

Direct observation of hydration and dehydration behaviors of poly(*N*-isopropylacrylamide) on silica beads through infrared spectroscopy

Stimuli-responsive polymers have attracted attention for several decades as functional materials in the field of materials chemistry. In particular, poly(*N*-isopropylacrylamide) (PNIPAAm) has been extensively studied owing to its remarkable temperature-dependent hydrophobicity and conformational alterations. PNIPAAm exhibits hydration and dehydration phenomena below and above its lower critical solution temperature (LCST) of 32°C, resulting in a conformational transition, referred to as the coil-globule transition (Fig. 1(a)). This transition induces expansion and contraction of the PNIPAAm chain. These thermally modulated modifications of the properties of PNIPAAm have significant applications in various biomedical fields, such as temperature-modulated bioseparation induced by temperature-modulated hydrophobic interactions (Fig. 1(b)) [1,2].

To investigate the PNIPAAm hydration behavior, infrared (IR) spectroscopy has proven to be a highly effective approach for examining the chemical environment of PNIPAAm functional groups. Changes in the amide bands of the IR spectrum during heating provided valuable insights into the variations in hydrogen bonding between the C=O and N–H bonds. Furthermore, the presence of hydrogen bonds between most carbonyl groups and water molecules in the hydrated coiled state at low temperatures, as well as the formation of intrachain and interchain cross-

linkages through hydrogen bonding between C=O and N–H groups in the dehydrated globular state at high temperatures, can be inferred from IR spectroscopy. However, characterizing grafted PNIPAAm on base materials presents challenges compared to PNIPAAm in aqueous solution or bulk form because of the relatively small amount of grafted PNIPAAm compared to the base materials, resulting in weak signals from the grafted PNIPAAm.

To address this issue, IR spectroscopy was performed at the IR beamline, SPring-8 BL43IR. The high brilliance of infrared synchrotron radiation is an advantage of SPring-8, and its ability to focus light on small areas using an infrared microscope can be applied to BL43IR. PNIPAAm modified silica beads were prepared by silane coupling reaction for modifying the atom transfer radical polymerization (ATRP) initiator on silica beads and subsequent of ATRP of PNIPAAm [3]. The hydration behavior of the PNIPAAm brush on the silica beads with changes in relative humidity and temperature was observed by IR spectroscopy of the IR beamline BL43IR at the SPring-8 synchrotron facility [3].

The FT-IR spectrum of the grafted PNIPAAm on the silica beads was observed by changing the humidity at 10°C to investigate the hydration behavior of PNIPAAm (Fig. 2). The height of the broad peak increased with increasing relative humidity (Fig. 2(a)). This broad peak was attributed to the O–H stretching of the water molecules. Generally, PNIPAAm hydrates at 10°C; thus, the grafted PNIPAAm on silica beads absorbed water molecules from humidified air, resulting in an increased absorbance at 3700–3000 cm⁻¹ with higher relative humidity.

We investigated the changes in the FT-IR spectrum containing the peaks of the amide I and II bands as the relative humidity varied (Fig. 2(b)). The PNIPAAm-grafted beads exhibited peak shifts with increasing relative humidity. In particular, with increasing relative humidity, the amide I and II peaks, attributed to C=O stretching and N–H bending vibrations, shifted to lower and higher frequencies, respectively. These peak shifts were attributed to the hydration of PNIPAAm. The C=O of PNIPAAm forms hydrogen bonds with the neighboring N–H and water. Thus, C=O acts as an acceptor of hydrogen bonds. On the contrary, the N–H group acts as a hydrogen bond donor. With increasing relative humidity, the quantity of water surrounding PNIPAAm increased, resulting in an increased number

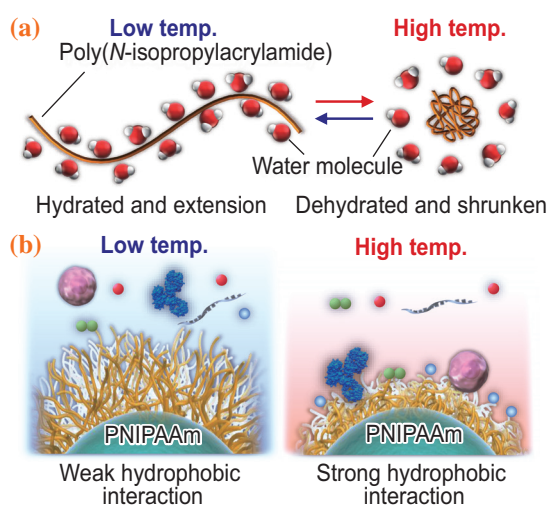


Fig. 1. Thermoresponsive polymer poly(*N*-isopropylacrylamide) and its application in temperature-responsive chromatography. (a) Thermoresponsive polymer PNIPAAm properties. (b) Concept of temperature modulated bio-separation using PNIPAAm with temperature-modulated hydrophobic interaction.

