-sized aggregates (peak III) actually increased here after. In the trimodal stage (Figure 3.9c), the intensity of the compact globules (and also the aggregated chains) started to change gradually. It is clearly shown in Figure 3.9e that the intensity of the small globules, with $d_H = 10$ nm suddenly increased to 8 (from 4 in Figure 3.9c); this sudden increase in intensity suggests simultaneous formation of both small and large aggregate masses from the collapse of single polymer chains which can be of high or low density, yielding an average $<d_H> = 250$ nm for the larger size masses and for the smaller size masses a broader distribution curve was obtained. This can be supported by the fact that, every polymer chain after undergoing a transition from good solvent to poor solvent condition, shows fast chain collapse (crumpling/globulization) and slow aggregation (entanglement) which was proved by de Gennes [109].
Figure 3.9. Intensity autocorrelation function and distribution of the hydrodynamic dia. (inset), from the CONTIN analysis of solution of PNIPAM $M_n = 32$ kg/mol, $\Phi_p = 0.16$ wt%, $\Phi_L = 0.4$ in [BMIM][BF$_4$/H$_2$O, as computed at different temperatures: (a) 30°C (b) 45°C; (c) 56°C; (d) 59°C; (e) 65°C; (f) 80°C.
The large-sized aggregates could be seen only till 65°C. With the rise in temperature the single PNIPAM chains were undergoing transition from the coil to the collapsed globule, simultaneously the entangled coil in the aggregates could be undergoing further contraction as hypothesized earlier [106]. As shown in the transition from parts e to f of Figure 3.9, the intensity of peak III lessens significantly and the aggregates decreased in size from 250 to less than 50 nm (Figure 3.4) as they began-to contract. The contraction of aggregates became apparent only after a threshold temperature value of 65°C at a later stage of DLS. During the phase transition process for 0.16 wt% (Mₙ = 10.3 kg/mol, Φ_IL = 0.2) PNIPAM, similar behavior as discussed above was seen (Figure 3.10). Similar coil-to-globule type transition and aggregation process could be interpreted from the intensity correlogram results, as in the previous analysis for 0.16 wt% (32 kg/mol) sample. Except in these scattering experiments we observe the peak III appearing at even lower temperatures and remaining throughout. This might be because of the low CP temperature of 0.16 wt% -32K sample (44.6°C) as compared to 0.16 wt% -10.3K sample (57°C).
Figure 3.10. Intensity autocorrelation function and distribution of the hydrodynamic dia. (inset), from the CONTIN analysis of solution of PNIPAM $M_n = 10.3$ kg/mol, $\Phi_p = 0.16$ wt\%, $\Phi_H = 0.2$ in [BMIM][BF$_4$]/H$_2$O, as computed at different temperatures: (a) 39°C; (b) 45°C; (c) 68°C; (d) 71°C; (e) 80°C.
After completing the Dynamic light scattering (DLS) experiments of PNIPAM in aqueous solutions of Ionic liquid [BMIM][BF₄] the mechanism for the interactions between the salt ions and PNIPAM were discussed from a structural point of view. There were findings by DLS measurement that the polymer chain conformations; sizes \(d_\text{H} = 1-10 \text{ nm}\) and large clusters/aggregates; sizes \(d_\text{H} = 100 \text{ nm}\) coexist at temperatures near the CP and beyond. At temperatures much higher than the CP there is enough evidence of chain aggregation of PNIPAM from the sizes. The \(<d_\text{H}>\) of these aggregates increases with temperature. Increase of \(<d_\text{H}>\) is accompanied by an increase of scattered light that clearly indicates the aggregation of PNIPAM chains in water. However at temperatures much higher than the CP, DLS measurement becomes erroneous because of wide scale turbidity and the \(<d_\text{H}>\) starts to decrease. Even in the presence of IL, below phase transition temperature the \(<d_\text{H}>\) is very small which may be due to contribution from the individual PNIPAM chains present in it. After the phase transition or CP, PNIPAM chains start to aggregate forming large particles and with increasing temperature the size of the aggregates further increases due to increased hydrophobic interaction of PNIPAM chains (Figure 3.4, 3.5 and 3.6). Dawson [1, 110] and Wu [4, 74] have observed similar reversible volume transition for dilute PNIPAM solutions undergoing LCST and tentatively propose that the formation of chain steric-assemblies, such as crumpled globules of chains, could compete with aggregation and precipitation. To characterize such LCST type behavior, we had recourse to dynamic light scattering (DLS) and turbidimetry measurements (described in Chapter-2). DLS was primarily used to measure the hydrodynamic diameter of the polymer chains below the CP, and to confirm the onset
of aggregation at the CP. We believe that without collapse of the PNIPAM chains in experimented IL/H₂O solutions, it is unlikely that the phase transition, as observed by the aggregation of the PNIPAM molecules would occur.

3.3.3 Size Distribution in Aqueous solutions of Ionic liquid [BMIM][BF₄]

We also conducted experiments to find the particle size distribution in [BMIM][BF₄]/H₂O mixtures at IL volume fractions, Φₜₐₜ = 0.2 and 0.4 without any PNIPAM. And surprisingly we found that IL solutions without PNIPAM at higher temperatures (> 45°C) showed aggregates of larger average size as compared to that in presence of PNIPAM in mixtures. James Bowers and colleagues [111] have proposed the aggregation behavior in aqueous solutions of 1-alkyl-3-methylimidazolium cation based ILs by means of surface tension, conductivity, and small angle neutron scattering (SANS) measurements. They outline based on their SANS experiments that the shapes and sizes of the aggregates of the short-chain 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF₄] system behave as a “dispersion of polydisperse spherical aggregates” that form above a certain critical aggregation concentration. All the ILs they studied are completely miscible with water over the entire composition range at the temperature of study (298 K). They even reported that [BMIM][BF₄] + water has a critical aggregation concentration of 800 mM at a temperature of 298 K and the IL volume fractions corresponding to their reported critical aggregation values are around 15 vol% for the [BMIM][BF₄] system. Such aggregation behavior of the [BMIM][BF₄] IL should have an effect on our DLS results. Our results confirm that there is some inherent degree of aggregation in the aqueous solutions of IL-[BMIM][BF₄] with the increase of
temperature. Aggregation also depends on the nature of the anion. Aggregates of sizes as big as 200-300 nm average diameter ($<d_H>$) were found in mixtures of IL/water with $\Phi_{IL} = 0.2-0.4$ at 60°C (Figures 3.11 and 3.12). The effect of the anion nature on aggregation has also been studied by Wang et al. [112]. They demonstrated that the anionic effect correlates well with the Hofmeister series of the anions. [BF$_4$]$^-$ anion is known to be a weak kosmotrope and at intermediate IL concentrations $\Phi_{IL} = 0.2-0.5$, [BF$_4$]$^-$ begins to accept protons from water due to hydrogen bonding and may form [BF$_3$OH]$^-$ and HF [113]. It has also been showed that imidazolium ring protons may act as hydrogen-bond donors to F atoms of the counterion in ionic liquids with fluorine containing anion [114]. In our case, the presence of water is likely to replace progressively the C(sp$^2$)-H····F interactions with hydrogen bonds involving water as an acceptor towards the cation and as a donor towards the [BF$_4$]$^-$ ion. The structure of pure ionic liquid is thus changed towards a different organization with a lower degree of ring stacking. In other words, the presence of water makes the imidazolium–imidazolium associations looser, with consequent increase of intermolecular distances between ring protons. At higher temperatures however these [BF$_4$]$^-$-H$_2$O hydrogen bonds get disrupted, which might result in aggregation between IL molecules. Qualitatively similar behavior of coil-to-globule transition and aggregate formation above the CP temperatures could also be found in case of [BMIM][OAc]/H$_2$O systems. However we don’t discuss the data for [BMIM][OAc] systems here since our main focus is to correlate how the observed LCST type phase transition for an IL/H$_2$O system correlates with increase in particle size and aggregate formation above the CP.
Figure 3.11. Displays the z-average particle size as a function of temperature for $\Phi_{IL}$ ([BMIM][BF$_4$]) = 0.4 obtained from cumulant fits, (a) without PNIPAM. (b) with PNIPAM-32 KDa, $\Phi_p = 50 \mu$M (0.16 wt%).
Figure 3.12. Displays the z-average particle size as a function of temperature for $\Phi_{\text{IL}}$ ([BMIM][BF$_4$]) = 0.2 obtained from cumulant fit, (a) without PNIPAM. (b) with PNIPAM-10.3 KDa, $\Phi_{p} = 250$ $\mu$M (0.26 wt%).

