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Thermoresponsive Interpolyelectrolyte Complexation: Application to Macromolecular Assemblies

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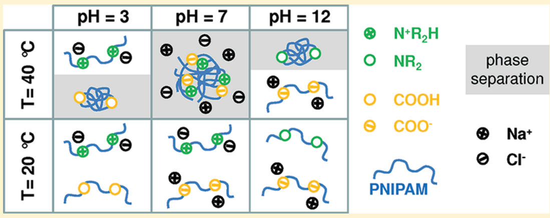
S *Supporting Information*



ABSTRACT: pH and thermoresponsive polymers have been prepared by copolymerizing *N*-isopropylacrylamide (NIPAM) with various amounts of ionizable comonomers, either acrylic acid (AA) or *N*-[3-(dimethylamino)propyl]methacrylamide (MADAP). In aqueous solution, the LCST-type phase transition of these copolymers studied by differential scanning calorimetry is strongly influenced by the comonomer ratio. Under un-ionized conditions, the phase transition temperature progressively increases with MADAP content at pH 12 while it remains unchanged or slightly decreases with AA at pH 3 due to the formation of hydrogen bonds between AA and NIPAM units. When the copolymer chains are progressively charged by tuning the pH, the phase transition of PNIPAM-AA and PNIPAM-MADAP is shifted at higher temperature and is no longer observable below 60 °C when the ionic content exceeds 10%. By comparison with these single systems, where the association properties can be finely adjusted by coupling hydrophobic attractions and electrostatic repulsions, we also investigate the possibility to couple hydrophobic interactions with electrostatic attractions by mixing oppositely charged copolymers: PNIPAM-AA and PNIPAM-MADAP. This study was carried out with the copolymer pair containing 10 mol % of ionizable groups (A10 and M10) which was the most adaptable one from the point of view of responsivity. At pH 7, when AA and MADAP units are ionized, the copolymer chains are separately soluble in water in the whole temperature range while their mixture, also soluble at room temperature, phase separates upon heating. The original feature highlighted in this work is that the phase transition proceeds through a selective mechanism between complementary chains (formation of a reversible interpolyelectrolyte complex) and that this selectivity can be switched with the pH. Indeed, starting at room temperature with the copolymer mixture (A10 and M10), which remains homogeneous at all pH, we demonstrate that the association process can be switched by increasing the temperature and that the pH can be used to specifically address these associations: from A10/A10 at low pH to A10/M10 at pH 7 up to M10/M10 at high pH. Finally, the responsive precursors A10 were grafted onto a polyacrylamide backbone, and the viscoelastic properties of graft copolymers were studied in the semidilute regime and compared with calorimetric data. In this way, we show that the same set of interactions can be readily applied to more sophisticated macromolecular assemblies with the responsive formation of physical gels under pH and temperature control.

1. INTRODUCTION

Responsive polymers have attracted considerable attention during the past 20 years due to their high potentiality in tailoring smart materials and complex formulations. It is the subject of a large number of scientific papers dedicated to theoretical aspects of the transition (thermodynamics and kinetics) and to potential applications in the field of mechanical transducers (artificial muscles), microfluidics, reversible adhesion, or drug delivery systems.[1−7] Among these polymers, poly(*N*-isopropylacrylamide) (PNIPAM), which phase separates in aqueous media above its lower critical solution temperature (LCST) close to the body temperature, is certainly the most popular. While its solubility in water strongly depends on environmental conditions, such as temperature, pressure, added salt, cosolvent, surfactants, etc.,[2,8−12] its properties can be finely tuned by playing with the chemical composition of the polymer chain. For instance, the solubility of PNIPAM in water can be easily reduced or enhanced by introducing hydrophobic or hydrophilic comonomers, respectively.[13−15] Moreover, the introduction of ionizable groups, acids or bases, gives rise to multiple responsive systems with a strong coupling between temperature, pH, and ionic strength.[16−23] This set of interactions, which has been initially applied to control the swelling behavior of covalent hydrogels,[1] has been extended later on to a large number of nanostructured stimuli-responsive polymer materials. The responsive properties of these materials rely on the phase behavior of macromolecular assemblies that takes place either in solution (formation of physical gels, micelles, nanogels, capsules, vesicles, etc.) or at interfaces in thin films (polymer brushes, multilayered films, membranes, etc.), emulsions, or foams.[24] By changing its conformational state under given stimuli, the responsive systems can strongly modified their macroscopic swelling/collapse behavior) or at interfaces where they are able to switch their interactions and to bind reversibly specific molecules.

