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Inverse thermally-reversible gelation of aqueous *N*-isopropylacrylamide copolymer solutions

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A batch of poly(*N*-isopropylacrylamide-co-acrylic acid) synthesized in benzene shows distributions in molecular weight as well as polymer composition. The copolymer turns out to be a blend of polymers having various lower critical solution temperatures. An aqueous solution of the copolymer above a critical concentration experiences four distinct phases upon heating. One of the phases is a gel phase that shows reversibility in gel–sol transition without noticeable hysteresis or syneresis. Once the gel is formed, it does not dissolve or change in water content until the temperature reaches another transition point. This particular process is related to the molecular weight, the chain entanglement, the molecular transition at elevated temperatures from expanded coils to collapsed globules of a portion of the polymers, and the subsequent aggregation of the collapsed globules to make weak physical junctions. The junctions can be discriminated from the physical cross-linking involved in gelation processes known so far. The thermally induced molecular aggregation of the collapsed polymer chains may be responsible for the gel opacity, negligible gel induction time, lack of hysteresis, and non-elastic deformation.

(Keywords: reversible gelation; no hysteresis; no syneresis)

INTRODUCTION

The thermal phase transition from a polymer solution to a gel by decreasing temperature is commonly found and applied to various areas, particularly in the food industry[1]. Tacticity controlled synthetic polymers, such as polystyrene[2–5], poly(methyl methacrylate)[6], and poly(vinyl chloride)[7] in organic solutions, and many natural polymers of polysaccharides[8,9] and proteins[10] in aqueous solutions undergo gelation when the temperature decreases below its gelation temperature. This process was explained either by solvent-induced or crystallization-induced gelations, and the common features involve reversible formation of local physical cross-linking induced by specific interactions between polymer and solvent or between polymer and polymer segments. Most polymeric systems showing physical gelation are characterized by strong hysteresis between the gelation and gel melting temperature, with a considerable induction time period for gelation, and sometimes with syneresis[2,11–13].

Thermally reversible gelation upon heating (inverse gelation) is relatively rare and is only reported with modified polysaccharides, such as an aqueous solution of methyl cellulose, the gelation mechanism of which is not yet clearly understood[1,14]. Some aqueous solutions of A–B–A-type block copolymers composed of poly(ethylene oxide) (A block) and poly(propylene oxide) (B block), known as Pluronic or Poloxamer, exhibit gelation at elevated temperatures above a critical concentration[15]. Pluronic gel, however, erodes with an excess amount of water from the gel surface. The gelation is explained by temperature-dependent micelle formation and packing of micelles of the amphiphilic block copolymers, resulting in loss of micelle mobility.

This paper reports the unique inverse gelation process of an aqueous solution of poly(*N*-isopropylacrylamide-co-acrylic acid). Poly(*N*-isopropylacrylamide) (polyNiPAAm) is a representative of polymers exhibiting a lower critical solution temperature (LCST) around 32°C in distilled water[16–18]. The LCST has been manipulated by copolymerizing more hydrophilic or more hydrophobic comonomers and is influenced by salts and other additives[19–24]. The related polymers have been widely applied in biomedical and pharmaceutical fields[25–28] as well as in biotechnology[23,29–33], including surface modification, cell culture matrix, drug delivery, drug targeting, enzyme modification and immobilization, and molecular separation. These applications are related to the modulated hydrophilicity of polyNiPAAm by temperature, especially around its LCST.

Unlike polyNiPAAm, of which an aqueous solution precipitates at a low concentration or results in a shrunken mass at higher concentrations when the temperature is elevated above its LCST, the copolymer synthesized in benzene experiences gelation above a critical concentration and this gelation is characterized by no hysteresis and negligible gel induction time. This novel gelation process and associated solution properties have been investigated to elucidate, at least partially, the gelation mechanism and to propose a probable model for the gelation.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NiPAAm; Acros Organics, Belgium) was purified by recrystallization from *n*-hexane

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and dried under vacuo for four days. Acrylic acid (AAc; Acros) and ethylene glycol dimethacrylate (EGDMA; Acros) were distilled at 39°C/10 mmHg and 60°C/0.1 mmHg, respectively. 2,2′-Azobisisobutyronitrile (AIBN; Acros) was purified by recrystallization from methanol. All solvents used in this experiment were reagent grade and were used as received.