This special behavior of IL/water mixtures corresponds with the results of our recent study of volumetric effects in [BMIM][BF$_4$] water mixtures deduced from rheology measurements (see chapter-4). This may also be explained by the formation of strong
hydrogen bonded water networks incorporating PNIPAM chains and IL ions. As the mobility of such hydrogen bonded water clusters with cosolutes in the mixture is lower than that in pure molecular water, the diffusion of any aggregates or clusters of the IL should be observable with heating as those found here and also contributes to \(<d_H>\) [112]. Qualitatively, the link between the volume LCST type behavior and the aggregation comes from the hydrodynamic size measurements. The hydrophilic to hydrophobic transition is the main cause of cloud point behavior of PNIPAM in aqueous solution. It has been stated in other studies that single chains are accordingly less prone to random aggregation and have an increased tendency to crumple or collapse first before forming aggregates [106]. In other terms, the formation of large aggregates above the CP competes with the formation of intra-cluster hydrophobic associations leading to collapsed/dense coils. There still needs to be further understanding on PNIPAM’s LCST transition. It is generally thought that the conformational transition from the extended to the collapsed states is entropy driven [15]. The detailed mechanism of the structural LCST transition is not the subject of this thesis. Instead, the goal here is to discuss the effect of different IL anions on phase transition temperature of PNIPAM solutions.

3.4 Conclusions

DLS was performed on PNIPAM solutions in [BMIM][BF₄]/H₂O mixtures at various concentration of the polymer from 0.16 wt% to 0.26 wt%. The size distribution of the dilute polymer solution was monomodal at T < CP, but broadened when T > CP and bimodal as well as trimodal distribution was observed. The average hydrodynamic size \(<d_H>\) increased with increasing temperature. The intensity of the light scattered by the
aggregates increased at CP, thus indicating that the average density of the chain clusters increased. However, after a particular peak value of average hydrodynamic size upon phase transition, $<d_H>$ starts to decrease. This suggests that the aggregates are metastable and become heavier and settle, though this requires further experimentation. The thermal properties and the aggregate formation of the PNIPAM aqueous solutions depend on the IL and PNIPAM concentration. When phase separation occurs during heating, the size distribution of the aggregates broadens. First we investigated the well known coil-to-globule transition for PNIPAM chains with concentrations lesser than the overlap concentrations ($\Phi_p^*$. The collapse and aggregation of the chains was observed as the solutions approach their CP, shown in a manner similar to that previously reported for PNIPAM [106, 110]. We were able to see up to a 20 times increase in the average hydrodynamic size $<d_H>$ after the CP transition where the scattering intensity due to chain aggregation was maximum. However, further increase in temperature leads to a well known combined collapse-aggregation process where individual polymer chains collapse and aggregate simultaneously to form small, relatively compact globules [16]. These aggregates are mesoscopic in nature, and in order to indicate that each average aggregate consists of many collapsed polymer chains, we have shown intensity size distribution and correlation decay functions following similar literature studies [106]. Upon further raising the temperature, the aggregates got denser and slightly decreased in size and finally became essentially unstable at high temperature. Our additional experiments have shown that globules of PNIPAM for $M_n = 10.3$ kg/mol are constant in size up to 65°C after surpassing CP. To be precise, a range of temperatures up to 65°C
were studied in each experiment. We hypothesize this based on the fact that, beyond the CP in dilute aqueous solution of PNIPAM, the chains are metastable in form of individual collapsed coils. However, at higher temperatures the collapsed chains become denser and compact, which are composed of a number of polymer chains, with relatively multi-modal size distribution. Our observations of average hydrodynamic size and size distribution confirm aggregation with temperature rise that are in good agreement with the literature DLS data for similar PNIPAM systems, and suggest LCST type phase separation and clouding.
CHAPTER 4

VISCOELASTIC BEHAVIOR OF PNIPAM IN AN IL/WATER MIXTURE UPON LCST TYPE PHASE TRANSITION

4.1 Introduction
Rheology of polymer/IL solutions has received considerable attention recently in order to understand various macromolecular interactions in IL [49] [66]. There are numerous reports on the use of these polymer systems in the development of novel “smart” gels, which have applications in the controlled drug delivery, bioseparations and robotics [115]. In view of their potential practical applications, understanding the solution behavior becomes essential. To gain further knowledge on PNIPAM phase behavior in [BMIM][BF₄]/H₂O mixtures, we perform rheological experiments on PNIPAM solutions at different temperatures and focus on the viscoelastic response of these systems with respect to applied frequency. Such solubility behavior using ILs have been reported not only for synthetic polymers but also for protein based solutions [66]. We show that, at all concentrations and temperatures viscosity clearly dominates over the elasticity and exhibits an onset to an almost frequency independent plateau, characteristic of viscoelastic polymer solution. Similar viscoelastic measurements have been done by Senff and Richtering [116] in the low-shear viscosity range of PNIPAM microgel particles as a function of temperature. Lately, Zeng et al.[17] investigated the network formation in the system water/PNIPAM by dynamic viscoelastic measurements. We are interested in ionic liquid (IL): [BMIM][BF₄] and its aqueous mixtures as the solution
media for PNIPAM. Nevertheless, the details of the rheological behavior leading to homogenous single phase or phase separation (aggregation) in ILs remain unclear and there has been little rheological study reported for PNIPAM in IL/H$_2$O mixtures.