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In this regard, the coupling between the thermosensitivity of PNIPAM and electrostatic interactions highly enhances the responsiveness of the macromolecular structures with the occurrence of discontinuous phase transitions[10,18] and opens the way to the development of responsive interpolyelectrolyte complex (IPEC) based materials with a broad spectrum of applications. For example, it has been shown by Tanaka and co-workers[25] that PNIPAM gels, containing a small amount of cationic monomer units (1/30), could have very different affinity for polyanionic species depending on the gel state: collapsed or swollen. According to the authors, thermoresponsive cationic gels were able to recognize and capture a target molecule by multiple-point interaction and can reversibly change their affinity to the target by more than 1 order of magnitude. Similarly, Kono et al.,[26] who worked on responsive microcapsules formed by polyelectrolyte complexation, have shown that the introduction of a large amount of NIPAM units into one of the polyelectrolyte could provide an original temperature dependence of the permeability of these capsules with controlled release properties. On the other hand, Dautzenberg et al.[27] have mixed PNIPAM copolymer chains containing either sulfonic or quaternary ammonium monomer units (10−30 mol %). They show that the complex formation between these oppositely charged PNIPAM copolymers gives rise to nearly stoichiometric complex particles on a 100 nm scale and that these particles show a strong temperature dependence of their swelling ability. This situation is somewhat equivalent to chemically cross-linked nanogels, but the covalent bonds between PNIPAM chains are replaced here by electrostatic interactions. Similarly, a lot of work is actually devoted to the ability of polycations to bind and condense DNA into submicrometer complexes (polyplexes) that protect the DNA from nucleases during transit to the nucleus. However, while polyplexes have demonstrated potential to transfect a wide variety of cells *in vitro*, they do not attain adequate expression levels *in vivo* to be therapeutically useful. In particular, the inability of condensed DNA to efficiently dissociate from the polycation during cytoplasmic transport has been identified has a major limitation for *in vivo* applications, and thermoresponsive cationic systems could have a positive effect with regard to this problem.[28] For all these systems, coupling hydrophobic and electrostatic forces, the main difficulty is generally to reach a good equilibrium between the interactions in order to get an accurate switch of the properties. Within this framework, the initial goal of this work was to use the chemical toolbox of NIPAM to develop a mechanism of thermoresponsive selectivity between two complementary PNIPAM copolymers. In other words, the idea was to find the right balance of interactions in order to design two oppositely charged copolymers, soluble in water at all temperatures, but that could be able to form a LCST-type complex when they are mixed together. Using NIPAM for its temperature responsivity and a weak acid (A) or a weak base (M; see Figure 1) for their pH responsivity, we have synthesized two series of copolymers, PNIPAM-A and PNIPAM-M, with various amounts of ionizable comonomers and studied their solubility in aqueous media by calorimetry as a function of pH and temperature. Among these systems, one pair of oppositely charged copolymers has been selected for its high versatility toward environmental conditions and was used to demonstrate the concept of thermoresponsive selectivity based on the formation of a reversible interpolyelectrolyte complex. Finally, these responsive precursors have been applied in the framework of macromolecular self-assemblies in order to test the potentiality of this interplay between hydrophobic and electrostatic interactions to switch sol/gel transitions in aqueous media.

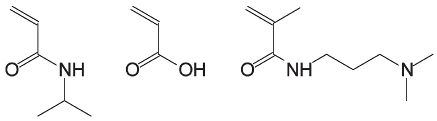

Figure 1. Chemical structure of *N*-isopropylacrylamide (NIPAM), acrylic acid (A), and *N*-[3-(dimethylamino)propyl]methacrylamide (M).

2. EXPERIMENTAL SECTION

2.1. Synthesis of Amino-Terminated Telomers. The synthesis of functional chains was achieved by radical polymerization using an efficient chain transfer agent (cysteamine). This kind of reaction, also called telomerization, allows controlling the end group of the polymer as well as its molar mass. The same protocol, detailed in ref 29, has been applied for all the copolymers taking care that the reaction takes place in acidic conditions; typically at pH 3−3.5 (experimental details are given in Supporting Information). The composition and molar mass of the copolymers were determined by potentiometric titration, ^{1}H NMR, and SEC (Table S1 in Supporting Information). Two series of PNIPAM copolymers have been prepared, with a molar percentage of ionizable units (y), acid or basic, of 5, 10, 20, and 50 mol %. The copolymers will be named PNIPAM-Xy, where X is A for AA or M for MADAP and y is the molar percentage of ionizable groups in the copolymer chain. PNIPAM-A5 is therefore equivalent to P(NIPAM$_{95}$-AA$_5$), i.e., a copolymer containing 95 mol % of NIPAM and 5 mol % of AA. All the copolymers are characterized by a similar molar mass distribution with M_n = 13 ± 2 kg/mol ($Đ \cong 1.4$) that is only slightly higher for the sample PNIPAM-A50 (M_n = 20 kg/mol; $Đ$ = 1.8). These results are in good agreement with similar telomerizations carried out with NIPAM and other hydrophilic monomers like acrylamide or sodium acrylamido-2-methylpropane sulfate.[14] Potentiometric titration of copolymers in water was carried out to estimate the pK_a values and the variation of ionization with pH (see Figure S2 in Supporting Information). For copolymers PNIPAM-M10 and PNIPAM-M50 the same pK_a was determined at 8.9. This value could be slightly underestimated due to the titration of the amino end group (AET pK_a = 8.4), but this terminal group remains negligible in number compared to the tens or 50 MADAP units present in the two copolymers. By comparison, the pK_a of MADAP monomer is 9.25.[30] In the case of PNIPAM-A10 and PNIPAM-A50 the pK_a were estimated as 5.1 and 5.5, respectively. This difference could be explained by a stronger polyelectrolyte effect for PNIPAM-A50. These values are in good agreement with the literature data: 5.1 for PAA chains,[31] 4.9 for PNIPAM-AA[19] (including 0.7% molar of AA units), the pK_a of AA monomer being 4.5.[31]