Polymer preparation

High molecular weight polymers of NiPAAm with AAc (2 mol%) or without AAc were synthesized by free radical polymerization in benzene (10 wt% monomer concentration) with AIBN as an initiator (7×10^{-3} moles/mole monomer). The monomer solutions were bubbled with dried nitrogen gas for 30 min. After adding AIBN, the mixture was degassed for 30 min by applying a vacuum. The polymerization was performed at 60°C for 16 h under dried nitrogen gas pressure. The polymers precipitated in the solvent as polymerization proceeded. The precipitated products were dissolved in acetone/methanol mixture (90/10 v/v%) by warming, precipitated in an excess amount of diethyl ether, and then dried in vacuo for 3 days. The copolymers were obtained at various conversions by changing the reaction temperature and time. Low molecular weight NiPAAm homopolymer and copolymers with AAc were synthesized in 1,4-dioxane. All polymer products were dialyzed using 15 000 molecular weight cut off dialysis tubing against distilled water for at least 4 days to remove low molecular weight compounds. The crosslinked poly (NiPAAm-co-AAc) was prepared with identical monomer concentration in benzene, except that 5×10^{-3} moles of EGDMA per mole of monomer were added as a crosslinker. The monomer mixture was poured between two glass plates which were separated with a flat silicon rubber gasket (3 mm in thickness). After polymerization for 16 h at 60°C, the glass plates were separated in an ultrasonic bath and the crosslinked polymers were purified by successive soaking in a series of methanol/water mixtures (100/0, 75/25, 50/50 and 25/75 v/v) for one day in each mixture. The polymer was finally purified by soaking in deionized water for 7 days.

A single batch of the copolymer synthesized in benzene was fractionated by a successive solution fractionation method[34,35] at 25°C. The copolymer sample (10 g) was dissolved in 200 ml of acetone to make a starting solution. To this solution, 3 l of diethyl ether was added to cause phase separation. The polymer-lean phase was separated as the first fraction. Acetone was added to the polymer-rich phase to give a solution with the same total volume as the first cycle and this solution was used as a starting solution in the next cycle. This procedure was repeated four times to obtain five polymer fractions.

Characterization

Absolute molecular weights of the polymers were determined by dynamic light scattering (Malvern: S4700 Version PCS). The polymer solutions were prepared in methanol at concentrations of 0.1, 0.2, 0.3, and 0.4 w/v% and filtered through a 0.2 μm pore size membrane. The light scattering measurement was carried out at 488 nm wavelength at 25°C. The incident beam (He/Ne laser) was unpolarized and measuring angles between 40 and 140 degrees were used to construct Zimm-plots.

The compositions of the polymers were estimated by a titration method. Polymer samples (100 mg) were dissolved in 15 ml of 0.15 M NaCl aqueous solution. All AAc groups were ionized by adding 3 ml of 0.015 M NaOH and titrated with 0.015 M HCl using a phenolphthalate solution as an indicator. The temperature was kept constant at 20°C for 30 min prior to and during titration using a thermostated water bath.

Cloud temperature

The polymer solutions were prepared in phosphate buffered saline (PBS: pH 7.4; ionic strength 0.2 M). The temperature of the solutions was raised from 5 to 60°C at a rate of 0.1°C min^{-1} and the transmittance at 500 nm was recorded by a Varian UV-VIS spectrometer (CARY 1E). The cloud temperature was defined as the temperature at which the transmittance becomes 50% of the initial value at 5°C.

Gelation process

The gelation and gel dissolution were examined by a vial inversion method as increasing or decreasing temperature. Polymer solutions (1 ml) at various polymer concentrations in glass vials (7 ml vol., 1.2 cm dia.) were kept in a thermostated water bath for 5 h prior to inverting the vials. The experiment was carried out at 1°C intervals. The gelation temperature was determined visually when the polymer solutions did not flow by inverting the vials. The reversibility in the gelation was confirmed by measuring flow time through a Cannon–Fenske viscometer tube (ASTM size 350) at different temperatures. Five ml of polymer solution (5 w/v%) was poured into a viscometer tube. The temperature was kept constant for 30 min prior to and during flow time measurement in a water bath. The flow time of the solution was measured from 20 to 37°C at 1°C interval.

