

Photophysical Investigation of Poly [N-isopropylacrylamide-co-Acrylic Acid] Copolymers

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Abstract

Poly[n-isopropylacrylamide], Poly[NIPAM], and Poly[n-isopropylacrylamide-co-acrylic acid], poly[NIPAM-co-AA] copolymers exhibit the interesting property of size contraction with increasing temperature. Photophysical probe, ruthenium-tris-phenanthroline has been used to monitor the above changes and the studies are correlated with independent study state fluorescence data.

Keywords: *Polymers, Poly[n-isopropylacrylamide] and Poly[n-isopropylacrylamide-co-acrylic acid], Ruthenium-tris-phenanthroline, Fluorescence, Single Photon counting*

Introduction

Photophysical studies have been widely used to investigate the lower critical solution temperature of many stimulus responsive polyacrylate polymers and polyelectrolytes. The basic concept is to incorporate a molecule with selected photophysical properties into the system. This probe molecule, via its spectroscopic properties, reports back on the nature of its environment, and on the access of other quencher molecules, from various regions of the system, to the probe molecule¹. Thermoresponsive poly[n-isopropylacrylamide] and poly[n-isopropylacrylamide-co-acrylic acid] copolymers have been prepared according to the free radical polymerization technique described in our previous report². These polymers have an open coil like structure when dissolved in water at lower temperature [i.e. below 32°C] however, when heated above 32°C, they contract (reversibly) and change to globules. This contraction is believed to be a consequence of a decrease in the polymer solvent interaction with increasing temperature³.

Polymers, responsive to external stimuli such as pH, temperature, ionic strength and electric field, have been the focus of many studies in view of their potential applications in diverse fields. Poly(N-isopropylacrylamide) has become an important and the most popular member of a class of polymers that possess inverse solubility upon heating, a property contrary to the behavior of most polymers. It exhibits thermoreversible phase separation behavior in aqueous solution, which makes the polymer useful as a thermoresponsive material and is characterized by a lower critical solution temperature (LCST). Experimentally, the LCST can be detected between ca. 30 and 35°C, the exact temperature being a function of the detailed microstructure of the macromolecule. The reason for this sharp phase transition is a good balance between hydrophilic and hydrophobic interaction in the polymer⁴. The phase transition behavior of PNIPAM in aqueous solution has been widely investigated by calorimetric, turbidimetric and spectroscopic techniques. Among the techniques, fluorescence spectroscopy is the most extensively used and the most informative. This can be attributed to the high sensitivity of fluorescence measurement and the strong dependence of the fluorescence behavior of probe molecules on the microenvironment. An interesting feature common to other thermosensitive polymers lies in the possibility of tuning the LCST by adding cosolvents, salts, surfactants or polyelectrolytes to the PNIPAM solutions or by

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incorporating comonomers with variable degree of hydrophilicity, in fact increasing or decreasing the hydrophilic content of a copolymer will usually result in an increase or decrease, respectively, of its LCST. Furthermore, polymerizing N-isopropylacrylamide (NIPAM) with weakly ionisable comonomers permit to obtain stimulus responsive polymers capable of responding to both temperature and pH variations; interestingly, owing to the variation of their degree of ionization with pH, systems with pH dependent LCST are obtained^{5,6,7}.

The general application of fluorescence as a probe to characterize the polarity, mobility, and other properties in diverse media has been already described in many literatures. Various molecules can be used in free solution or covalently bound to ascertain details of polymer solution behavior^{8,9}. In this work, a chemically non-attached metal complex, ruthenium(II)-tris-phenanthroline has been used as a photophysical probe to investigate the LCST behaviour of poly(NIPAM) and its copolymers with pH sensitive group.

Experimental Methods

Materials and Methods

The polymers used in this study were synthesized according to our previous report². All commercial chemicals were purchased from BDH Inc., unless otherwise noted. Bidistilled water was used throughout the experiment. Three polymers, poly [n-isopropylacrylamide], PNIPAM, poly[n-isopropylacrylamide-co-acrylic acid], poly[NIPAM-co-AA] with 1 and 2 mol % of AA were used for fluorescence measurements. The structure of the employed polymers and other compounds is shown in Figure 1.

