

Measuring LCSTs by Novel Temperature Gradient Methods: Evidence for Intermolecular Interactions in Mixed Polymer Solutions

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Polymers exhibiting a lower critical solution temperature (LCST) are of interest in contexts as diverse as catalysis,¹ green chemistry,² drug delivery,³ sensor design,⁴ and permeability control in thin film composites.⁵ Aqueous solutions of polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) are common examples of such thermoresponsive materials in that they precipitate above their LCSTs and redissolve below this temperature.⁶ Many proteins display similar behavior.^{7,8}

Although the precipitation mechanisms are often complex, it is generally accepted that the desorption of water molecules from the hydrophobic portions of the macromolecule causes the formation of a more compact macromolecular structure and is directly associated with the phase transition.⁹ Beyond this, there exists a measure of uncertainty about the mechanism of the LCST phase behavior. For example, it has been suggested that the clouding of a polymer solution proceeds in two cooperative steps with the first stage involving the folding of individual macromolecules and the second being aggregation of the folded polymers.¹⁰ The details of the process whereby soluble polymers become precipitated aggregates, however, are unknown.

Existing experimental methods have afforded many examples of LCST behavior and have shown that LCST phenomena in poly(*N*-alkylacrylamide)s in particular are dependent on the identity of the *N*-alkyl group and other solution components (e.g., salts). However, while there are many studies of LCST phenomena, efforts employing existing techniques can suffer from experimental difficulties. For example, if the reversibility of the process is being studied, prolonged temperature ramping is required to minimize hysteresis.¹¹ Furthermore, the speed of data collection may be limited by the detection method. Typical studies employ transmittance, light scattering, and differential scanning calorimetry (DSC) for measuring the LCST. These methods involve taking data sequentially as a function of temperature. This Communication describes a new high throughput approach for studying LCSTs that only requires a few microliters of sample and provides excellent temperature resolution capabilities.

Previous measurements of the LCSTs for PNIPAM and poly(*N,N*-diethylacrylamide) (PDEAM) show that these macromolecules have very similar clouding points (31 °C (±1 °C) and 30 °C (±1 °C)), respectively.^{12,13} We have measured these as well, using a temperature gradient device¹⁴ developed in our laboratory, which collects data as a function of *position* rather than *time* (Figure 1). This device allows us to make multiple measurements of LCST phenomena in a single experiment with microliter sample volumes. Significantly, this device allows us to study mixed polymer systems with sufficient resolution to reveal mechanistic insights into the LCST behavior of mixed polymer systems.

In our setup, hot and cold brass tubes were placed in parallel on a glass slide.¹⁴ A linear temperature gradient forms between them in accordance with the Fourier heat diffusion equation.¹⁵

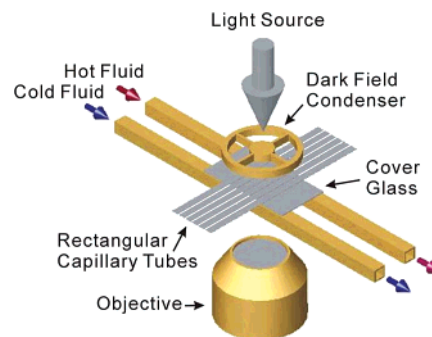


Figure 1. Schematic drawing of a temperature gradient device. Each CCD image fits six capillary tubes simultaneously under a 2× objective.

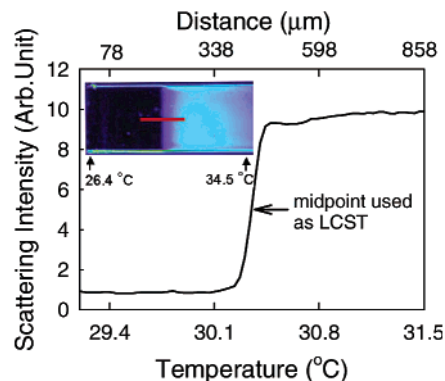


Figure 2. Clouding curve for PNIPAM (10 mg/mL, M_w 340 kDa, LCST 30.5 °C) in DI water solution. Inset: CCD image of the PNIPAM clouding. The temperatures at the two ends were at 26.4 and 34.5 °C.

Rectangular borosilicate capillary tubes with a high aspect ratio (100 $\mu\text{m} \times 1 \text{ mm} \times 2 \text{ cm}$) were placed parallel to the direction of the thermal gradient; therefore, determining the temperature along the tube was a simple matter of measuring the lengthwise position. As a demonstration, a tube was filled with an aqueous PNIPAM solution. Since the cloudy portion of the solution scattered significantly more light than the clear portion, the clouding process could be observed under an optical inverted microscope by employing dark field microscopy (Figure 2). Interestingly, although the solution has a typical polydispersity (1.89 for PNIPAM), the temperature range of the phase transition only spanned about 0.2 °C. The LCST of PDEAM with a lower degree of polymerization ($M_w = 230 \text{ kDa}$) and a comparable polydispersity (2.09) was similarly precise, indicating that a narrow polydispersity is not necessarily required to obtain such well-defined phase transitions.

