Targeted Hydrogel Synthesis

A New Route to Crystalline Hydrogels, Guided by a Phase Diagram**

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Monodisperse colloidal systems have been used as models for the study of phase transitions and as templates for the fabrication of photonic crystals.[1–7] Most investigations on colloids are primarily focused on hard-sphere-like particles such as polymethylmethacrylate, silica, or polystyrene.[7] It was found recently that wet and soft poly-*N*-isopropylacrylamide (PNIPAM) nanoparticles can self-assemble into a crystalline array in water.[8–14] However, uses of PNIPAM colloidal dispersions that exploit their crystalline structure are limited because the structure can be easily destroyed by any external disturbance, such as small vibrations. To solve this problem, Asher et al. developed a method for embedding a polystyrene colloidal crystalline array inside a PNIPAM gel.[8,14] Hu et al. demonstrated that the crystalline structure of PNIPAM nanoparticles can be stabilized by bonding the particles into a network.[15,16] The latter approach has the restriction that the particle-linking reaction must be carried out in a harsh environment (pH 12) that could encumber loading with protein drugs. Furthermore, the mechanical strength of the bonded particle assembly was low due to its low polymer concentration. Here we show a new route for the fabrication of crystalline hydrogels that follows a phase diagram. The central idea is to synthesize monodisperse nanoparticles of PNIPAM-*co*-allylamine and measure the phase diagram of their water dispersion. By exploiting the fact that thermally sensitive nanoparticles in colloidal glasses can be converted into ordered crystals by particle-based volume transition, as reported by Lyon et al.,[10] a crystalline hydrogel could be obtained by initiating the crystallization process near the colloidal crystal melting temperature while subsequently bonding the PNIPAM-*co*-allylamine particles below the glass-transition temperature. This can increase the polymer concentration and result in a crystalline hydrogel with higher mechanical strength. Furthermore, allylamine has amino groups that can be covalently bonded in water at neutral pH, but it does not significantly affect the volume phase transition of the PNIPAM polymer. Hydrogels are well known for their unique hydrophilic and environmentally responsive properties, which lead to fascinating applications that include controlled drug delivery, artificial muscles, devices, and sensors.[17–25] Tailoring hydrogels with periodic

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structures under mild synthesis conditions could open a new avenue for these applications.

Monodisperse *N*-isopropylacrylamide (NIPA)-*co*-allylamine colloidal spheres were synthesized by using a precipitation polymerization method.[26] These particles showed phase behavior similar to that of pure PNIPAM gel[27] with a slightly higher volume phase transition temperature around 35 °C. The average hydrodynamic radius of the particles is about 140 nm at 23 °C and shrinks to about 80 nm at 36 °C.

The dispersions with polymer concentrations ranging from 2 to 4 wt % exhibit iridescent colors (Figure 1 a), which

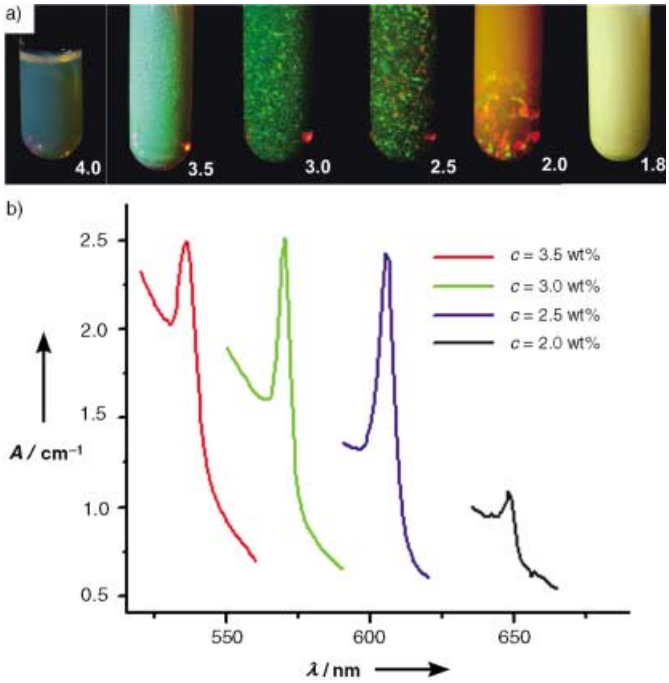


Figure 1. a) Photographs of PNIPAM-*co*-allylamine nanoparticle dispersions at various polymer concentrations at 23 °C. From left to right: 4.0, 3.5, 3.0, 2.5, 2.0, and 1.8 wt %. Here the average hydrodynamic radius of the particles in water at 23 °C is 140 nm. The diameter of the tubes is 1 cm. b) Turbidity versus wavelength curves measured with a UV/Vis spectrophotometer. The Bragg diffraction peak shifts to lower wavelength as the polymer concentration increases. From left to right: 3.5, 3.0, 2.5, and 2.0 wt %.

indicate that the particles self-assemble into an ordered arrangement. As the polymer concentration increases, the crystalline grain size decreases, and the color shifts to shorter wavelengths. When the concentration exceeds 4.5 wt %, the crystals are too small to be observed, and the assemblies are in a glass phase. When the concentration is below 1.8 wt %, the dispersions become cloudy because the particles are well separated and scatter light randomly.