So far, we have studied the CST type behavior of PNIPAM solution in ILs by using DLS and turbidimetry. We then explore rheology to characterize the viscoelastic nature of chains and the aggregation of PNIPAM in a widely used hydrophilic IL, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF$_4$]). In chapter-3 we proposed that there is a hysteresis for the phase transitions as measured by DLS and by visual cloud point observations. Which is a consequence of rearrangements that may occur in the collapsed state of PNIPAM, as segmental mobility is expected to be high since the solutions are still dilute. The above mechanisms describe the interactions between segments in the same macromolecule. Although such interaction in PNIPAM has been studied extensively previously, there needs to be more insights on their temperature dependent viscoelastic nature. Polymer solutions can undergo dramatic changes when subjected to shear flow like from turbid to clear, transparent to cloudy and undergo phase separation and precipitation. The influence of shear, oscillation frequency and stress on the viscoelastic behavior of polymer solutions has a direct relevance to the application of polymers. Therefore, we make an attempt to study these phenomena here in IL/H$_2$O mixtures, on passing through the CP of a high $M_n$ (186.8 kg/mol) linear PNIPAM solution at 0.8% w/w concentration, a medium $M_n$ (32 kg/mol) linear PNIPAM solution at 1.5% w/w concentration, as a function of increasing temperature.
We have studied a range of polymer concentrations covering the dilute solution regime ($\Phi_p \ll \Phi_p^*$ (chain overlap concentration)), where coil overlap is absent, and semi-dilute concentrations ($\Phi_p > \Phi_p^*$), with an idea of characterizing the formation of chain networks through rheology. Therefore, based on the rheological behavior any chain hydrophobic entanglements formed at the lowest polymer concentrations should involve widespread intramolecular associations. However, we do not predict based on our experimentation whether at the lowest concentrations the polymer exists as single chains or aggregates. When the polymer concentrations exceed $\Phi_p^*$ both intra- (topological) and intermolecular associations can happen due to hydrophobic interactions [17]. Also, at higher concentrations of linear PNIPAM (1 wt%), solutions develop extensive aggregation after the CP. This is shown in our viscoelastic measurement data. LCST type phase separation and clouding is due to unfavorable solute-solvent interaction (i.e., it results from the formation of PNIPAM-interchain linkages which arise due to a net attractive force between chains). When such linkages are formed extensively and span the sample volume, the result is large scale globular aggregation giving a visibly cloudy solution (i.e., a three-dimensional network of PNIPAM chains) [16, 117]. During the process of phase separation, there is a transition of the initial homogenous PNIPAM solution into a self-aggregated chain system which exhibits inhomogeneous viscoelastic characteristic. Since many of the applications of PNIPAM revolve around its volume phase transition properties, an understanding of the parameters which control the solvent quality change from good to poor is important in understanding its solubilization. The volume phase transition of PNIPAM has been intensely studied for macroscopic gels and
also for microgels and linear PNIPAM chains where the coil to globule transition is of particular interest [16, 117, 118]. However, there are only very few studies on rheological properties of PNIPAM with ILs as solution media. We believe that such temperature dependent volume phase transition of the PNIPAM chains give rise to changes in the polymeric network and subtle alterations in their flow behavior [119, 120].

4.2 Rheology - Materials and Measurement Protocols

Rheological measurements were performed on a stress controlled rheometer (AR2000ex, TA instruments, USA), with a cone-plate sensor over an extended time at different temperatures. Conventional cone-and-plate geometries were employed for the measurement: cone angle of 2°, acrylic cone diameter of 40 mm. Temperature control was achieved with a circulating water bath. The measuring unit was equipped with a temperature unit (Peltier plate) providing a rapid change of the temperature and temperature control (± 0.05°C). The gap between the center of the cone and the plate was 0.105 mm. To prevent the loss of water due to evaporation and the absorption of atmospheric moisture, a thin film of light silicone oil was applied around the outer radial edge of the platens to prevent contact of the test fluid with air. The effect of this oil film on the measured rheological properties was assessed by testing pure ionic liquid with and without the applied oil film, and was found to be negligible.

To probe the volume phase transition and hence the viscoelastic interactions present in our polymeric solutions, we undertook rheological studies under steady and dynamic shear [121]. In steady state shear rheology, the (apparent) viscosity was determined as a
function of shear rate under flow conditions. In dynamic rheology on the other hand, the sample was subjected to a low-amplitude oscillatory shear, and the response of the material was recorded in terms of two parameters: the elastic modulus ($G'$) and the viscous modulus ($G''$). The variation of $G'$ and $G''$ with oscillation frequency provided a signature of the viscoelastic property of the system [122]. In dynamic rheology the solutions can be probed in their at-rest state without causing any changes in their solubility behavior [121]. Thus, dynamic rheology might offer a more direct correlation with temperature dependent viscoelastic properties of PNIPAM than steady state rheology. To erase any previous shear histories and allow the samples to equilibrate, a steady pre shear was applied at a shear rate of $1 \text{s}^{-1}$ for 60 s followed by a 120 s rest period before each dynamic rheological measurement. Measurements were made at a series of temperatures over the range of $25\text{°C}$ to $70\text{°C}$, with equilibrated heating. $5\text{°C}$ temperature steps were applied at low and high temperatures, smaller steps were applied for the measurements taken around the transition temperature ($30–40\text{°C}$). For the measurements at a constant shear, a stress of $0.01 \text{Pa}$ was applied at various temperatures, ranging from $25\text{°C}$ to $70\text{°C}$. The viscosity of samples were measured using a steady state shear flow protocol over a range of shear rates from $10 \text{s}^{-1}$ to $1000 \text{s}^{-1}$ and each stress applied for 180 s in order to obtain steady flow. Since all the sample solutions were dilute, the region of linear viscosity response to shear rate was taken to be the viscosity of sample.

The same rheometer was also used in the oscillatory shear mode. For the present measurements a frequency range of $0.01$-100 Hz was chosen. To compute the elastic
modulus \((G')\) and viscous modulus \((G'')\), two types of measurements were made at each
temperature, i) a sweep of increasing oscillatory stress \((\sigma)\) at constant frequency then, ii)
a frequency sweep at a single stress. In oscillatory shear measurements, one initially
fixes the frequency \((\approx 0.1 \text{ Hz for dilute, semidilute solutions})\) and measures the
rheological parameters as a function of the stress amplitude [119]. To determine the
linear viscoelastic limit in our samples, a dynamic measurement as a function of stress
amplitude (typically, 0.1-15 Pa) was conducted before each dynamic frequency sweep
measurement. The frequency sweep was performed at constant oscillatory stress
amplitude (typically between, 8-10 Pa). Evaporation of water was minimized by using a
beaker containing water to ensure a saturated environment around the sample. As the
viscosity of the samples is in general very low (0.5 to 10 mPa.s), particular care was to be
taken to avoid measurement artifacts and so studies were made only at low stress. The
data were recorded after 300 s thermal equilibration time. Data recorded during the first
180 s of application of stress were not used for fitting.