RESULTS AND DISCUSSION

A typical gelation process of an aqueous polymer solution (5 wt%) of poly(NiPAAm-co-AAc) (2 mol% AAc in feed), polymerized in benzene by a conventional free radical polymerization method, in phosphate buffered saline (PBS; pH 7.4; ionic strength 0.2 M) is shown schematically in *Figure 1*. The clear polymer solution (phase 'A') at low temperatures becomes cloudy as temperature increases to 27°C (defined as the cloud temperature), but it is freely mobile (phase 'B') even with slightly reduced viscosity (see *Figure 2*). With further increased temperature, the opaque polymer solution subsequently becomes immobile at 35°C (defined as the gelation temperature) as demonstrated in *Figure 1* (phase 'C'), resulting in gelation without a

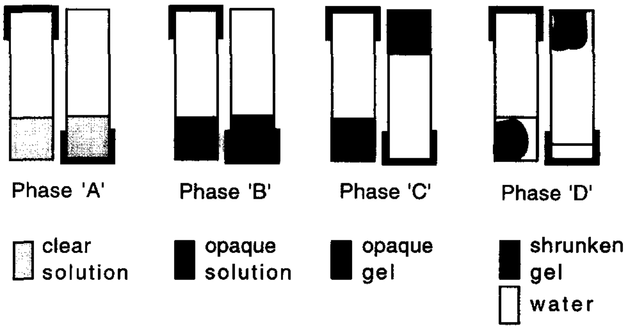


Figure 1 Phase transitions and gelation process of an aqueous solution (PBS, pH 7.4, ionic strength 0.2 M) of poly(NiPAAm-co-AAc) (synthesized in benzene with 2 mol% AAc in feed) with increasing temperature. (Refer to the experimental section for the conditions of gelation process)

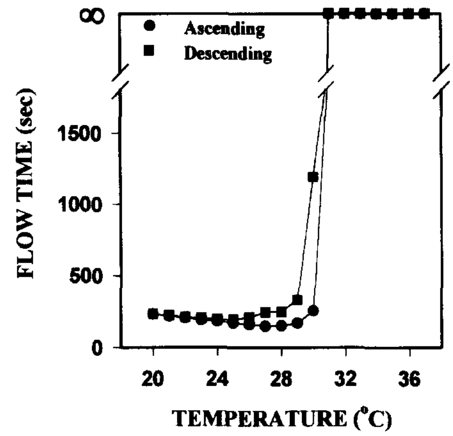
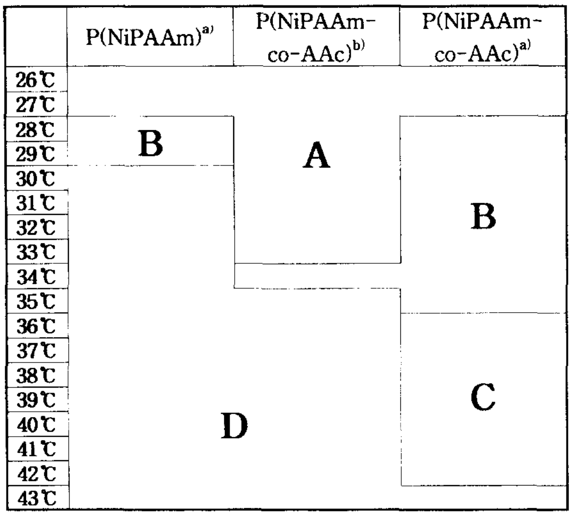


Figure 2 Flow time (viscosity) of poly(NiPAAm-co-AAc) (synthesized in benzene with 2 mol% AAc in feed) solution (PBS, pH 7.4; ionic strength 0.2 M; 5 wt% polymer) as a function of temperature through a Cannon–Fenske viscometer tube (ASTM#350)



a) Synthesized in benzene
b) synthesized in 1,4-dioxane

Figure 3 Phase transition tempertures of poly(NiPAAm) and poly(NiPAAm-co-AAc) solutions (PBS, pH 7.4; ionic strength 0.2 M; 5.0 wt% polymer). A: Clear solution phase; B: Cloud solution phase; C: Gel phase; D: Shrunken gel phase