2.2. Synthesis of Grafted Copolymers. In order to investigate the responsive properties of PNIPAM derivatives, in the framework of macromolecular assemblies, a polyacrylamide backbone (PAM) with pendant PNIPA-A10 side chains was prepared. Starting from the PNIPAM-A10 precursor, the synthesis follows a two-step procedure, already described in refs 32 and 33, with (1) a peptide coupling between acrylic acid and the amino-terminated telomer and (2) the radical copolymerization of the resulting macromonomer with acrylamide (see Supporting Information). The grafted copolymer named PAM-*g*-PNIPAM-A10 was characterized by SEC (M_n = 470 kg/mol and $Đ$ =1.9) and ^{1}H NMR. The weight fraction of PNIPAM-A10 side chains introduced in the copolymer is 0.34, which gives an average number of 13 grafts per macromolecule.

2.3. Differential Scanning Calorimetry (DSC). Responsive properties involving PNIPAM phase transition were studied by differential

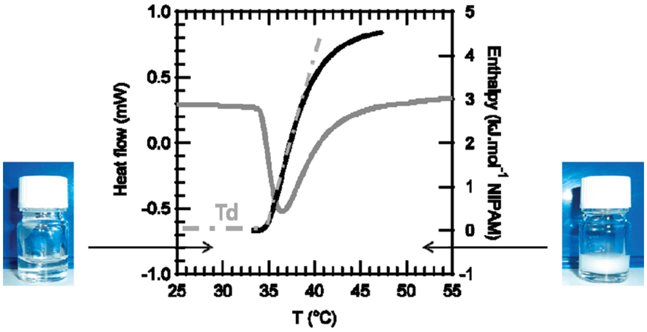

Figure 2. DSC experiment of PNIPAM telomer in aqueous solution (C_p = 1 wt % and pH = 7). Heat flow (gray line) and cumulated enthalpy (ΔH: black line) are plotted as a function of temperature. The determination of T_d, the beginning of the transition, is shown in gray.

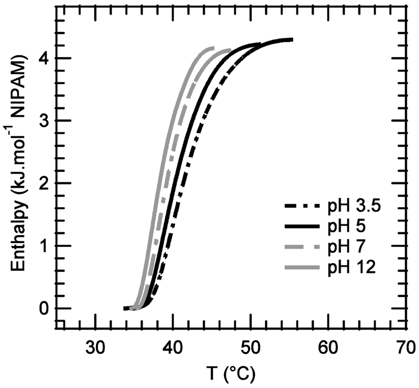

Figure 3. Influence of pH on the phase transition of PNIPAM in water (C_p = 1 wt %).

scanning calorimetry (DSC) with a microDSCIII (Setaram). Polymer solutions ($V \cong$ 0.8 mL), equilibrated with a reference filled with the same quantity of solvent, were submitted to temperature cycles between 10 and 70 °C with heating and cooling rates of 1 °C/min.

2.4. Rheology. The viscoelastic properties of the graft copolymers were studied in aqueous solutions, in the semidilute regime, using a stress-controlled rheometer (AR 1000 from TA Instruments) equipped with a cone/plate geometry (diameter 40 mm, angle 2°, truncature 55.9 μm). The experiments were performed in the linear viscoelastic regime which was established for each sample by a stress sweep at the lowest frequency. The temperature was controlled by a high power Peltier system that provides fast and precise adjustment of the temperature during heating and cooling stages. The experimental conditions were fixed at constant frequency (1 Hz) and shear stress (2 Pa). A particular care was taken to avoid the drying of the sample by using a homemade cover which prevents from water evaporation during experiment. In these conditions, dynamic moduli (G' and G'') as well as complex viscosity (η^*) were recorded between 20 and 60 °C by applying heating and cooling scans of 2 °C min^{-1}. This rate was chosen to avoid problem of drying at high temperature, and we have checked that in these conditions the macroscopic properties are under thermodynamic control, i.e., independent of the scanning rate.