4.3 Results and Discussions
Concentration dependence of linear viscoelastic response: Dynamic rheology and
Steady state shear rheology

4.3.1 Dynamic- Elastic and Viscous Modulus
To determine the mechanical properties of our dilute solutions, we perform oscillatory
rheology in the linear stress regime and determine the viscous \((G'')\) and elastic moduli
\((G')\) as a function of frequency \(\omega\) for constant oscillatory stress values. Figures 4.1 and
4.2 present the frequency dependence of the dynamic viscoelastic properties of PNIPAM
of $M_n = 32$ kg/mol polymer in [BMIM][BF$_4$]/H$_2$O mixtures for $\Phi_{\text{IL}} = 0.3$ and 0.4 at constant polymer concentration $\Phi_p = 1.5$ wt%. As clearly shown in Figure 4.1a at 35 °C the solution containing 1.5 wt% of the PNIPAM (CP $\approx 40$ °C) exhibits a frequency-dependent viscous modulus ($G''$) that is higher than its elastic modulus ($G'$) by 1 order of magnitude at lower frequencies; the system behaves as a dilute solution (polymer chains in coil conformation). This indicates that the PNIPAM chains are stable with the $G''$ is higher than $G'$ over the low frequency range (0.1-2.5 Hz) with a strong frequency dependency as expected for a non-aggregated solution containing 1.5 wt% of the PNIPAM which is substantially over the overlap concentration $\Phi_p^* = 1.1$ wt%. At about 2.5 Hz there is crossover of the two moduli and $G'$ is higher than $G''$. This indicates a transition from purely viscous-like behavior to partially elastic-like behavior at higher frequencies of oscillation. Consistent with this, the solution also develops aggregation or chain collapse at higher temperature (Figure 4.1c) marked by the decrease in crossover frequency of $G'$ and $G''$ with increasing temperature. We interpret this decrease in crossover frequency as the seeding of PNIPAM chain aggregates upon passing through CP (CP $\approx 40$ °C) and the increase in viscous modulus is a measure of the structural relaxation of the solution. Figure 4.2 shows similar behavior as in Figure 4.1. Except that this solution has a higher cloud point of 50 °C.
Figure 4.1. Storage/elastic ($G'$, filled symbols) and loss/viscous ($G''$, hollow symbols) moduli as a function of frequency for solutions of PNIPAM with $M_n = 32$ kg/mol at $\Phi_p = 1.5$ wt% in [BMIM][BF$_4$/H$_2$O mixtures at constant IL volume fraction $\Phi_{IL} = 0.3$ (CP $\approx 40$ °C) at (a) 35°C, (b) 40°C, (c) 50°C.
Figure 4.2. Storage/elastic ($G'$, filled symbols) and loss/viscous ($G''$, hollow symbols) moduli as a function of frequency for solutions of PNIPAM with $M_n = 32$ kg/mol at $\Phi_p = 1.5$ wt% in [BMIM][BF$_4$]/H$_2$O mixtures at constant IL volume fraction $\Phi_{IL} = 0.4$ (CP $\approx$ 50°C ) at (a) 30°C, (b) 50°C.

Similar dynamic moduli measurements were determined in case of higher molecular weight polymer of $M_n = 186.8$ kg/mol, $\Phi_p = 0.8$ wt%, $\Phi_{IL} = 0.4$ (Figure 4.3). For the 0.8 wt%, $\Phi_{IL} = 0.4$ (CP = 37 °C) sample in the low frequency region ($\omega<1$), $G'$ and $G''$ are proportional to $\omega^{1.9}$ and $\omega^1$ respectively, before and after CP, which is characteristic of a viscous liquid [123]. This is also close to the theoretical low-frequency limit of $\omega^2$ and $\omega^1$ for $G'$ and $G''$ respectively in case of Maxwell’s fluid. At such dilute regime, the solutions exhibit the terminal behavior expected for a Newtonian fluid: $G'' > G'$, at low $\omega$; $G' > G''$, at high $\omega$. On the other hand, for $\omega>1$, as shown in Figures 4.1-3 there is a viscoelastic transition. This reflects a transition from liquid like to partially elastic like behavior at higher $\omega$. 

90
Figure 4.3. Storage/elastic (G', filled symbols) and loss/viscous (G'', hollow symbols) moduli as a function of frequency for solutions of PNIPAM with Mn = 186.8 kg/mol at Φp = 0.8 wt% in [BMIM][BF4]/H2O mixtures at constant IL volume fraction ΦIL = 0.4 (CP ≈ 37°C) at (a) 30°C, (b) 50°C.

There is a shift in the critical crossover frequency as seen for the case of the high molecular weight polymer, PNIPAM-186K at Φp = 0.8 wt% and ΦIL = 0.4 (v/v), for the response of G' and G'' with the applied oscillatory frequency. The crossover frequency before the cloud point transition (30°C) is more than twice at f = 5.011, compared to the value after the cloud point transition (50°C) at f = 1.995. Also, the ratios G''/G' at high and low temperatures just before crossing over are G''/G' = 1.424 and G''/G' = 1.217 respectively. Since, G''/G' > 1 the solution has dominantly a viscous behavior.

4.3.2 Dynamic Viscoelastic response

It is interesting to note that along with the frequency-dependent behavior of G' and G'', the effect of the increasing IL content can be seen for solutions by plotting their crossover
frequencies. This is clearly shown in Figure 4.4, where the crossover frequency \( f \) of both moduli is plotted as a function of temperature at different \( \Phi_p \) and \( \Phi_{IL} \). Since the increase in \( G' \) is much more sensitive to the [BMIM][BF₄] content than that of \( G'' \), the moduli of the mixtures at \( \Phi_{IL} \geq 0.3 \) further cross at lower frequencies. The following conclusions can be drawn from this: (a) at fixed \( \Phi_p \) and \( \Phi_{IL} \), crossover frequency \( f \) decreases with increase in temperature; (b) at fixed \( \Phi_p \) and \( T \), crossover frequency increases with increase in IL volume fraction. The viscoelastic response for intermediate frequencies \((0.1-2 \text{ s}^{-1} < \omega < 10 \text{ s}^{-1})\) exhibits a much stronger frequency dependence as for a viscous non-elastic polymer suspension. Such a behavior characterizes the viscoelasticity of the polymer/IL/water mixtures, and it is generally explained by assuming the existence of a strong volume-spanning hydrogen bond network [124]. In contrast with these systems, however, we do not notice a clear low-frequency plateau for \( G' \) and \( G'' \) even after the CP temperature. This suggests that the whole phase of our solution might be forming mesoscopic aggregates slowly and continuously with increasing temperature but the aggregation phenomenon is not so strong to be reflected in modulus values.
Figure 4.4. Comparison of crossover frequencies of \( G' \) and \( G'' \) (frequency at \( G' = G'' \)), their variation with temperature, \( \Phi_{IL} \) and \( \Phi_P \) being 1.5 wt% for 32K and 0.8 wt% for 186K.