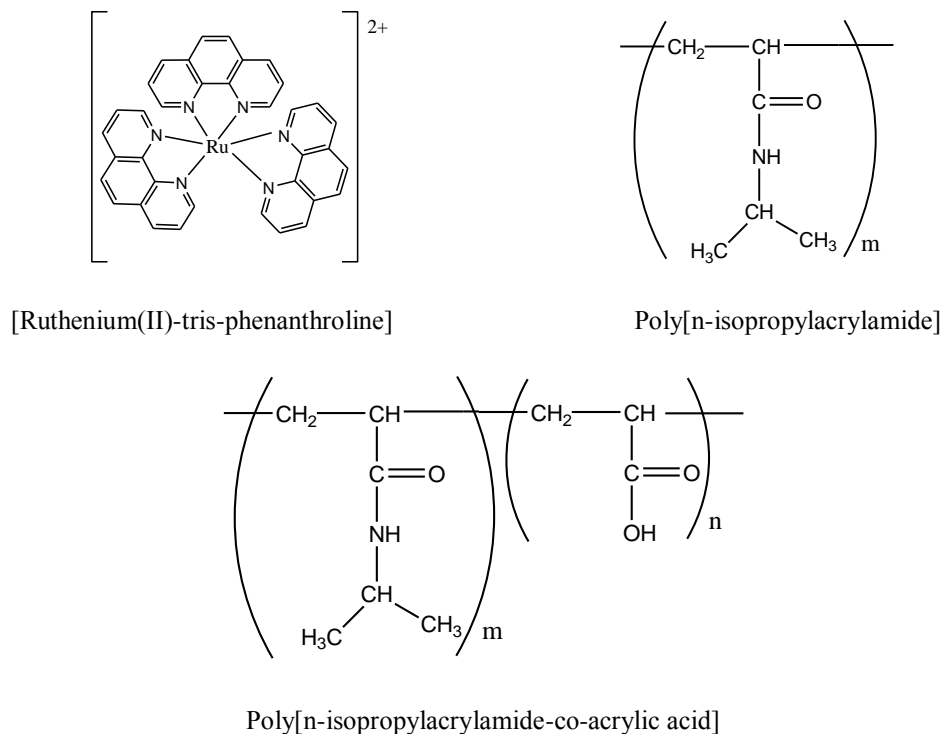


Figure 1: Structure of employed polymer and other compounds

Fluorescence Spectroscopy

Steady state fluorescence measurements were performed using single photon counting equipment EAI-FS/FL 900 (Edinburg Analytical Instruments, UK) working in the lifetime range of 500 ps to 500 μ s. The temperature of the water jacketed cell holder was controlled with a circulating bath. All measurements were performed in aerated solutions. The concentration of polymer solution in bidistilled water was 0.18g/L. The emission spectra were recorded in a temperature range of 26 to 42°C. Ruthenium [II]-tris-phenanthroline [1.25×10^{-5} M in bidistilled water] was used as a photophysical probe. The stock solution of aluminium sulphate [1×10^{-2} M] was prepared in bidistilled water and 100 μ L of this solution was used for each steady state fluorescence measurement. The excitation and emission wavelengths were 450 nm and 620 nm respectively. The method of analysis of steady state data has previously been described¹⁰.

Results and Discussion

The study of microheterogenous and heterogeneous structures through their interaction with metal complexes has been successfully pursued for the past three decades and is a widely accepted and highly developed method. The technique relies on the dependence of luminescence lifetimes and intensities in the ³MLCT (metal to ligand charge transfer) excited states of ruthenium(II)-polypyridyl complexes upon their local microenvironment and on the effect of quenchers such as cobalt (III)-polypyridyl complexes. The basic concept is to incorporate a molecule with selected photophysical properties into the system. This probe molecule, via its spectroscopic properties, reports back on the nature of its environment, and on the access of other quencher molecules, from various regions of the system, to the probe molecule^{11,12}. Photophysical probe ruthenium(II)-tris(phenanthroline) has been used in this study to monitor the conformational change (size contraction) and to investigate the LCST behaviour of poly[NIPAM] and its copolymers featuring pH sensitive group.

Photophysical studies of poly[NIPAM] and poly[NIPAM-co-AA] using ruthenium(II)-tris (phenanthroline) as a photophysical probe

In Figure 2 a typical series of emission spectra of ruthenium(II)-tris (phenanthroline) in aqueous solution of poly(NIPAM-co-AA), (98:2) as a function of temperature is shown ($\lambda_{ex} = 450$ nm). The spectra composed of the broad emission at around 600 nm. Temperature changes above LCST trigger changes of the polymer conformation from coil to globules form.