3. RESULTS AND DISCUSSION

3.1. Phase Behavior of PNIPAM Telomers. *HomoPNIPAM.* PNIPAM chains are known to undergo a phase separation upon heating. This transition is related to the dehydration process of the polymer that occurs with a rearrangement of water molecules. Below the critical temperature, a coiled structure is promoted as this allows maximum interactions between the polymer chain and water molecules. The macromolecule undergoes a sharp coil-to-globule transition in water at about 32 °C, changing from a hydrophilic state below this temperature to a hydrophobic one above it. The LCST phase transition of PNIPAM is an endothermic process (energy is needed for disrupting hydrogen bonds between water molecules and amide group) that can be followed by DSC. A typical thermogram is given in Figure 2 for an aqueous solution of PNIPAM at pH 7 (C_p = 1 wt %). In the present case, where short PNIPAM chains are investigated, the transition zone is rather broad with a dehydration process that spans over 10 °C. The coil–globule transition of PNIPAM begins at T_d = 34 °C that typically corresponds to the cloud point of the solution (see Figure 2). This value is in good agreement with other transition temperatures reported in the literature for PNIPAM in water, higher temperatures and broader transitions being generally correlated to lower molecular weights.[34] In order to facilitate the analysis of the whole phase separation process, the transition enthalpy (ΔH in kJ/mol of NIPAM) has been calculated by integrating the endotherm. It allows to estimate the yield of the transition versus the temperature and to calculate the enthalpy of the whole phase transition (ΔH_T). In this way, a transition enthalpy of ΔH_T = 4.3 kJ/mol is obtained, in good agreement with the literature data concerning the dehydration process of PNIPAM chains from aqueous solution (ΔH_T = 4–6 kJ/mol[34–36]). An exothermic peak with the same associated energy is obtained when cooling down the solution with almost no hysteresis.

As it can be observed in Figure 3, a similar phase transition is observed for PNIPAM in both acidic and basic media with a very small sensitivity to pH. While the transition enthalpy (ΔH_T) remains independent of the pH, the transition temperature increases very slightly from 36 to 38 °C with increasing the pH from 3 to 12, respectively, the most visible difference being the width of the transition decreases with increasing pH. These small differences can be attributed to the contribution of the ionizable amino group introduced at the end of the PNIPAM telomer. Under basic conditions (pH 12), the functional group is not ionized and does not interfere with the dehydration process of PNIPAM.

Conversely, below the pK_a of the amine (8.05–8.35), the ionic end group $-NH_3^+Cl^-$ acts again the phase separation for entropic reasons and slightly delays the transition process at higher temperature. This phenomenon has already been described in the case of poly(ethylene oxide-*co*-propylene oxide) oligomers (M_n = 2.5 kg mol^{-1}), the influence of end groups being obviously more pronounced with short polymer chains.[37] Although the ionizable end group slightly impacts the phase transition of the telomer, we can nevertheless consider as it is reported in the literature that the pH does not really interfere with the dehydration process of NIPAM units, at least in the pH range investigated.

PNIPAM Copolymers. The solubility of PNIPAM copolymers in aqueous solution has been investigated at different pH, and we will look first at the phase behavior of un-ionized copolymer chains, i.e., pH 12 for the PNIPAM-My series and pH 3.5 for the PNIPAM-Ay samples (Figure 4). The general comparison immediately points out differences between the two series that have to be considered separately. In the case of PNIMAM-My samples (Figure 4a), the introduction of MADAP units, which are more hydrophilic than NIPAM, increases the overall solubility of the

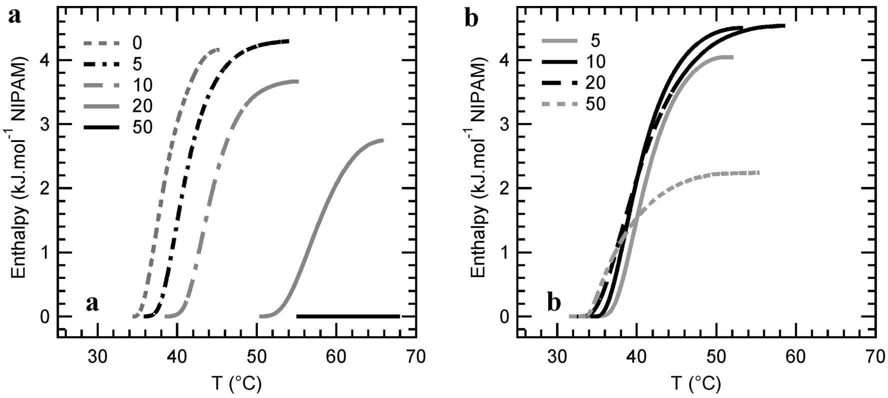

Figure 4. Phase transition of aqueous solutions of PNIPAM-My at pH 12 (a) and PNIPAM-Ay at pH 3.5 (b) for various content of comonomers *y* (C_p = 1 wt %).

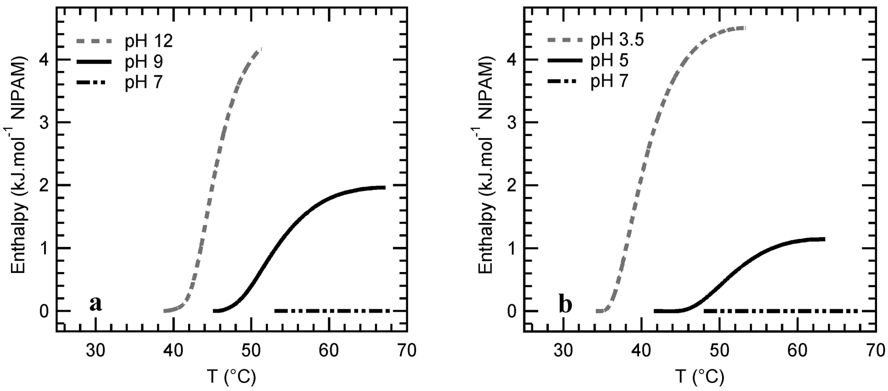

Figure 5. Phase transition of PNIPAM-M10 (a) and PNIPAM-A10 (b) aqueous solutions at different pH (C_p = 1 wt %).

copolymer chain. The phase transition is then shifted at higher temperature with increasing amount of MADAP and the enthalpy of transition, calculated taking into account only NIPAM units, progressively decreases.