### 4.3.3 Steady State Shear and Viscosity Responses

There are certain factors which play an important role during the imposed deformation/shear on entanglements of the polymer chains. For LCST type systems, there are reportedly two principally different modes of energy storage under shear: \[125\] The normal mode, which results from chain entanglements (entropic in nature) and another mode (of enthalpic nature) related to particular chemical structures. In the former case, the first experimentally observable effect upon a gradual increase of shear rate results in shear-induced mixing whereas in the latter case, shear-induced de-mixing is observed. However at higher PNIPAM concentrations, there should be contribution from both chain entanglements and hydrophobic associations to the network. Therefore, the response of the system under steady shear is a net result of the loss of the steady-state chain
entanglement density and the formation of any intermolecular hydrophobic associations [126]. Loss in the density of topological entanglements often gives a shear thinning behavior to solution [127]. We observed similar shear thinning behavior of the PNIPAM solutions at lower rates of shear, and further increases with increase in PNIPAM concentration. At higher rates of shear our PNIPAM systems typically exhibited a linear (Newtonian) response. At higher rates of shear, the time scale of the subjected shear might be small, compared to the rate of formation of hydrophobic associations. Hence there might not be enough time for the chains to interact with each other.

The shear-rate responses of viscosity for the PNIPAM solution are shown in Figures 4.5 and 4.6. The shear influence on the flow behavior of a moderately concentrated aqueous solution of PNIPAM is shown in Figure 4.5. Even at T < 40 °C, well below the phase separation temperature of the polymer (i.e. 55-60 °C) for different volume fractions of IL the solutions show initial shear thinning. There is some shear thinning at shear rates > 10 s⁻¹, similar to polymer solutions at concentrations near the chain overlap concentration due to chain disentanglement processes [127]. Qualitatively it could arise due to preference for inter-segmental interaction over interaction between solvent molecules and polymer chains at low shear. However, this behavior became steady at higher shear rates. At higher shear rates, the steady state shear data (Figure 4.5) showed a Newtonian type plateau, also the value of zero-shear viscosity was low (< 0.1 Pa s).
Figure 4.5. Shear rate dependence of steady shear viscosity of PNIPAM solutions with $M_n = 32$ kg/mol and constant polymer concentration of $\Phi_p = 1.5$ wt% measured at temperatures of 30°C, 35°C, 40°C, 50°C and 55°C for 2 different IL volume fractions. (a) $\Phi_{IL} = 0.3$ and (b) $\Phi_{IL} = 0.4$. 
Figure 4.6. Shear rate dependence of steady shear viscosity of PNIPAM solutions with $M_n = 32$ kg/mol and constant polymer concentration of $\Phi_p = 0.13$ wt% measured at various temperatures of run for different IL volume fractions. (a) $\Phi_{IL} = 0.1$, (b) $\Phi_{IL} = 0.2$, and (c) $\Phi_{IL} = 0.3$. 
We also observe an initial decrease in the viscosity (shear thinning) at lower shear rates in case of some samples (Figure 4.6) over a narrow range of shear rates. This can be explained by considering the fact that in this sample, $\Phi_p (0.13 \text{ wt\%}) \ll \Phi_p^* (1.1 \text{ wt\%})$. At lower shear rates the primary chains of PNIPAM are disrupted by application of shear. One could speculate that this observation is due to the formation of comparatively stable clusters of PNIPAM chains upon prolonged standing at the beginning. Such inhomogenities may result from the clusters of PNIPAM chains that are more compact and dense compared to the rest, resulting in a microphase separated solution comprising of a polymer rich phase and a polymer lean phase and thus have a lower viscosity than a completely homogeneous mixture. On the basis of these hypotheses one could understand the findings if one postulates that at high enough shear rates such clusters are disrupted and that the solution shows steady Newtonian behavior. Such observations in our system clearly indicates the existence of both PNIPAM chain entanglements and covalent/non-covalent interactions such as H-bonding and hydrophobic interactions, which are responsible for the flow properties.

Figure 4.7 displays the flow curves at temperatures of 30–50°C, i.e. through the CP, for different volume fraction of [BMIM][BF$_4$]/H$_2$O mixtures with a high $M_n$ PNIPAM-186K. The viscosity at 30 °C is always higher compared to the viscosity at 50 °C, and also at all temperatures the viscosity at the lower frequency is comparatively more than that measured at higher frequencies until it starts to plateau, meaning that the low-shear viscosity steadies with frequency of shear. At a particular volume fraction, $\Phi_{IL}$, the viscosity of the PNIPAM solution was evaluated by performing steady-state shear tests,
and the frequency independence of the viscosity reflects the Newtonian behavior of solution. On increasing temperature, the shear rate dependence of viscosity falls more rapidly as for any Newtonian solution. The slight variation in viscosity and shear thinning behavior at low shear rates for 0.13 wt% PNIPAM suggests that since [BMIM][BF₄] successfully competes for hydrogen bonds to dissolve PNIPAM and is hygroscopic, perhaps differences in water of hydration might account for the unusual observations.
Figure 4.7. Shear rate dependence of steady shear viscosity of PNIPAM solutions with $M_n = 186$ kg/mol and constant polymer concentration of $\Phi_p = 0.8$ wt% measured at temperatures of 30°C, 40°C and 50°C. (a) $\Phi_{IL} = 0.3$, (b) $\Phi_{IL} = 0.4$, and (c) $\Phi_{IL} = 0.6$. 
However, several investigators noted the stability against macroscopic aggregation/precipitation of PNIPAM clusters or multimolecular aggregates in much more concentrated solutions [1, 128]. For example, Chan et al. [128] reported the formation of colloidally stable dispersions of microphase separated PNIPAM ($M_w = 547,000$ g mol$^{-1}$) in solutions of concentration up to 1.0 g L$^{-1}$. The diameter of their PNIPAM aggregates increased as a function of the cube root of PNIPAM concentration. Based on their observations, one could account for the unexpected stability of aggregates similar to our system. One may hypothesize depending on the solution concentration that, individual globules are not stable; they coagulate, yielding larger sized globules. These grow in size until a stable population of chains is reached. This results in increased Van der Waals forces of attraction between particles due to an increase in particle size and after some extent the aggregation becomes entropically unfavorable. So, although macroscopic aggregation takes place due to LCST type phase separation, at such dilute solution conditions PNIPAM in [BMIM][BF$_4$]/H$_2$O mixture behaves dominantly as a viscous fluid.

4.3.4 Influence of Temperature – Viscoelasticity of the Bulk Solution

We also studied the interactions between macromolecules on passing through the cloud point. We reported the viscosity of a low molecular weight PNIPAM ($M_n = 32$ kg/mol) solution at concentration of 0.13 % w/w as a function of increasing temperature. As, can be seen from Figure 4.8, the viscosity ($\eta$) of the solution is at 0.0013 Pa.s at $T < CP$, there is a steady decline in $\eta$ to a value of 0.001 Pa.s as the solution approaches its CP $\approx 36^\circ$C, with a sudden jump down in viscosity at 36$^\circ$C. This indicates the volume phase transition
of the solution due to insolubility of PNIPAM chains in IL/H$_2$O mixture. Our main investigations have focused on steady shear viscosity, while the non-linear variations in viscosity behavior have been interpreted qualitatively. As a result of the coil to globule type transition through CP, PNIPAM chains might form mesoscopic aggregates which reduces the bulk viscosity of solution upto some extent.