The color observed in the dispersions is due to diffraction from ordered colloidal arrays with a lattice spacing on the order of the wavelength of visible light according to the Bragg law: $2nd\sin\theta = m\lambda$, where n is the mean refractive index of the dispersion, θ the diffraction angle, d the lattice spacing, m the diffraction order, and λ the wavelength of diffracted light.[8] Figure 1 b shows the turbidity of the PNIPAM-*co*-allylamine nanoparticle dispersions as a function of wavelength. The sharp peak is due to Bragg diffraction and shifts from 640 to 530 nm as the polymer concentration increases from 2.0 to 3.5 wt %. This shift is due to the decrease in the interparticle distance with increasing polymer concentration.

Figure 2 a and b show the temperature-dependent turbidity of the PNIPAM-*co*-allylamine colloidal dispersions at 2.5 and 3.0 wt %, respectively. The initial increase in the intensity of the Bragg peak when the temperature is increased above

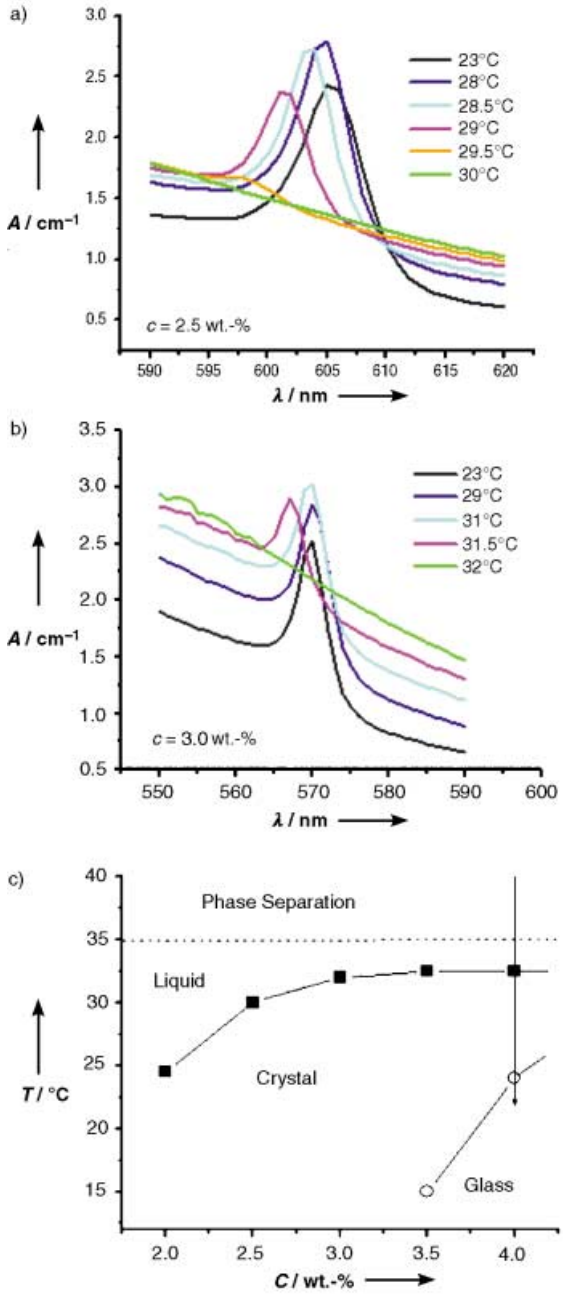
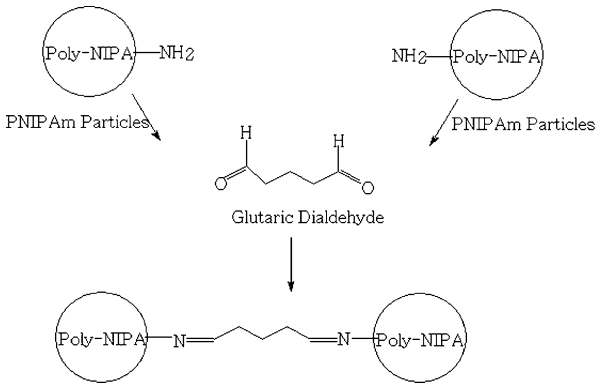