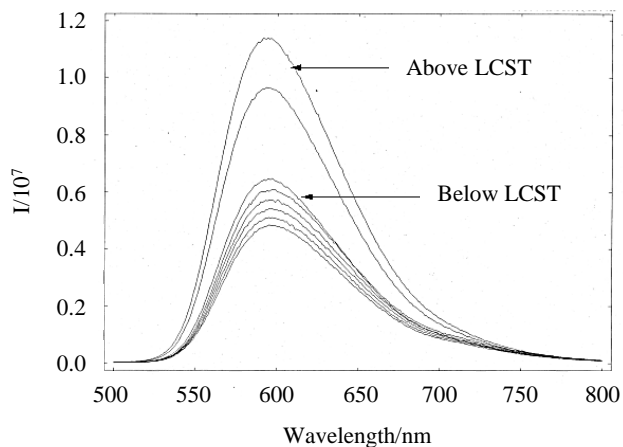


Figure 2: Fluorescence spectra of ruthenium[II]-tris-phenanthroline above and below the LCST of Poly[n-isopropylacrylamide-co-acrylic acid], Poly[NIPAM-co-AA] copolymer.

These changes lead to the protection of probe molecule within the hydrophobic nano-domain formed by globules and decrease the quenching of its fluorescence by water molecules. As a result, the fluorescence spectra show a tremendous increase in fluorescence above the LCST of polymer solution¹³.

Figure 3 shows the plot of fluorescence intensity of ruthenium(II)-tris-phenanthroline in presence of poly(NIPAM) and poly(NIPAM-co-AA) polymers as a function of temperature. When a solution of poly(NIPAM) and poly(NIPAM-co-AA) in water containing ruthenium(II)-tris-phenanthroline was heated from 26°C to higher temperatures several events occurred. In all cases the intensity decreases slightly to reach a minimum value then increases sharply to reach a limiting value with an increase in temperature.

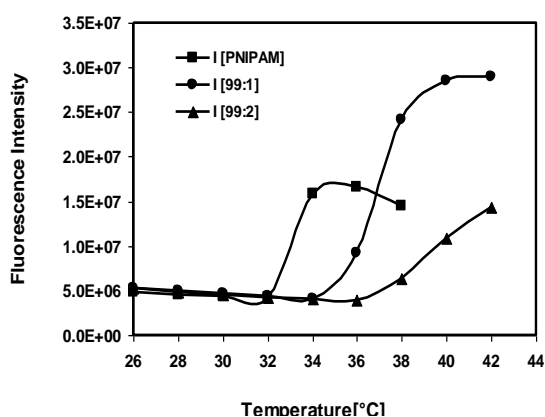


Figure 3: Fluorescence intensity of ruthenium [II]-tris-phenanthroline above and below the LCST of Poly[NIPAM], Poly[NIPAM-co-AA, 99:1 and 98:2] copolymers. Effect of pH sensitive acrylic acid group on LCS

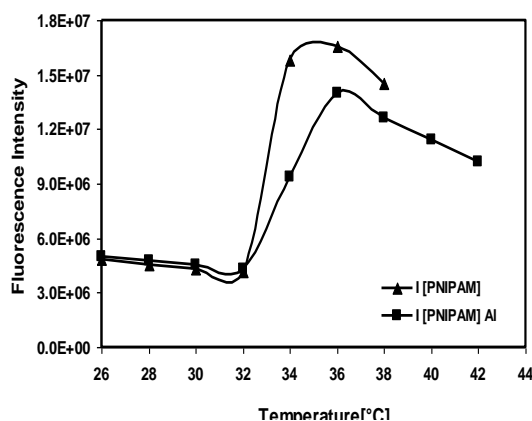


Figure 4: Fluorescence intensity of ruthenium [II]-tris-phenanthroline above and below the LCST of Poly[NIPAM]. Effect of Al³⁺ ion on LCST of poly[NIPAM] solution

These changes were completely reversible, upon slow cooling of the solution, the intensity decreased and return to its initial value. The sudden increase in intensity after 32°C, 34 °C, and 36°C directly reflects the conformational transition of these polymers chain from an isolated coil to compact globular structure. These temperatures, i.e. 32°C, 34°C and 36°C are called the lower critical solution temperature for poly(NIPAM) and poly(NIPAM-co-AA) polymers in aqueous solution. For poly(NIPAM) it is 32°C, whereas for copolymers: poly(NIPAM-co-AA) (99:1) and poly(NIPAM-co-AA), (98:2) it is 34°C and 36 °C respectively.

In Figure 4, the effect of Al³⁺ ion on LCST of poly(NIPAM) in aqueous solution is shown. Here the fluorescence intensity of probe molecule is plotted as a function of temperature for the polymer solutions with and without Al³⁺ ion.