![Viscosity-Temp](image)

**Figure 4.8.** Variation of viscosity of PNIPAM solutions with temperature for $M_n = 32$ kg/mol, constant polymer concentration of $\Phi_p = 0.13$ wt% and $\Phi_{IL}$ ([BMIM][BF$_4$]) = 0.2 measured from 25°C to 40°C.

Rheological properties of 0.13 wt% PNIPAM in [BMIM][BF$_4$]/H$_2$O mixtures shows that, while all the solutions exhibited a shear thinning behavior at low shear rates, for higher shear rates there existed partial shear thickening region under all temperature conditions. There might be partial chain aggregation, even in dilute solutions, due to strong hydrogen bonding interactions among PNIPAM chains. For example Figure 4.6 displays flow curves for a 0.13% w/w PNIPAM-32K solution upon heating within a range of applied
stresses. In Figure 4.6, from low to high temperature runs, the solution is Newtonian and the viscosity decreases with increasing temperature, behavior that is in agreement with expectations at such low ionic liquid concentration (i.e. volume fraction) and the continuous phase (water) viscosity decrease is the major contributor. It is important to note that the same behavior is obtained with shear rate sweeps as well as upon decreasing the applied stress. Similarly, at temperatures well above the CPs, the rheology is Newtonian too but the fall in viscosity with temperature is less as the PNIPAM chains have already collapsed into aggregates. The fall in viscosity is rather continuous for all the PNIPAM solution systems under consideration with the increment in temperature; the expected behavior of any Newtonian solution. At higher polymer concentrations i.e. > 0.5 wt% (Figure 4.5) but same shear stress regime, we see a weak shear-thinning behavior followed by a constant viscosity behavior, extending over a wide range of applied shear stress for different volume fraction of IL. It is possible that both chain entanglements and hydrophobic associations persist in this concentration regime. Hydrophobic associations lead to structuring in chains, while loss of entanglements with shear results in shear thinning generally seen in polymeric systems [127]. The competition between these two phenomena should result in the observed shear thinning behavior followed by a viscosity plateau.

We now resort to qualitative explanation of the solution rheology, highlighting the changes in network structure that may occur on variation of the polymer concentration, IL/H₂O mixture ratio and under shear. In the absence of an externally imposed deformation, and below the CP, PNIPAM chains may be considered to adopt a random
coil-like conformation. At such conditions and lower concentrations of PNIPAM, any hydrophobic chain association present is due to intramolecular interactions. However on application of a steady shear, these coils elongate and the elongated coils tend to favor the formation of intermolecular associations over intramolecular interactions. This might be the reason for unsteady viscosity behavior upon initial shear. However on increasing PNIPAM concentrations, both chain entanglements and hydrophobic interactions contribute to the network structure formation. Therefore, the net response of the system under steady shear is the cumulative result of loss of the steady-state chain entanglement populations and the formation of intermolecular hydrophobic associations. The resulting shear thinning behavior is driven by loss of PNIPAM chain entanglements [127]. This effect is observed here at lower shear rates and is more apparent at higher temperatures as seen for Figures 4.6a-c i.e. the dip in the steady state shear curves increases as the sample solution is sheared at higher temperatures.

4.4 Conclusions

The interrelation between the phase separation behavior and the rheological properties of [BMIM][BF₄]/H₂O mixtures of a low and high molecular weight (Mₙ = 32 kg/mol and 186 kg/mol) PNIPAM was investigated. Rheology have been performed on PNIPAM solutions in varying [BMIM][BF₄]/H₂O mixtures with a concentration range of 0.13, 0.8 and 1.5 wt% of PNIPAM with Mₙ of 32 and 186 kg/mol at different temperatures. The steady shear viscosity of PNIPAM solution increased with the increase of solution concentration and simultaneously decreased with the decrease in temperature. The effect of temperature on viscosity of PNIPAM in [BMIM][BF₄]/H₂O solutions show that
increase of solution temperature would lower the viscosity of the solution in accordance with Arrhenius relationship. The viscosity of the ILs is sensitive to temperature, e.g., the viscosity sharply changes when the ILs are at lower temperatures, the viscosity of the ILs with high viscosity values is especially sensitive to temperature. Viscosities of IL/H$_2$O mixture decrease dramatically when increasing temperature. While the evolution of the volumetric properties of ILs in the presence of water is almost negligible (1–2%), the presence of water strongly decreases the viscosity of the samples. Such a sensitivity of ILs viscosity to temperature has been indicated in other literatures [6, 129, 130]. From the concentration dependence of steady state shear viscosity of PNIPAM in [BMIM][BF$_4$]/H$_2$O solutions at different temperatures, it can be concluded that 0.13-1.5 wt% PNIPAM solutions are considered as dilute solutions due to almost linear viscosity increase with increasing concentration. The linear viscous behavior at higher shear rates indicates that PNIPAM-solvent interactions are more dominant than PNIPAM inter chain interactions in this concentration range. Both storage modulus ($G'$) and loss modulus ($G''$) increased with the increase in PNIPAM concentration. The crossover frequency of $G'$ and $G''$ is shown to decrease as the concentration and volume fraction of IL increased, which indicates that the chain entanglement or cross-links enhanced with the increased PNIPAM or IL concentration. The increasing value of frequency-dependent $G'$ is the most important meaningful parameter because the increasing $G'$ is a dynamic parameter that is relevant to interactions in the solution system. Indeed, the variation of $G'$ for the dilute PNIPAM solutions was strongly correlated with the purely viscous interaction between chains.
In conclusion, one can state that the rheological behavior in its nature is determined by physical associations between the polymer segments. The formation of these associations results from the fact that PNIPAM contains both hydrophilic (–CONH) and hydrophobic (–CH₃) groups. The interaction parameter between these polymer segments and water is made up of both contributions. At low temperatures, polymer segment and solvent molecule interactions are sufficiently favorable (because of the supporting enthalpy) and the polymer is completely soluble. However, upon an increase of T the entropic penalty for the formation of a particular arrangement of the components in the mixture, required for the favorable interaction between them becomes so large that the system separates into the two-phase state showing LCST type behavior.