In that case we can argue that the dehydration process involving NIPAM sequences in the copolymer chain becomes less effective in the presence of hydrophilic monomers; fewer NIPAM units take part in the association. When 50 mol % of MADAP is introduced into the PNIPAM chain, the resulting copolymer does not phase separate anymore in water—at least below 70 °C. Very similar results have been obtained on NIPAM/MADAP copolymers by Bokias et al.[20]

Unlike the PNIPAM-My series, the phase transition of PNIPAM-Ay samples is still observed for 50 mol % of AA units, and the transition temperature slightly decreases with the amount of added AA (see Figure 4b). By comparison with MADAP, acrylic acid can also be considered as hydrophilic, even more hydrophilic than MADAP, but the main difference arises from the possibility for acrylic acid to form hydrogen bonds with NIPAM at low pH.[38] Considering that the transition enthalpy mirror the dehydration process at the molecular level, we can assume that the formation of hydrogen bonds between NIPAM and AA units allows to keep a high dehydration level in the collapsed state. Indeed, the enthalpy remains at its maximum level (~4 kJ/mol) up to 20% of AA in chains and then decreases down to 2.5 kJ/mol for 50% of AA. We have also to be aware that in this range of low pH hydrogen bonding shows a strong dependence with temperature.[39] For instance, at pH 2.5, the sample PNIPAM-A50 starts to phase separate below 30 °C (T_d = 27 °C) with a transition enthalpy as high as 4 kJ/mol. As shown in Figure 4, the introduction of hydrophilic comonomers in the PNIPAM chain differently impact the phase separation behavior. While MADAP shift the "hydrophilic—hydrophobic" balance of PNIPAM by promoting polymer—solvent interactions, AA plays on both polymer—solvent interactions (hydrophilic monomer) and hydrogen bonding between NIPAM and AA, the latter prevailing at low pH.

By comparison with this preliminary study, the solubility behavior of PNIPAM copolymers in water is strongly modified as soon as ionization takes place. These modifications are exemplified in Figure 5a,b, with two homologous PNIPAM involving the same content of ionizable comonomers: 10 mol %.

In the case of PNIPAM-M10 (Figure 5a), the amount of ionized group is negligible at pH 12 (see Figure S2 in Supporting Information) and the phase transition is mainly controlled by PNIPAM as it was previously described. By decreasing the pH to 9, almost half of the MADAP units are charged and the ionic contribution starts to impact significantly the solubility behavior. The transition enthalpy decreases, and the beginning of the association (T_d) is shifted toward higher temperatures. Assuming that the enthalpic response is mainly due to the dehydration of NIPAM units, we can deduce that the fraction of NIPAM involved in the transition process tends to decrease proportionally. Finally, at pH 7, when all tertiary amines are protonated, the polymer chains no longer phase separate in water, and the solution

Table 1. pH Dependence of the Transition Temperature of Aqueous Solutions of PNIPAM Copolymers (C_p = 1 wt %)[a]

Samples / **pH**	**2.5**	**3.5**	**5**	**7**	**9**	**12**
PNIPAM-M50	sol below 70 °C					
PNIPAM-M20						52,3
PNIPAM-M10					47.5	40.7
PNIPAM-M5				45.5	42.7	38.0
PNIPAM		37.3	37.3	36.3		35.2
PNIPAM-A5	36.5	36.9	41.4	45.5		
PNIPAM-A10		36.5	46.1	sol below 70 °C		
PNIPAM-A20	34.5	35.2	55.0			
PNIPAM-A50	27.0	33.9				

[a] The pH domains are highlighted in gray when phase separation occurs below 70 °C, and T_d values are quoted when determined.

remains homogeneous in the whole range of temperature investigated (up to 70 °C). In that case, the ionic contribution dominates over the hydrophobic one. Very similar results were obtained with solutions of PNIPAM-A10, but conversely, the association occurs at low pH, when acrylic acid remains uncharged, and disappears at pH 7, when all carboxylic groups are charged (see Figure 5b and Figure S2 in the Supporting Information).

As shown in Table 1, the same holds for all telomers, PNIPAM-Ay and PNIPAM-My, but with different dependences according to their comonomer content. For the lowest modification extent (y = 5 mol %), we can note that the phase transition temperature increases very smoothly with increasing degree of ionization, and both copolymers keep their transition at pH 7 and beyond, when all the comonomer units are charged.