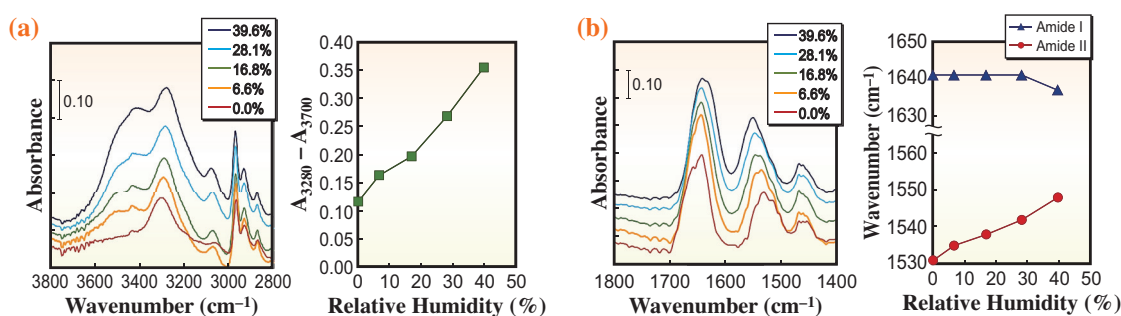


Fig. 2. FT-IR spectrum of the prepared PNIPAAm-grafted beads at a relative humidity of 10°C, (a) showcasing the absorbance difference ($A_{3280}-A_{3700}$) and (b) the peak shifts of amide I and II with changing temperature.

of hydrogen bonds within the PNIPAAm amide bond. Consequently, amide I (C=O) and amide II (N-H) experienced shifts toward lower and higher frequencies, respectively, indicating the hydration of PNIPAAm owing to elevated humidity levels.

Temperature-dependent IR spectral changes were observed at a relative humidity of 39.9% (Fig. 3). A significant change in the IR spectrum with temperature was observed at 3700–3000 cm^{-1} and 1800–1400 cm^{-1} (Fig. 3(a)). At 3700–3000 cm^{-1} , the intensity of the broad peaks decreased with increasing temperature because of temperature-dependent hydration and dehydration. With increasing temperature, PNIPAAm was dehydrated, significantly changing the IR spectrum from 3700 to 3000 cm^{-1} .

A notable shift in the peaks corresponding to amide I and II was observed between 1800 to 1400 cm^{-1} as the temperature increased (Fig. 3(b)). This shift was attributed to the temperature-dependent formation of hydrogen bonds involving the carbonyl

bonds of PNIPAAm, particularly at relatively high humidity levels. At 10°C, PNIPAAm formed hydrogen bonds with water. The C=O group of PNIPAAm formed hydrogen bonds with both water molecules and adjacent N-H groups, resulting in a shift toward lower frequencies in the amide I peak. In contrast, the N-H group formed hydrogen bonds with water, and the number of hydrogen bonds decreased with increasing temperature. Thus, the amide II peak shifts to a higher frequency with increasing temperature. Additionally, conformational changes in PNIPAAm induce temperature influence the C-N stretching vibrations of amide II. Increasing the temperature caused a significant peak shift for amides I and II.

These results suggest that the hydration and dehydration behaviors of the grafted PNIPAAm on the silica beads can be observed using FT-IR spectrometry by controlling the humidity and temperature. These findings have practical applications for the design of biomedical materials utilizing PNIPAAm.

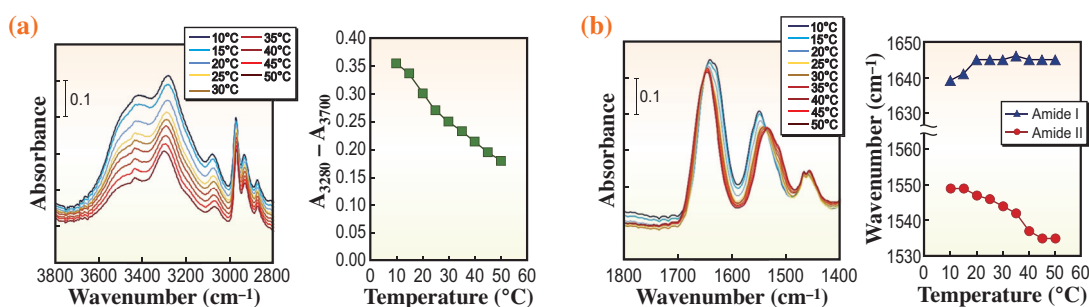


Fig. 3. FT-IR spectrum of the prepared PNIPAAm-grafted beads at various temperature at relative humidity of 39.9%, (a) showcasing the absorbance difference ($A_{3280}-A_{3700}$) and (b) the peak shifts of amide I and II with changing temperature.

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References

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