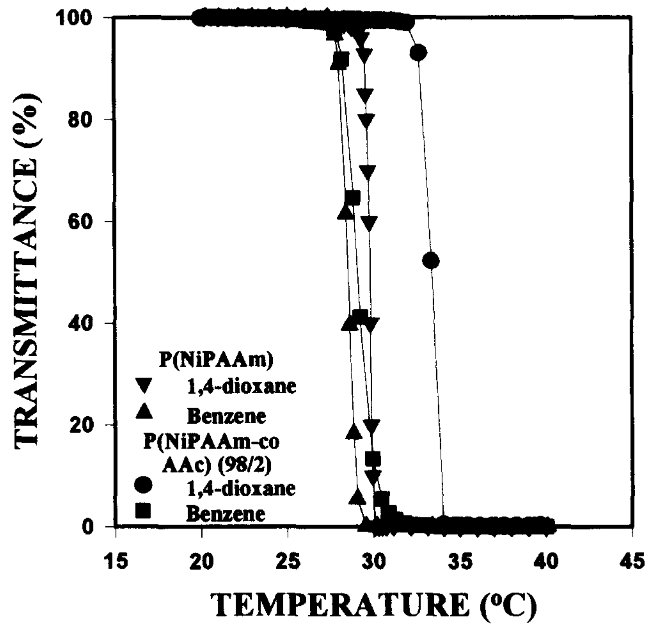


Figure 4 Temperature-dependent transmittance of poly(NiPAAm) and poly(NiPAAm-co-AAc) solutions (PBS, pH 7.4; ionic strength 0.2 M; 5.0 wt% polymer)

significant gel induction time. The time required for the gelation is apparently of the same order as the thermal induction time. The initially formed gel is translucent and becomes more opaque with increasing temperature. At 43°C (defined as the gel-shrinking temperature), the gel starts to shrink by expelling water (syneresis). When being exposed to a mechanical shear stress, the gel deforms without shape memory and can be considered as a non-elastic material which is different from other physical gels. Once the gel is formed, it does not dissolve or change its water content on adding additional water. Upon cooling the gel, it becomes mobile at the same temperature as the gelation temperature, indicating no hysteresis between gelation and gel flowing temperatures. This reversibility is confirmed by measuring the temperature-dependent viscosity (flow time) through a viscometry tube and the result is shown in *Figure 2*. The solution viscosity in phase 'A' slightly decreases before clouding. When the polymer chains become compact globules, there is a strong tendency to aggregate with each other, resulting in a cloudy solution. The decrease in the viscosity in phase 'A' may indicate that there is an intermediate state between fully expanded and completely collapsed polymer chains and in this state the chains remain distinct. The viscosity of the cloudy solution sharply increases and eventually becomes a non-flowing mass, at around 31°C in the flow time measurement. On decreasing temperature, the polymer solution starts to flow at the same temperature. Although a small hysteresis loop in viscosity below the gelation temperature was found, there is no noticeable discrepancy between the gelation temperature upon heating and the gel-flowing temperature upon cooling. The hysteresis is always observed with known natural and synthetic gelling polymers in either an aqueous or organic environment, with the smallest hysteresis being for *i*-carrageenan among other gelling polymeric systems, where the gelation occurs at about 65-68°C by decreasing temperature and melts above 2-5°C[2]. The lack of hysteresis might be a unique observation with the particular gelling system used in this study.

For reference, we have examined the gelation with polyNiPAAm synthesized in benzene and poly(NiPAAm-co-AAc) (2 AAc mol% in feed) synthesized in 1,4-dioxane which is a common solvent for NiPAAm copolymer synthesis in the literature and the result is presented in *Figure 3*. Phase 'C' is only found with the copolymer synthesized in benzene. When being polymerized in 1,4-dioxane, the polymer has a lower molecular weight (MW) (M_W~100 000) than that (M_W~1 000 000) polymerized in benzene because of a radical chain transfer reaction to the solvent molecules during polymerization[36]. Surprisingly, its aqueous solution at the same concentration does not form a gel and becomes a shrunken mass at elevated temperatures, although the average chemical composition of the two copolymers are close to each other, confirmed by ^1H NMR spectra and elemental analysis. Another unexpected observation is that the cloud temperature of the copolymer synthesized in 1,4-dioxane is 33°C which is consistent with the results in other reports[19,20], considering the AAc content in the copolymer and the salt effect on LCST, but the cloud temperature of the copolymer synthesized in benzene is close to polyNiPAAm. *Figure 4* shows the detailed experimental results from the measurement of light

Table 1 Molecular weights and relative acrylic acid content of fractionated poly(NiPAAm-co-AAc) (2 mol% AAc in feed) synthesized in benzene

	Frac. #1	Frac. #2	Frac. #3	Frac. #4	Frac. #5
Av. Mw[a]	2 180 000	1 050 000	550 000	300 000	68 000
Relative AAc content[b]	1.42	1.37	1.21	1.05	1

[a] Determined by the dynamic light scattering method.