By increasing the comonomer content, the transition temperature increases and disappears for most of the copolymers when full ionization is achieved. This phenomenon is clearly evidenced in the case of the PNIPAM-Ay series. Indeed, while similar transition temperatures are observed at pH 3.5 (T_d = 34–37 °C), the differences become very large at higher pH (5 or 7) when half or all the comonomer units are ionized, respectively. Interestingly, while the sample PNIPAM-M50 is fully soluble below 70 °C at all pH, the sample PNIPAM-A50 is clearly able to phase separate at low pH due to the formation of hydrogen bonds between NIPAM and AA units. For instance, at pH 2.5, the copolymer phase separates well below the reference temperature of homoPNIPAM.

A summary of these results is given in Figure 6 where the transition temperature of copolymer solutions has been plotted versus the molar ratio of ionized units (f^- or f^+) in the macromolecular chain. In order to highlight the influence of the ionic content only, and to minimize the impact of other types of interactions (hydrogen bonds for AA or hydrophilicity for MADAP), ΔT_d instead of T_d was used in Figure 6. ΔT_d is the difference between the transition temperature of the copolymer solution at a given pH (given level of ionization) and the un-ionized state that is used as reference, i.e., pH = 12 for PNIPAM-My and pH = 3.5 for PNIPAM-Ay.

In this way, we can observe that all the data follow the same trend; the degree of ionization being clearly the key parameter for controlling the phase transition of PNIPAM copolymers. All these results are qualitatively in good agreement with the literature data,[21,40] but a quantitative comparison cannot be reasonably carried out as differences are expected due (1) to the nature of terminal end groups, (2) to the molar mass of the macromolecular species (oligomers, polymers, 3D networks), (3) to the concentration of polymer solutions or gels, and (4) to the characterization method itself (cloud point, conformational transition, calorimetry, etc.).

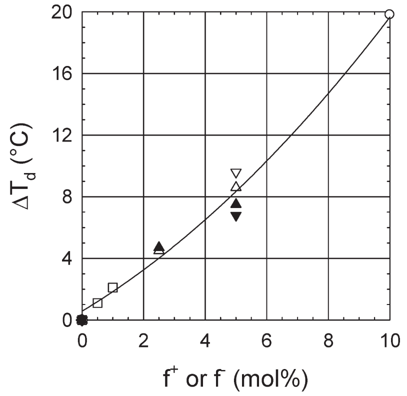

Figure 6. Variation of the transition temperature with the percentage of charged units in the copolymer backbone. ΔT_d is the difference of transition temperature between ionized and un-ionized states. PNIPAM (□) and PNIPAM-Xy: A5 (△), A10 (▽), A20 (○), A50 (◇), M5 (▲), M10 (▼), M20 (●).

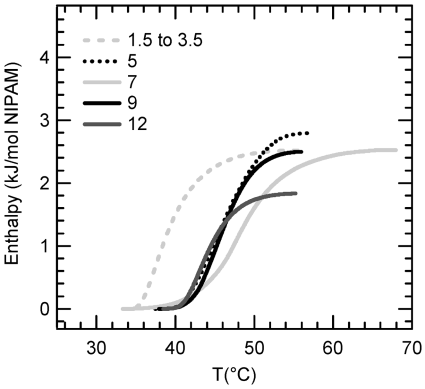

Figure 7. Influence of pH on the transition enthalpy of copolymer mixtures PNIPAM-M10/PNIPAM-A10 in water (C_p = 1 + 1 wt %).

Mixture of Copolymers. In the framework of this study, where the main objective was to finely tune the association behavior in water by playing with electrostatic and hydrophobic interactions, different possibilities arise if one aims to explore mixtures prepared with homologous copolymers (see Table 1). Indeed, while the mixture M50/A50 will be dominated by electrostatic interactions (formation of a nonsoluble interpolyelectrolyte complex at room temperature), the mixture M5/A5 will behave more or less like PNIPAM itself even if some electrostatic attractions could interfere around pH 7. The two intermediate mixtures, namely M10/A10 and M20/A20, seem clearly more adequate in this playground of interactions, but as the latter already forms a weak complex at pH 7 and room temperature we specially choose the more versatile system M10/A10. The phase transition behavior of this mixture has been studied at different pH using the same relative concentration for each copolymer, i.e., close to the stoichiometry of ionizable units: PNIPAM-M10 (C_p = 1 wt %) and PNIPAM-A10 (C_p = 1 wt %). As we can see in Figure 7, several situations (at least two) can be distinguished.