*Supplemental Information*

**Oscillatory stress Measurements**

Subsequent to steady state flow experiments, we applied oscillatory stress fields of varying magnitude to the samples. Figure S1 shows evolution of the viscous (G″) and the elastic (G′) modulus at a particular temperature for different shear stress amplitudes for a system with PNIPAM of M_n = 32 kg/mol, Φ_p = 1.5 wt% and Φ_IL = 0.3 of [BMIM][BF_4]. At low stress amplitudes, the elastic modulus (G’) gradually increased with respect to stress whereas the viscous modulus (G″) remained steady constant. This means that, in this limit, the solution is primarily a viscous fluid and evolution of viscous modulus does not depend on the magnitude of oscillatory stress. It can be seen that G″ is always higher than G’ for a particular value of stress. As the stress amplitude increased, evolution of G’ with stress showed progressively sluggish increase at larger stress amplitudes.
Interestingly at around 10 Pa oscillatory stress amplitude, both the moduli $G'$ and $G''$ become steady and almost are parallel to each other with stress. Figures S1- S3 compare the stress dependence of the elastic and viscous moduli at temperatures of 30 and 50°C, i.e. through the LCST type phase transition. At 30°C, the moduli at lower oscillatory stress measurements are the same as measured at 50°C, meaning that the low-stress modulus is independent of temperature. Newtonian behavior becomes obvious: the low stress elastic modulus ($G'$) increases between values of 0.3 and 10 Pa for both 30 and 50°C while the high-stress modulus continues to become steady after 10 Pa and approaches a steady value. The region where $G'$ and $G''$ are independent of stress is clear for the Newtonian behavior, as seen for temperatures of 30°C and 50°C.
**Figure S1.** Oscillatory stress dependence of storage/elastic ($G'$, filled symbols) and loss/viscous ($G''$, hollow symbols) moduli of PNIPAM solutions with $M_n = 32$ kg/mol and constant polymer concentration of $\Phi_p = 1.5$ wt% measured at temperatures of (a) 30°C and (b) 50°C for IL volume fraction $\Phi_{IL} = 0.3$ (CP $\approx 40$ °C).
Figure S2. Oscillatory stress dependence of storage/elastic ($G'$, filled symbols) and loss/viscous ($G''$, hollow symbols) moduli of PNIPAM solutions with $M_n = 32$ kg/mol and constant polymer concentration of $\Phi_p = 1.5$ wt% measured at temperatures of (a) 30°C and (b) 50°C for IL volume fraction $\Phi_{il} = 0.4$ (CP ≈ 50 °C).
Figure S3. Oscillatory stress dependence of storage/elastic ($G'$, filled symbols) and loss/viscous ($G''$, hollow symbols) moduli of PNIPAM solutions with $M_n = 186$ kg/mol and constant polymer concentration of $\Phi_p = 0.8$ wt% measured at temperatures of (a) 30°C and (b) 50°C for IL volume fraction $\Phi_{IL} = 0.4$ (CP $\approx 37$ °C).
Time sweep experiments- viscoelastic moduli variations

We also conducted Low-frequency ($\omega = 0.063$ rad/s) time-sweep experiments to investigate the temporal evolution of the viscoelastic properties for PNIPAM-32K, $\Phi_p = 0.13$ wt%, $\Phi_{IL} = 0.3$ (v/v) (Figure S4). The relaxation behavior of the samples were evaluated by means of oscillatory shear frequency scans in the linear regime. To extend the analysis over longer time scales, stress relaxation experiments were also performed. The hydrodynamic size of the PNIPAM chains in the corresponding solution was determined using DLS. As shown in chapter-3 PNIPAM of $M_n = 32$ kg/mol, $\Phi_p = 0.13$ wt% in different [BMIM][BF$_4$]/H$_2$O mixtures consist of polydisperse sized clusters/aggregates interspersed in the polymer solution. Each cluster might consist of several primary PNIPAM chains, which are densely packed inside the aggregate complex. The clusters are nonequilibrium structures rearranging towards a more favorable thermodynamic state during a subsequent aging above the CP temperature (CP $\approx 45 \, ^{\circ}\text{C}$). We tried to evaluate such internal rearrangements by imposing a low-frequency ($\omega = 0.063$ rad/s) oscillatory shear in the linear regime and measuring the elastic and viscous moduli as a function of time for more than 2 hr. The weak time dependence of the shear moduli as well as the DLS data (chapter-3) indicates that the structure of the chains is not changing drastically with time. Our results suggest that a saturated solution of PNIPAM in aggregate state forms rapidly at $T > CP$ and does not rearrange significantly over time. Overall, in such dilute regime the chains have enough space to diffuse freely as individuals or clusters, which produces no change in bulk elasticity. Flow curves of the steady shear viscosity plotted against shear rate closely
follow the frequency dependence of the complex viscosity acquired using oscillatory shear.

**Figure S4.** Time dependence of storage/elastic ($G'$, filled symbols) and loss/viscous ($G''$, hollow symbols) moduli of PNIPAM solutions at fixed oscillatory stress value with $M_n = 32$ kg/mol and constant polymer concentration of $\Phi_p = 0.13$ wt% measured at 50°C for IL volume fraction $\Phi_{IL} = 0.3$. 
CHAPTER 5

CONCLUSION

In this dissertation the experimental values of phase transition temperatures of the ternary system \{H2O + IL + PNIPAM\} were measured at different PNIPAM concentration (\(\Phi_P\)) and volume fraction of I onic liquid (\(\Phi_{IL}\)). A comparison between [BMIM][BF\(_4\)] and [BMIM][OAc] shows that the former produces an extensive LCST type region till \(\Phi_{IL} = 0.45\). The difference between these two salts resides only in their chemical structure, which suggests an important role played by their hydration structure in aqueous solutions. Although both [BMIM][BF\(_4\)] and [BMIM][OAc] are miscible with water in any proportion, each IL demands a different type of hydration structure. Because the [OAc]\(^{-}\) anion has a stronger dipole moment, its hydration process probably leads to a less significant disturbance in water structure and promotes a less extensive dehydration of PNIPAM segments. On the other hand [BF\(_4\)]\(^{-}\) anion has a weaker dipole moment. This means that the hydration shell involving [BMIM][BF\(_4\)] is less stable and it is more likely to create a larger disturbance in the structure of water molecules when compared with [BMIM][OAc], thus promoting an extensive LCST type behavior. Also compounds like [BMIM][BF\(_4\)] are considered to be weaker kosmotropes (structure makers) and interact more favorably with water limiting the stability of hydrogen-bonded water structure. However, the effect of any co-solute as a ‘structure maker’ is due to the formation of its favorable hydration structure with water, beyond which the so-called hydrophobic interaction may be dominant, subsequently leading to phase separation. In contrast to the above works that were restricted to the water rich regime, the phase behavior in the IL-
rich regime depends much more strongly on the nature of the IL anion. In neat [BMIM][OAc] we find that PNIPAM is completely soluble in the temperature range studied (Chapter-2, Figure 2.5(a)). Similar behavior is observed for neat [BMIM][SCN] (data not shown). On the other hand, in neat [BMIM][BF₄], PNIPAM is only soluble above 85°C thus showing an UCST type behavior. Whereas at lower temperatures in neat [BMIM][BF₄], PNIPAM appears as an insoluble dispersed phase (Chapter-2 Figure 2.5(b)). Thus in [BMIM][BF₄]–water mixtures, the phase behavior of PNIPAM depends in a complex manner on the combination of temperature and IL volume fraction Φ_{IL}.