[b] Obtained by the acid–base titration method.

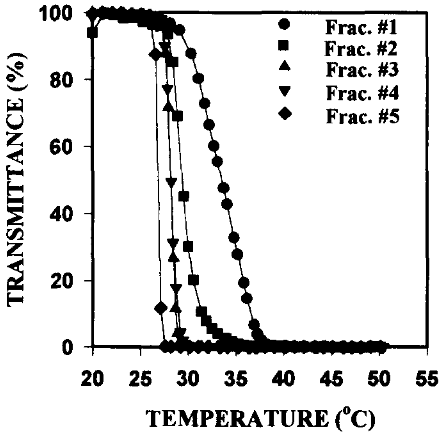


Figure 5 Temperature-dependent transmittance of fractionated poly(-NiPAAm-co-AAc) (synthesized in benzene) solutions (PBS, pH 7.4; ionic strength 0.2 M; 5.0 wt% polymer)

transmittance. This result implies that another factor rather than molecular weight may influence the gelation.

To investigate this in more detail, we attempted to fractionate the polymer synthesized in benzene. When the copolymer was divided into five different fractions by a solvent fractionation technique, it was revealed that the copolymer has distributions not only in molecular weight but also in composition (see *Table 1* for composition and molecular weight for each fraction), indicating a blend of different polymers despite being synthesized in one batch. The fraction of higher MW has the higher content of AAc. The AAc distribution was also confirmed by polymerization at low conversions. Chemical analysis demonstrated 4.4 and 2.7 mol% of AAc on average for 8 and 40% conversion polymers, respectively. The cloud temperature of the polymer solutions increased to 40°C (8% conversion) and 34°C (40% conversion). These results indicate that the polymer composition varies with conversion, which may result from a higher reactivity ratio of AAc than that of NiPAAm in benzene. The reactivity ratios of the two monomers in benzene and dioxane are under investigation. The clouding behaviours of the fractionated polymers examined by measuring transmittance of the visible light at 500 nm as a function of temperature are presented in *Figure 5*. The light transmittance reflects the degree of aggregation of the collapsed polymer chains with the higher transmittance for more transparent solutions. The transmittance from the fraction #1 decreases in a 10° range starting from 28°C. The sharpness in the transmittance change increases with lower molecular weight polymers and less AAc content. The relatively shallow decrease for higher molecular weight polymers is an unusual result, considering the similar experimental results for NiPAAm copolymer reported in the literature[33,37], where the transmittance drops sharply in a 1-2° range, as in the cases of the fractions #3, #4 and #5 in this experiment. This smooth decrease may be attributed to the high molecular weight and the higher AAc content which give more swelling power to the chain.

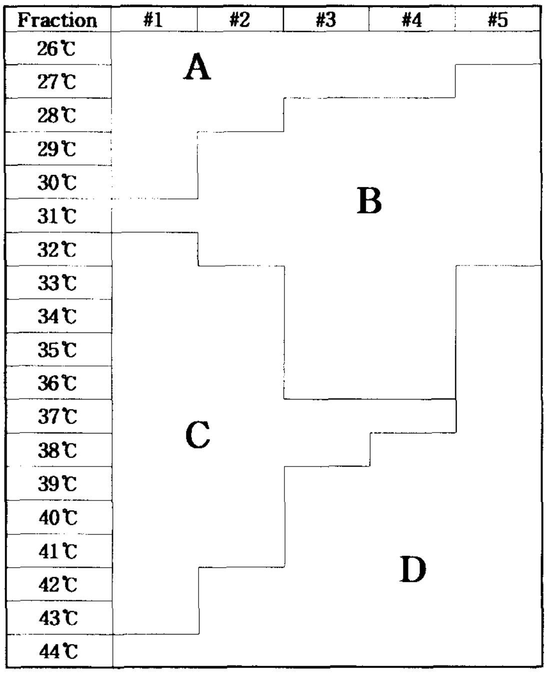


Figure 6 Phase transitions of fractionated poly(NiPAAm-co-AAc) (synthesized in benzene) solutions (PBS pH 7.4; ionic strength 0.2 M; 5.0 wt% polymer). A: Clear solution phase; B: Cloud solution phase; C: Gel phase; D: Shrunken gel phase