At pH 3.5 and 12, the telomers behave as they were alone in solution without interfering with each other. The neutral oligomer (PNIPAM-M10 at pH 12 or PNIPAM-A10 at pH 3.5) self-assemble

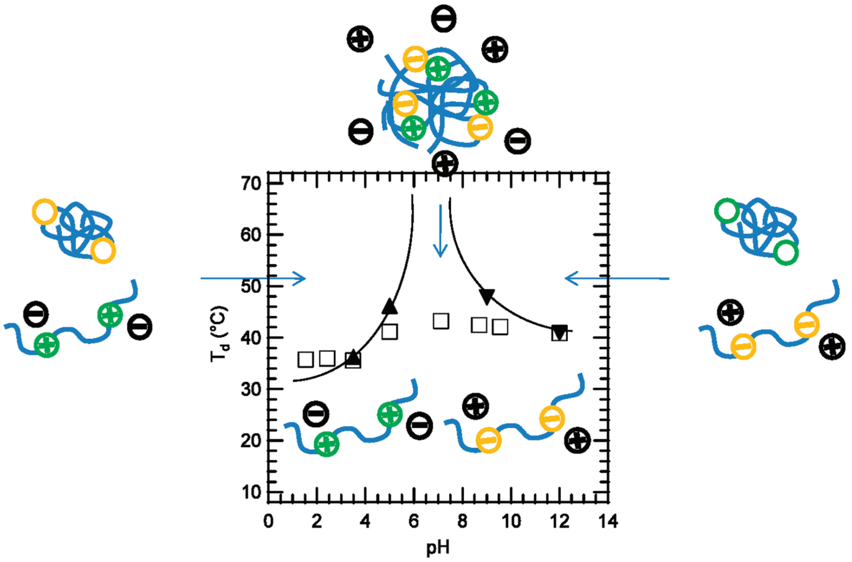

Figure 8. Schematic representation of the phase transition in mixtures of oppositely charged PNIPAM copolymers. This cartoon is superposed to the pH dependence of the transition temperature (T_d) observed for aqueous solutions of PNIPAM-M10 (▼, C_p = 1 wt %), PNIPAM-A10 (▲, C_p = 1 wt %), and a mixture PNIPAM-M10/PNIPAM-A10 (□, C_p = 1 + 1 wt %).

upon heating while the charged copolymer remains soluble and does not participate to the aggregation process. For that reason the overall transition enthalpy (ΔH_T), calculated on the basis of the total number of NIPAM units in the solution, is about half of the value determined previously in similar conditions for single copolymer that entirely participates to the phase transition. A schematic representation of this behavior is sketched in Figure 8.

By comparison with these boundary situations, where the two copolymers behave independently in the whole range of temperatures, a strong synergy is clearly evidenced at intermediate pH when the two copolymers are partially or fully charged. This is more particularly the case at pH 7 where the stoichiometric mixture clearly displays a LCST-type phase behavior although fully ionized copolymers (PNIPAM-A10 and PNIPAM-M10) are separately soluble in water at all temperatures (see Table 1). As shown in Figure 8, this feature necessarily involves the formation of a responsive interpolyelectrolyte complex between carboxylate and ammonium groups. Because of the low content of ionic groups in PNIPAM-X10 chains, some electrostatic interactions can take place at room temperature between the two copolymers, but the mixture remains fully soluble in these conditions. It is only by increasing the temperature that the coupling between electrostatic and hydrophobic interactions becomes effective giving rise to the dehydration of the PNIPAM chains with the release of mobile counterions in the outer medium. A similar behavior has been reported with partially ionized PNIPAM architectures (linear, grafted, blocks, or 3D microgels) by adding oppositely charged surfactants or proteins.[41−45] In that case, the large electrostatic barrier that initially prevents the ionic PNIPAM swollen structure from shrinking, self-assembling, and/or demixing with increasing temperature is progressively reduced due to the cooperative binding of oppositely charged species. The LCST behavior can be recovered, but it remains strongly dependent from the hydrophilic/hydrophobic balance of the complex that can be insoluble in the whole temperature range in the vicinity of the charge stoichiometry.[41] Moreover, as ionic surfactants can interact at room temperature both electrostatically with oppositely charged units of the macromolecule and hydrophobically with NIPAM units,[8] it is generally more difficult to fully and reversibly control the level of associations.

In the case of the symmetric mixture PNIPAM-A10/PNIPAM-M10, the phase transition proceeds through a selection mechanism between polymer chains that can be readily controlled by the pH and switched by the temperature. As a matter of fact, starting at room temperature with a mixture of A10 and M10, homogeneous at all pH, it is possible to trigger the association mechanism by increasing the temperature, and by choosing the pH, one can specifically address the associations: from A10/A10 telomers at low pH to A10/M10 at pH 7 up to M10/M10 at high pH. At pH 7, the dehydration of the PNIPAM complex occurs over a broader range of temperatures, compared to single uncharged copolymers, but the transition range remains however narrower if partially ionized copolymers (PNIPAM-M10 at pH 9 or PNIPAM-A10 at pH 5) are considered (see Figure 5). It is also interesting to notice that at pH 7, where the LCST-type phase transition proceeds through the formation of an electrostatic complex, the transition enthalpy is about 2 kJ/mol, i.e., half of the dehydration process determined for homoPNIPAM chains (4−4.5 kJ/mol). On that point, some comments can be drawn considering that (1) only half of the chains participate to the aggregation process forming dehydrated domains while the others remain in equilibrium in the solution, (2) the dehydration is less effective in the presence of ammonium carboxylate ion pairs and "hydrated" clusters are formed, and (3) a combination of these possibilities.