Although the cononsolvency or LCST type effect occurred upon addition of any of the tested ILs to PNIPAM–water solutions, only [BMIM][OAc] systems exhibited a single LCST type behavior. The effect of the molecular weight (M_n) in our experiments is to decrease the CP, which agrees with the trend for the “critical point” LCST as predicted from a modified Flory-Huggins solution theory. This theory also predicts an initial decrease of LCST with polymer concentration and M_w, which agrees well with the observed trend in CP over the concentration range studied. We found that an increase in IL content increases CP. However, this trend does not continue indefinitely. From this perspective, it is seen that PNIPAM is generally soluble at intermediate IL–water compositions. Interestingly, for [BMIM][BF₄]/H₂O mixtures, the width of this solubility window decreases with increasing molecular weight (M_n), suggesting an eventual convergence of the two insoluble regimes. Such tunability in aqueous solutions of IL potentially allows for new approaches to tune macromolecular phase behavior, and
therefore merit exploration. We have identified PNIPAM as such a polymer in certain imidazolium based ionic liquids from a practical perspective.

In our light scattering experiments we have qualitatively established the relation between chain collapse aggregation and LCST type phase transition through hydrodynamic size measurements. There were findings by DLS measurement that the polymer chain dispersion; sizes $d_H = 1$-$10$ nm (highly solvated state) and large clusters; sizes $d_H = 100$ nm (poorly solvated state) coexist at temperatures near the CP and beyond. At temperatures much higher than the CP we showed enough evidence of chain aggregation of PNIPAM. The $<d_H>$ of these aggregates increases with temperature. Increase of $<d_H>$ is accompanied by an increase of scattered light intensity that clearly indicates the aggregation of PNIPAM chains in water. These aggregates arise from the amount of PNIPAM chains that have undergone the well known coil-to-globule transition. At temperatures above the CP, the size of the aggregates was determined from cumulant fit. Since, a transition from monomodal distribution (at $T < CP$) to bimodal and trimodal distribution (at $T > CP$) with final $<d_H> > 100$ nm was observed, the formation of well-defined aggregates of collapsed polymeric chains was confirmed. For all PNIPAM/[BMIM][BF$_4$] aqueous systems with $\Phi_{IL} < 0.45$, both scattering intensity and size results indicate that there is an LCST-type phase transition taking place on the basis of the CPs observed. Therefore, we conclude that the homo polymer dissolves at low temperature and forms aggregates above the CP as determined from the hydrodynamic size values in IL/H$_2$O mixtures. We have highlighted a model system comprising of IL in water, where change in IL concentration modified the CP values of PNIPAM irrespective
of polymer concentration or $M_n$. This suggests a future direction towards controlling the LCST values of aqueous polymers too. We conclude that all of our PNIPAM samples after undergoing cloud point (CP) behavior are indeed solutions which show aggregation of PNIPAM chains and phase separation. Such reversible volume transition of the LCST type behavior with dilute PNIPAM solutions might inspire the design of materials with chain steric-assemblies, such as crumpled globules of chains, with competition between aggregation and precipitation.
FUTURE PERSPECTIVES

Polymeric materials with core shell structures that respond to environmental changes have become of major interests in pharmaceutical applications. AB type copolymers with PNIPAM consisting of structural units which have varying/opposite solubility in IL/H₂O mixture can be studied for applications in drug delivery. Such copolymers form micelle like complexes with one block soluble and other block insoluble under a particular solvent condition. The soluble block generally forms the outer micellar shell whereas the insoluble one comprises the dense core of the micelle. Lodge et al. have studied PNIPAM from the point of view of targeted drug delivery forming micellar core shell structure when copolymerized with other polymers like PEO and PBnMA. The unique physicochemical properties of room temperature ILs have placed them amongst a select other solvents as a media for block copolymer self-assembly. It would be interesting to study the phase tunability of such micellar vehicles in IL/H₂O mixtures starting with model chaotropic and kosmotropic IL salt systems like ours. Recently Lodge et al. reported a doubly thermosensitive micelle-inverse-micelle self assembly of the same diblock copolymers with PNIPAM in IL [24] [23]. The thermo-responsivity of these materials arises due to either UCST or LCST type phase behavior in ILs upon heating. Therefore by selecting proper UCST or LCST type blocks with PNIPAM the double thermosensitivity can be created, where one forms a collapsed phase of the core above its LCST and the other forms a shell of freely soluble chains above its UCST, on increasing temperature and vice-versa [23]. Also the UCST or LCST temperature of a given polymer can be adjusted by changing the cation or anion of the IL or by varying the
composition of IL/H\textsubscript{2}O mixture. We have shown that PNIPAM shows LCST type behavior in aqueous solutions of IL [BMIM][BF\textsubscript{4}] and [BMIM][OAc], with the CP temperature varying over a range both above and below its CP temperature in pure water. We propose that a block copolymer of PNIPAM-PEO can form core-shell complex \cite{23} with a temperature tunable core in such [BMIM][BF\textsubscript{4}] and [BMIM][OAc] IL/H\textsubscript{2}O mixtures. During the collapse at CP PNIPAM chains form the core of dense spherical shaped aggregates shelled by hydrophilic soluble PEO chains. We can say that at each CP temperature there is a competition between the solubilising effect of the hydrophilic PEO segments and shrinking effect of the hydrophobic interactions among PNIPAM chains. The thermal properties and size of aggregates at particular polymer concentration and fixed PEO/PNIPAM ratio will depend on IL concentration \cite{24}.

Polymers with structural units of varying solubilities mimic the behavior of globular proteins \cite{131}. Like globular proteins it is possible to synthesize AB type block copolymers with both hydrophobic and hydrophilic residues using PNIPAM. Recently Virtanen et al. have synthesized PEO grafted PNIPAM copolymers to mimic the behavior of globular proteins at elevated temperatures \cite{132}. However it would be interesting to study the temperature driven phase transition of the copolymers in presence of IL/water mixtures, reason with kosmotropic and chaotropic effect of IL anions at fixed polymer concentration and correlate with the modified Flory-Huggins solution theory for the $M_w$ variations. Such AB block copolymers might undergo similar coil-to-globule transition like globular proteins and ELPs at higher temperature in IL/water mixtures. Like globular proteins one type of block would consist of the hydrophobic core upon temperature
induced chain collapse (globule), where as the other type of block thus hydrophilic would surround the core. Most of the activity of enzymes and proteins in bio-systems are based on their conformation. It would be challenging to study if such AB type block copolymers with PNIPAM remember the native conformation during their synthesis when subjected to temperature changes in presence of ILs. This would help in the design of novel bio-mimetic polymer systems which memorize their functional features based on a particular conformation.


84. C. Boutris, E.G. Chatzi, C. Kiparissides, "Characterization of the LCST behaviour of aqueous poly(N-isopropylacrylamide) solutions by thermal and cloud point techniques", Polymer 38, pp. 2567-2570(1997)