The phase transition behaviour of the fractionated polymer samples is summarized in *Figure 6*. There are interesting trends in the temperature ranges for each phase. With the decrease in molecular weight and AAc content, the cloud temperature decreases as presented in *Figure 5*, the gelation temperature increases, and the gel-shrinking temperature decreases. Thus, fraction #1 forms a gel in a border temperature range from 31 to 43°C, while fraction #5 does not form phase 'C' at all, the pattern of which is similar to the polymer synthesized in 1,4-dioxane. This suggests that the distributions in molecular weight and acrylic acid content can be critical factors in gel formation and its stability.

The gelation of fraction #1 at 31°C may be related to the result of *Figure 2*. In the flow–time measurement, the unfractionated polymer solution stopped flowing at the same temperature. Although the vial inverting method showed the gelation temperature at 34–35°C, the gelation trend of the polymer portion of the highest molecular weight and AAc may cause resistance of the unfractionated polymer solution in a small diameter capillary, although this resistance is not large enough to prevent the solution from flowing in a sample vial.

The construction of a phase diagram for the unfractionated polymer is presented in *Figure 7*. At a concentration lower or equal to 3 wt%, the solution does not form phase 'C', indicating that there is a critical concentration for this particular polymer. On increasing the concentration, the cloud temperature and gel-shrinking temperatures stay almost constant above 4 wt% solution. However, the

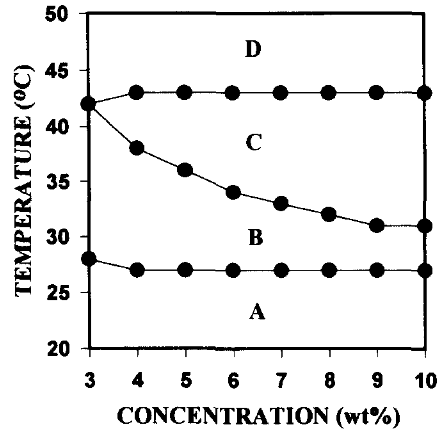


Figure 7 Phase diagram of poly(NiPAAm-co-AAc) (synthesized in benzene) solutions (PBS, pH 7.4; ionic strength 0.2 M). A: Clear solution phase; B: Cloud solution phase; C: Gel phase; D: Shrunken gel phase

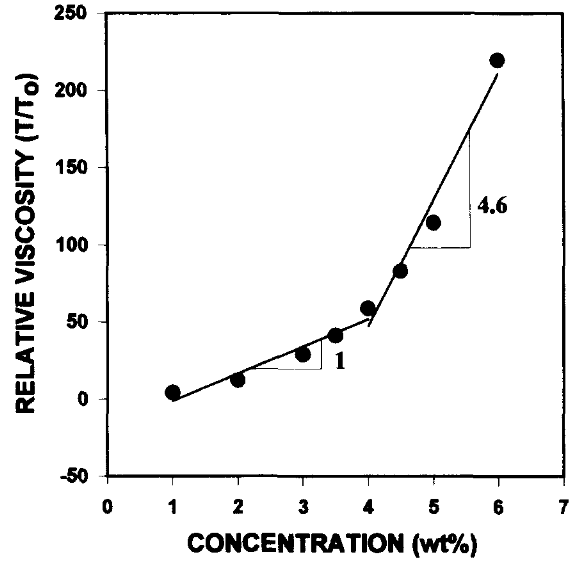


Figure 8 Concentration-dependent relative viscosity of poly(NiPAAm-co-AAc) (synthesized in benzene) solutions (PBS, pH 7.4; ionic strength 0.2 M; 5.0 wt% polymer)

gelation temperature decreases remarkably. This result suggests that in addition to the effects of molecular weight and AAc distribution on the gelation, the polymer concentration is another critical factor in the determination of each phase, especially the gel phase. To obtain more detailed information about the concentration effect, the viscosity of the unfractionated sample below its cloud temperature was measured as a function of concentration as shown in *Figure 8*, where the viscosity sharply increase at concentrations above 4 wt%, coinciding with the minimum concentration for gelation. This observation may result from considerable penetration among the polymer chain, thus leading to chain entanglement in aqueous solutions.