Figure 2. Temperature-dependent phase behavior of the PNIPAM-*co*-allylamine nanoparticle dispersions. a) Turbidity versus wavelength curves at various temperatures for 2.5 wt % dispersion and b) for 3.0 wt % dispersion. c) Phase diagram: the volume phase transition (T_c) of the PNIPAM-*co*-allylamine particles (-----), melting temperature (T_m, ■), and the glass-transition temperature (T_g, ○) are denoted.

room temperature indicates that heating can facilitate the formation of an ordered structure. As the sample is heated further, the Bragg peak broadens, shifts to shorter wavelength, and eventually disappears at the melting temperature T_m. The value of T_m increases with increasing polymer concentration. This phase behavior is summarized in Figure 2c. Here T_c (dashed line) is the volume phase transition temperature of PNIPA–allylamine particles, T_m (solid squares) is the melting temperature, and T_g (open circles) is the glass-transition temperature.

Guided by this diagram, we engineered a crystalline hydrogel with higher polymer concentration. We started with a PNIPAM-*co*-allylamine particle dispersion of 4.0 wt%, in which the viscosity is too high to allow the particles to form a periodic structure at room temperature. This system was heated from 23 to 40 °C and then cooled back to 23 °C at a rate of about 0.4 K min^{-1}. As indicated by the arrow in Figure 2c, when the sample was cooled to below T_m, crystallization started. Here the dispersion had low viscosity since the particles were only partially swollen. This gave the spheres enough freedom to self-assemble into an ordered array. When the sample was cooled further to below T_g, the particle size and viscosity increased considerably. As a result, the crystalline structure formed at the higher temperature was "frozen" or preserved. Then, the particle assembly with a crystalline structure was fully stabilized by a cross-linking reaction at neutral pH (Scheme 1) over about two days. As a result of the



Scheme 1. The amino groups on the PNIPAM-*co*-allylamine nanoparticles react with glutaric dialdehyde to form a network in water at neutral pH and room temperature.

higher mechanical strength of this crystalline hydrogel, it is easier to remove from the tube than the previous sample formed at room temperature in an aqueous solution at a high pH value.[16] It is apparent that a combination of a covalent bonding process and a heating–cooling process that yields a crystalline structure at a high polymer concentration can significantly improve the mechanical strength of a crystalline hydrogel.

This fabrication process leads to the formation of crystalline hydrogels with different iridescent color patterns. Figure 3 shows blue, green, and red iridescent hydrogels with polymer concentrations of 5.4, 4.0, and 3.5 wt%, respectively. With increasing polymer concentration, the interparticle

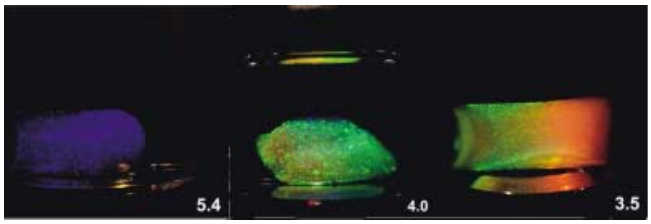


Figure 3. Iridescent colors of the PNIPAM-*co*-allylamine crystal hydrogels made according to Scheme 1 change with polymer concentration in water at pH 7. From left to right: 5.4, 4.0, and 3.5 wt%. The average hydrodynamic radius for the three samples in water at 23 °C is about 140 nm. The diameter of the vial is 2.73 cm.

distance decreases, and a blue shift in the iridescent color results. The angular dependence of the scattering was investigated. The color changes from red to blue when the angle varies from back-scattering to 90°.

Creating crystalline hydrogels allows us to obtain useful functionalities, not only from the periodic structure, but also from the constituent building blocks. Because the building blocks here are environmentally responsive colloidal spheres, their size as well as the lattice spacing should be tunable by external stimuli. Thus, the crystalline hydrogel can serve as an optical sensor to visually reveal environmental changes. An examples is shown in Figure 4a. The crystalline hydrogel at room temperature is bright green. With increasing temper-