The addition of Al³⁺ ion to the poly(NIPAM) solution causes no any shift of LCST to lower temperature. It may be due to the absence of ionisable groups in the poly [NIPAM] polymer.

Figure 5 and 6 represent the effect of Al³⁺ ion on LCST of poly(NIPAM-co-AA) copolymers containing 1 and 2 mol % of acrylic acid (NIPAM:AA mol ratio of 99:1 and 98:2). As in the case of poly(NIPAM), the fluorescence intensity of the probe decreases initially and reaches minimum at 34 °C for poly(NIPAM-co-AA, 99:1), and 36 °C for poly(NIPAM-co-AA, 99:2) then increases sharply to reach a limiting value with an increase in temperature. But after addition of Al³⁺ ion to the polymer solution, the LCST for both polymers shifted to lower temperatures, i.e. 32°C.

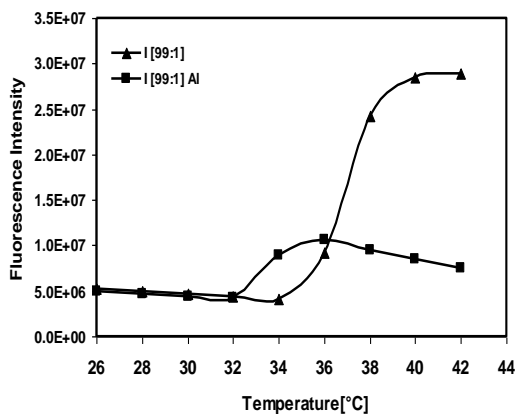


Figure 5: Fluorescence intensity of ruthenium[II]-tris-phenanthroline above and below the LCST of Poly[NIPAM-co-AA, 99:1]. Effect of Al^{3+} ion on LCST of Poly[NIPAM-co-AA, 99:1] solution.

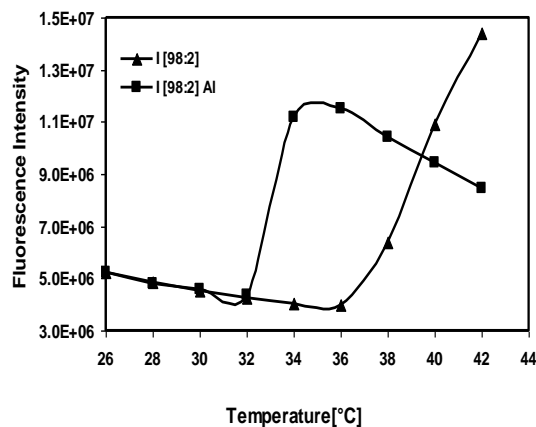


Figure 6: Fluorescence intensity of ruthenium[II]-tris-phenanthroline above and below the LCST of Poly[NIPAM-co-AA, 98:2]. Effect of Al^{3+} ion on LCST of Poly[NIPAM-co-AA, 98:2] solution.

The informations obtained from steady state fluorescence data can be explained as follows. The LCST of poly(NIPAM) copolymers is strongly influenced by the nature of the comonomer. Hydrophobic compounds lower the LCST and hydrophilic compound raise it. It has been shown that the LCST phenomenon disappears when a hydrophilic compound contains more than a certain amount of comonomer¹⁴.

The copolymerization of n-isopropylacrylamide with a monomer bearing a carboxylic acid group makes it possible to change the hydrophilicity of the polymer by altering the pH value resulting in a change in the LCST¹⁵. The hydrophilic character of the poly[NIPAM-co-AA] increases with increasing mol % of acrylic acid in it. This increase in hydrophilic character may be responsible for the increase in LCST of the copolymer solution.

The effect of salts on the solution behaviour of biological macromolecules was reviewed comprehensively by von Hippel and Schleich¹⁶. The general observation is that neutral salt can drastically alter the solution properties of the macromolecules, such as solubility, precipitation temperature, viscosity and the like. The magnitude of this change strongly depends on the nature of the individual ions¹⁷. By adding salt, the carboxyl ions of the copolymer will be condensed by cations and there is an increase in the shielding of the electronic charge to lower electrostatic interactions. There will be a continuous shift in the balance between the electrostatic and hydrophobic forces towards predominant hydrophobic interactions and the LCST of the copolymer solution decrease constantly. This interaction may be purely electrostatic and will increase with increasing the amounts of ionisable groups in the copolymers.

