Formation of a single poly(\(N,N\)-diethylacrylamide) microdroplet in water by coupling of photothermal effects and an optical force

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Abstract. Poly(\(N\)-isopropylacrylamide) (PNIPAM) exhibits phase separation with lower critical solution temperature (LCST). In the 1990s, Masuhara and co-workers reported the first demonstration of optical trapping of PNIPAM forming a micrometer-sized polymer droplet. Since then, this technique has attracted much attention to create a molecular assembly in a microspace. In the present study, we targeted poly(\(N,N\)-diethylacrylamide) (PDEA), which has an analogous chemical structure to PNIPAM. We demonstrated that optical tweezers formed the unique micro-morphologies of a phase separated PDEA droplet. Fluorescence microscopic images and Raman spectra of the PDEA droplet showed that a lot of smaller-sized water-rich micro-domains were inhomogeneously formed in the droplet. Such unique phase separation behavior was never observed in steady-state heating of an aqueous PDEA solution above its LCST. Our results indicate that a novel micro-structure can be formed by coupling of an optical gradient force and a local temperature elevation.

1. Introduction
Poly(\(N\)-isopropylacrylamide) (PNIPAM), which is a representative thermoresponsive polymer, is homogeneously dissolved in water at room temperature, taking hydrated random-coiled structures. The PNIPAM chains turn into globular structure accompanied by dehydration of the polymer chains upon heating above the lower critical solution temperature (LCST). Subsequently, these dehydrated globules aggregate with each other due to a hydrophobic interaction, resulting in micrometer-sized polymer assemblies or polymer-rich droplets. Such phase separation behavior of PNIPAM has been investigated over 50 years since the first demonstration by Heskins and Guillet [1,2], because PNIPAM has LCST near physiological conditions (c.a. 30 °C) thus rendering this water-soluble polymer suitable for biotechnological applications such as drug delivery system and artificial actuator. Moreover, PNIPAM has also attracted much attention to fundamental aspects such as understandings of protein folding and
DNA packing. However, little is known about their physical properties of the polymer-rich droplets such as their polymer concentration ($C_{\text{poly}}$), because it is hard to analyze individual droplet due to their rapid thermal fluctuation.

To address this issue, we focused on optical tweezers that is a powerful tool to trap micro-particles at the focal point of a tightly focused laser beam. In the 1990s, Masuhara and co-workers demonstrated that optical tweezers can trap PNIPAM chains dissolved in solution and form PNIPAM micro-droplet [3]. Inspired by these studies, we previously developed a method that combines optical tweezers with micro-Raman spectroscopy and determined $C_{\text{poly}}$ of the optically trapped droplet of PNIPAM (Figure 1) [4]. Moreover, we recently found that the molecular weight of PNIPAM chains had a strong effect on $C_{\text{poly}}$ [5].

![Figure 1. An illustration of optical tweezers combined with micro-Raman spectroscopy.](image)

In the present study, we targeted poly($N$,$N$-diethylacrylamide) (PDEA), which is another LCST-type thermoresponsive polymer. Although PDEA has an analogous chemical structure to PNIPAM, and both polymers have similar LCSTs in water, their phase separation behavior differs from each other [6]. We aimed at demonstrating optical trapping of PDEA and determining $C_{\text{poly}}$ of the optically trapped PDEA-assembly.

2. Experimental Section

2.1. Sample preparation and characterization

PDEA was synthesized by reversible addition-fragmentation chain transfer polymerization and was characterized in the same manner as our previous report [6]. The weight-averaged molecular weight ($M_w$) and the polydispersity ($M_w/M_n$) of the synthesized PDEA were evaluated using size-exclusion chromatography (SEC) to be 66000 and 1.4, respectively. The LCST of the aqueous PDEA solution was determined to be 29 °C.

2.2. Optical setup

We have already explained the details of the optical tweezers combined with a confocal micro-Raman spectroscopy in our previous report [4], and briefly explained here. All the experiments of optical tweezers were performed using an inverted optical microscope equipped with an objective lens ($\times100$, N.A. = 1.3), CMOS camera, and a thermostatic stage. For optical trapping, a continuous-wave (CW) near-infrared (NIR) laser beam ($\lambda = 1064$ nm) was focused into the sample solution. As a Raman excitation light source, a CW 532 nm laser beam was coaxially introduced with the NIR laser beam. The confocal system was arranged with a pinhole. Raman signals were detected with a cooled CCD.
3. Results and Discussion

3.1. Optical trapping of PDEA chains in water
Focusing the NIR laser beam into the dilute PDEA solution at room temperature, a single polymer-rich droplet was formed at the focal point (Figure 2a). The size of the particle became larger and reached a steady-state after several minutes. After switching off the NIR laser beam, the droplet immediately disappeared. The formation of the micro-droplet was due to a photothermal effect and an optical force [7].

To gain further insight into the micro-structures inside the droplet, we observed it under the fluorescence microscope. In this experiment, we used aqueous PDEA solution containing rhodamine B. Figure 2b shows a representative fluorescence microscopy image. There were a lot of water-rich domains fluctuating in the PDEA droplet. This inhomogeneous structure was never observed in steady-state heating of an aqueous PDEA solution above LCST. These results indicate that optical tweezers will open a new channel for the creation of ordered polymer structures based on the joint action of a photothermal effect and an optical force.

Figure 2. Bright-field (A) and fluorescence (B) optical micrographs of a single droplet formed by a focused near-infrared laser beam of 0.18 W into an aqueous PDEA solution (2.0 wt%). The droplets in the images are at steady state (10 min after the laser was turned on).

3.2. Determination of the polymer concentration of the PDEA droplet
To determine $C_{\text{poly}}$, we obtained the Raman spectra of the aqueous solution (without NIR laser irradiation) and the droplet of PDEA below the LCST (Figure 3). In the Raman spectra, two Raman bands appear: the C-H stretching vibrational mode of the polymers in 2800–3050 cm$^{-1}$ range and the O-H stretching vibrational mode of the water in 3100–3700 cm$^{-1}$ range. By the NIR laser irradiation, the intensity of the C-H band relatively increased, while that of the O-H band decreased because of the formation of the polymer droplet. Based on the comparison of integrated intensity ratio between the C-H band and the O-H band, we determined $C_{\text{poly}}$ to be 40 wt%. The $C_{\text{poly}}$ of PDEA was much lower than that of PNIPAM (60 wt%) in our previous report [4]. This means that in spite of the fact that PDEA (LCST = 29 °C) was slightly more hydrophobic than PNIPAM (LCST = 32 °C), the PDEA-droplet contained a substantial amount of water.

Figure 3. Raman spectra of an aqueous PDEA solution (2.0 wt%) below the LCST (Gray line) and of a PDEA droplet formed by a near-infrared laser beam of 0.16 W focused into the solution (Black line).
4. Conclusion
In this study, a single PDEA droplet was formed using optical tweezers. We found that the PDEA droplet had an inhomogeneous structure with a lot of water-rich domains fluctuating inside the droplet. Moreover, we determined $C_{\text{poly}}$ of the PDEA droplet based on micro-Raman spectra. We concluded that a novel micro-structure was formed in the PDEA solution by coupling of an optical gradient force and a local temperature elevation.

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