Taking the observations into account, one can expect that, with increasing temperature, the polymer chains with a lower AAc content precipitate first, while the polymers with higher AAc content remain as partially collapsed globules or in a fully expanded coil state, depending on the AAc content. The completely collapsed globules, entangled with expanded coils, may have a tendency to form hydrophobic aggregates. The aggregates of collapsed chains form physical junctions, but these are not strong enough to hold the gel shape under stress. This junction characteristic may explain the non-elastic deformation under mechanical shear stress and thermal reversibility. However, uncollapsed polymer chains entangled with globules may form the swollen matrix of the gel. The molecular weight effect of the polymer on gel formation may be related to the degree of chain entanglement at a fixed polymer concentration. This mechanistic model is presented schematically in *Figure 9*.

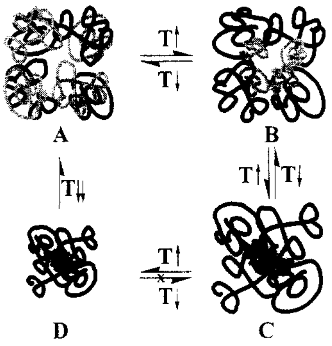


Figure 9 Schematic presentation of proposed mechanism for thermally reversible gelation of NiPAAm copolymer solution in water. A: Clear solution phase; B: Cloud solution phase; C: Gel phase; D: Shrunken gel phase

As a tool for the verification of the proposed model, a chemically cross-linked polymer network was synthesized in benzene under the same conditions, except using ethylene glycol dimethacrylate as a cross-linker at a concentration of 1 mol% of the monomers. After purification by extraction in water and drying, the network was allowed to imbibe PBS (pH 7.4, ionic strength 0.2 M) until the water content became 95% by weight, thus giving similar conditions of 5 wt% polymer solution. After complete equilibration at 23°C, the temperature was increased. The swollen gel became translucent at 32°C and started to shrink at 35°C, expelling water, which is the same as the gelation temperature of the uncrosslinked and unfractionated polymer at 5 wt% concentration. This network prevented possible migration of collapsed segment portions in the gel, preventing the formation of physical junctions.

This reversible gelation can be distinguished from other polymeric gelling systems of polysaccharides and proteins in aqueous solutions and tacticity controlled poly(methyl methacrylate), polystyrene, and poly(vinyl chloride) in organic solutions, in which gelation is induced physical crosslinking from either liquid–liquid phase separation followed by liquid–solid transformation or partial crystallinity, and characterized as a cold gelation with a strong hysteresis.

This unique reversible gel is currently applied to the design of extracellular matrices for a refillable cell transplantation system[38] which can supply specific cell products, such as insulin from islets of Langerhans, to the body. Since signals other than temperature can be utilized in the reversible gelation, a broad range of applications of this gelation is expected in biomedical and other industrial fields.

CONCLUSION

The composition of poly(NiPAAm-co-AAc) synthesized in benzene varies with conversion, resulting in a blend of polymers having various lower critical solution temperatures. An aqueous solution of the copolymer above a critical concentration experiences four distinct phases upon heating.

One of the phases is a gel phase but shows reversibility in the gel–sol or gel–sol transition without noticeable hysteresis or syneresis. This observation is unique for the copolymer synthesized in benzene. Once the gel is formed, it does not dissolve or change water content until the gel-shrinking temperature is reached. This particular process requires a high molecular weight for the chain entanglement and the AAc distribution in the polymer molecules for the early collapse of polymers having lower LCST with increasing temperature. This allows aggregation of the collapsed globules to make weak physical junctions, whereas the polymer with higher LCST are expanded. The junctions can be discriminated from the physical cross-linking involved in gelation processes known so far. The thermally induced molecular aggregation of the collapsed polymer chains may be responsible for the gel opacity, negligible gel induction time, lack of hysteresis, and non-elastic deformation.

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