Figure 7 shows the mechanism of phase separation of poly(NIPAM and its copolymers in aqueous solution at lower critical solution temperature. Based on the above steady state fluorescence data of ruthenium tris-phenanthroline (photophysical probe) the following two steps mechanism has been proposed for the phase separation.

During the heating of an aqueous solution, due to the destruction of hydrogen bonds and increasing hydrophobic interactions, the poly[NIPAM] chains first associate, then collapse and a phase separation

occurs. Steady state fluorescence intensity measurements of the non-attached metal complex [ruthenium(II)-tris-phenanthroline] have revealed a dramatic reduction in polymer's mobility at the LCST: a conformational transition from flexible coil to compact globular structure is apparent. Luminescence technique has helped to verify the mechanism of the LCST process: initially a conformational transition occur which is then followed by intermolecular aggregation.

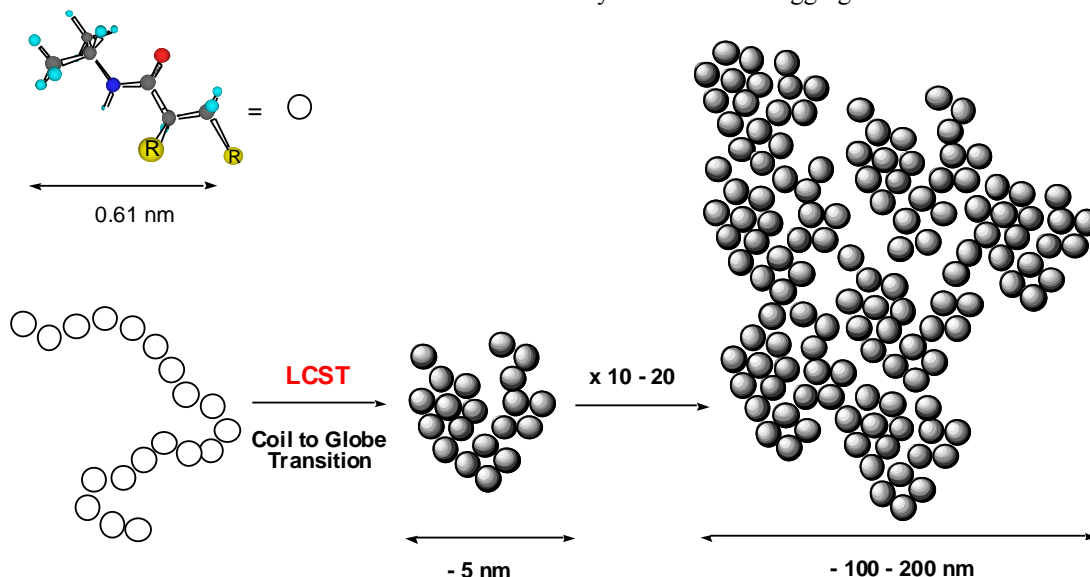


Figure 7. Mechanism of phase separation of Poly[NIPAM] and its copolymers at Lower Critical Solution Temperature (LCST), (1) Collapse of individual polymer chain ($T = LCST$) (2) Coagulation of the collapsed polymer chains ($T > LCST$)

Under certain conditions, hydrophobically modified polymers form microdomains isolating their hydrophobic chains against the polar environment. Fluorescence is a common method to investigate whether such kinds of microdomains are present in a polymer solution. In such experiments, a fluorescent dye is added either by covalent binding to the polymer or by simply dissolving the polymer in a solution containing the dye. In this work, a free photophysical probe, ruthenium-tris-phenanthroline was added to the polymer solution to investigate the LCST behavior of PNIPAM and its copolymers. The fluorescence intensity of this probe depends on the conformational change of the polymer chains.

Conclusions

Poly(NIPAM) and Poly(NIPAM-co-AA) polymers display lower critical solution temperature (LCST) behaviour. Steady state fluorescence intensity data of non-attached metal complex [ruthenium(II)-tris-phenanthroline] shows that the LCST occurs as a result of a two stage processes: initially an intramolecular conformational transition results in coil collapse. The second stage involves intermolecular aggregation between collapsed coils. The incorporation of ionisable monomer for e.g. acrylic acid to the thermosensitive polymer increases its lower critical solution temperature. The increase in LCST with the increasing mol-% of the acrylic acid may be due to the increase in hydrophilic character of the polymers. By adding salt to the poly[NIPAM-co-AA] solution, the carboxylic ions of the copolymer will be condensed by cations, this will cause a continuous shift in the balance between the

electrostatic and hydrophobic forces towards predominant hydrophobic interactions and the LCST of the copolymer solution is decreased.

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