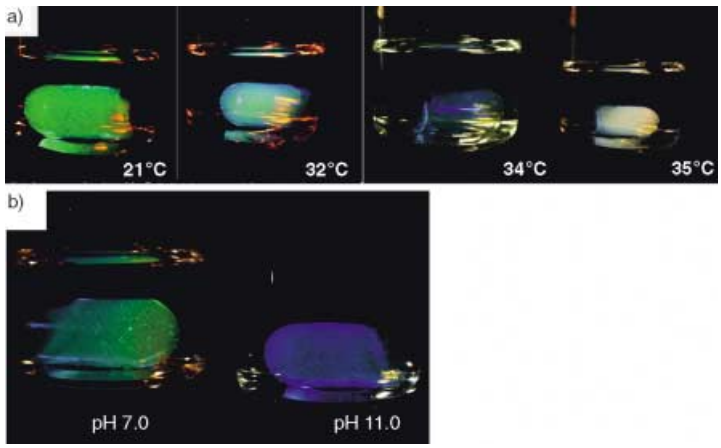


Figure 4. a) The PNIPAM-*co*-allylamine crystal hydrogel changes its iridescent color with temperature. The diameter of the vial is 2.73 cm. From left to right: 21, 32, 34, and 35 °C. b) pH-responsive property of the PNIPAM-*co*-allylamine crystalline hydrogel at pH 7 and pH 11.

ature, the color changes from green to blue at 32 °C, and eventually to milky white at 35 °C, just above the volume phase transition temperature of the particles. When the temperature is decreased to room temperature again, the color and volume of the gel are restored. This process is fully reversible. The color change is due to the decreasing particle size with increasing temperature, which causes a decrease in interparticle spacing. We note that the current hydrogel has iridescent speckles that indicate the existence of large

crystalline domains, in contrast to previous thermally tunable hydrogels, which have only a uniform color.[15,16]

As a consequence of the presence of basic NH_2 groups on the PNIPAM-*co*-allylamine building blocks, the swelling capacity of the hydrogel can be changed by controlling the pH of the medium. As shown in Figure 4b, the color of the hydrogel shifts from green to blue as the pH changes from 7 to 11. Partial ionization of the amino groups on the particles in water at neutral pH results in swelling of the particles. At higher pH values, ionization of the NH_2 groups is inhibited, and the particles shrink. This variation in size of the building block with pH value changes the lattice spacing and results in a color change.

In summary, monodisperse PNIPAM-*co*-allylamine colloidal spheres have been synthesized by precipitation polymerization. The phase diagram of this system was determined by UV/Vis spectrophotometry. By following the phase diagram, a new route to a crystalline structure with a high polymer concentration was discovered by initiating crystallization near T_m but stabilizing the crystalline structure below T_g. The stabilization is achieved by bonding the PNIPAM-*co*-allylamine spheres with glutaric dialdehyde as a cross-linker at room temperature at neutral pH. The hydrogel with a higher polymer concentration has better mechanical strength, while the mild synthesis at pH 7 makes this material particularly useful for biomedical applications, such as loading biomolecules between the particles for controlled drug delivery. The crystalline hydrogels exhibit iridescent patterns that are tunable by changing the temperature or pH value. Such soft and wet hydrogels with periodic structures may lead to new sensors, devices, and displays that operate in the aqueous solutions in which most biological and biomedical systems reside.

Experimental Section

Hydrogel nanoparticles: NIPA monomer (3.845 g), allylamine monomer (0.2 g, 10% molar ratio), methylene bisacrylamide (0.1315 g; cross-linker), sodium docecyl sulfate (0.0755 g; surfactant), and deionized water (230 mL) were mixed in a reactor. The solution was heated to 60 °C with nitrogen bubbling for about 40 min, and potassium persulfate (0.155 g) dissolved in deionized water (20 mL) was added to initiate the reaction. The reaction was carried out at 60 °C for 5 h. The resulting particle dispersions were dialyzed for 7 days to remove small molecules and surfactants.

Light-scattering: The temperature-dependent average hydrodynamic radius and its distribution of the PNIPAM-*co*-allylamine particles in dilute dispersions were measured on a light-scattering spectrometer (ALVCo., Germany) at a scattering angle of 60°.

UV/Vis spectrophotometry: The PNIPAM-*co*-allylamine particle dispersions were condensed in an ultracentrifuge at 40000 rpm for 2 h. The polymer concentration of a dispersion was obtained by completely drying the dispersion at 60 °C and then weighing it. The turbidity of the PNIPAM-*co*-allylamine nanoparticle dispersions as a function of the wavelength was determined on a UV/Vis spectrophotometer (Agilent 8453).[12]

Synthesis of crystalline hydrogels: A 4.0 wt% PNIPAM-*co*-allylamine particle dispersion was heated from 23 to 40 °C and then cooled back to 23 °C at a rate of about 0.4 K min^{-1}. Then glutaric dialdehyde (0.04 g) was added to the top of the dispersion to act as a cross-linker. This reagent diffused through the dispersion and covalently bonded the particles together at neutral pH as illustrated in Scheme 1. The particle assembly with a crystalline structure was stabilized by the cross-linking reaction over about 2 days, and removed from the test tube by injecting water into the bottom of the tube with a syringe.

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