LCSTs of PNIPAM and PDEAM measured with the device in Figure 1 were $30.5 \pm 0.16 \text{ °C}$ and $29.3 \pm 0.15 \text{ °C}$, respectively, and remained constant with time. Using this device, up to six samples of polymer solutions could be analyzed at once using two

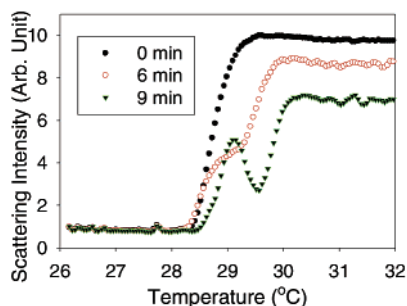


Figure 3. Kinetic studies of PNIPAM and PDEAM mixtures. PNIPAM and PDEAM were mixed as 1:1.5 molar ratio in DI water.

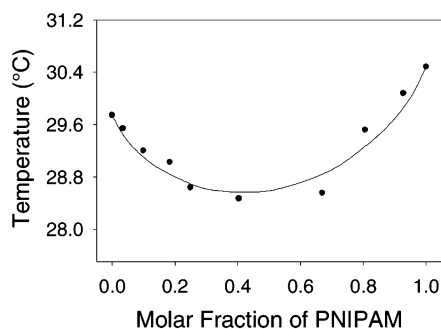


Figure 4. Plots of the initial LCST at different mole fractions of PNIPAM for PNIPAM and PDEAM mixtures. The solid line represents a fit to the data.

different polymer solutions with known LCSTs as internal controls to aid in temperature readout and prevent systematic errors between experiments.

Since the clouding curves of macromolecules in solution can be obtained with good temperature resolution over relatively short times, the time-dependent evolution of the clouding process was studied simply by recording a series of time-lapse images of a given sample with a standard CCD camera. When this was done for a mixed solution of PNIPAM and PDEAM, a series of time-dependent phenomena were revealed (Figure 3). The first image was taken immediately after the mixed polymer solution was placed over the temperature gradient. As can be seen, the original clouding curve of the mixture appeared to be smooth. After 6 min, however, a higher temperature kink appeared in the curve. This further evolved into a pronounced dip after 9 min. At this point, cycling between the latter two stages occurred; however, the initial sequence of clouding behavior as well as the migration of the LCST toward higher temperature were reproducible over many samples and within a given sample. Reversibility within the same sample was observed when the T_{Low} and the T_{High} sides of the tube were switched.

To investigate the significance of the 0 min conditions, experiments for mixtures of PNIPAM and PDEAM were carried out as a function of mole fraction of PNIPAM. A plot of the nascent LCST temperature is shown in Figure 4. The results bear a striking resemblance to the curve obtained for the Gibbs free energy of mixing of two substances: $G = \chi_1 G_1 + \chi_2 G_2 + nRT(\chi_1 \ln \chi_1 + \chi_2 \ln \chi_2)$. In this case, G_1 and G_2 are the Gibbs free energies of pure substances 1 and 2, respectively. χ_1 and χ_2 are the mole fractions of substances 1 and 2, respectively. This correlation implies that the aggregation process involves the rapid (i.e., kinetically controlled) formation of aggregates of a small number of polymer

chains that later segregate into pure PNIPAM and PDEAM. In other words, early aggregate formation most likely involves the mixing of PNIPAM and PDEAM. Such mixed aggregates would have higher entropy than the corresponding structures containing only one type of polymer. The difference in entropy between the pure and mixed systems should lead to a lower transition state free energy, ΔG^\ddagger , to precipitation for the mixed complexes. Interestingly, this initial precipitate then begins to phase segregate into pure PNIPAM and PDEAM particles presumably on enthalpic grounds. To verify these assumptions, DSC studies of PNIPAM and PDEAM were performed as a function of mole fraction of PNIPAM. The results showed that the onset to the phase transition came at a lower temperature for mixed systems by amounts that exactly matched the data in Figure 4. Furthermore, the overall enthalpy for the entire precipitation process rose continuously as the amount of PNIPAM was increased. This latter result is consistent with the notion that the two polymers must ultimately segregate upon undergoing the phase transition and that PNIPAM has the higher phase transition enthalpy. The DSC results are provided in the Supporting Information.

The methods and results described above provide a new and rapid protocol for studying the precipitation/solubilization behavior of thermoresponsive polymers with minute quantities of materials and excellent signal-to-noise ratios. Indeed, the high throughput potential for this method should make it possible in the near future to collect large amounts of data with great temperature precision quite easily.

Acknowledgment. We thank the National Science Foundation (DMR-9977911) (D.E.B.), the Army Research Office (DAAD19-01-1-0346) (P.S.C.), and the Office of Naval Research (YIP Award NOO014-00-1-0664) (P.S.C.). P.S.C. also acknowledges the following: Beckman Young Investigator Award, an Alfred P. Sloan Fellowship, and a Nontenured Faculty Award from 3M Corporation. H.M. acknowledges support from a Proctor & Gamble Fellowship.

Supporting Information Available: Experimental procedures and characterization data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA029691K