Finally, at intermediate pH (pH 5 or 9 in Figure 7) the phase separation phenomenon is more complex as it can proceeds through different mechanisms: the partially ionized copolymer (PNIPAM-M10 at pH 9 or PNIPAM-A10 at pH 5) is able to phase separate alone (see Figure 5) and/or with the other copolymer chains carrying opposite charges. As these different processes are expected to occur in the same temperature window, with T_d close to 40 °C, it is not possible here to favor one mechanism in comparison with the other.

These responsive interactions between complementary chains afford a very interesting tool at the molecular level which can be used to develop specific binding or selection between various entities, either in bulk or at interfaces. Such development is applied in the following section to the case of responsive networks.

3.2. Associating Properties of Grafted Copolymers. In order to investigate how these responsive precursors can be used to control macromolecular assemblies in aqueous solutions, we have specifically designed a grafted copolymer on the basis of a polyacrylamide backbone carrying PNIPAM-A10 side chains. As discussed in the synthesis part, this grafted copolymer mainly contains acrylamide (66 wt %) in order to keep a good water solubility in associating conditions and a sufficiently high number of side chains per macromolecule (here 13 in average) in order to provide the formation of multiple interchain associations with the formation of reversible networks. From SEC experiments, this copolymer was characterized by an intrinsic viscosity of 137 mL/g, which corresponds to a critical overlap concentration close to 1 wt % ($C^*[\eta] \cong 1$). All the studies reported in the following have been performed in the semidilute regime at $C_p = 8$ wt % and more exactly at the beginning of the entangled regime.

As shown in Figure 9, the copolymer solution clearly exhibits a thermothickening behavior at low pH; here at pH 3. At low temperature, below 27 °C, the backbone and the side chains are soluble in water and the grafted copolymer behaves like any conventional polymer in solution. The viscosity decreases smoothly between 20 and 27 °C, following an Arrhenius behavior ($\eta \sim \exp(E_a/RT)$) characterized by a low activation energy for viscous flow ($E_a = 19$ kJ/mol). This energy is close to the value of the solvent itself which is the main component at this concentration. As soon as the transition takes place (above $T_{as} = 27$ °C), PNIPAM-A10 side chains start to self-associate into microscopic domains; the solution retaining its transparency on the whole temperature range.

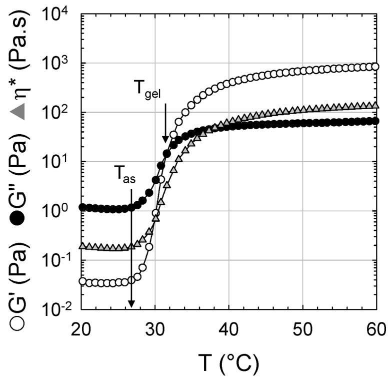

Figure 9. Temperature dependence of viscoelastic properties of a PAM-*g*-PNIPAM-A10 aqueous solution at pH 3 ($C_p = 8$ wt %, $f = 1$ Hz).

The formation of micellar junctions gives rise to a strong upturn of viscoelastic properties with temperature. The sol/gel transition (T_{gel}) can be estimated at about 32 °C from the crossover between G' and G''.[35] Under these experimental conditions ($f = 1$ Hz), the complex viscosity increases over 2–3 decades in less than 10 °C. The aggregation of PNIPAM-A10 stickers is confirmed by DSC in Figure 10a with the beginning of the endotherm detected at about 27 °C.

The transition enthalpy of the grafted copolymer solution ($\Delta H_T = 3.7$ kJ/mol) is close to the value determined for PNIPAM-A10 telomers under similar conditions of pH. This means that the dehydration process of PNIPAM grafts is almost quantitative (80–90%) and that the PAM backbone does not strongly impact the associating properties of responsive grafts. This behavior, which has been already reported with similar systems based on PAM backbone grafted with PNIPAM side chains,[33] is somewhat different from the one observed with thermothickening copolymers designed with polyelectrolyte backbones. For these latter, electrostatic repulsions between the main chains tend to delay the association temperature of responsive grafts and to decrease their transition enthalpy to about 50%. Above 45 °C, when the transition is over (Figure 10a), the physical network is formed and the viscoelastic properties (mainly G' and η^*) keeps on increasing, following a weaker thermothickening behavior. During and after the phase transition, the viscoelastic properties of the solution are strongly correlated to the dynamics of the stickers and to the conditions of analysis as the system is no longer Newtonian. We can also mention that this thermothickening behavior is totally reversible without any hysteresis. By comparison with the PNIPAM-A10 precursor, we can see that the association temperature of the grafted copolymer ($T_{as} = 27$ °C) is clearly lower than the cloud point of the telomer ($T_d = 36$ °C) determined in similar conditions of pH. This can be attributed at first to the higher relative concentration of PNIPAM-A10 in the case of the graft copolymer ($C_{PNIPAM\text{-}A10} = 2.7$ wt %). Indeed, complementary studies performed at different concentrations on graft copolymer solutions, between 2 and 10 wt %, show that the association temperature progressively decreases from 32 to 26 °C with increasing concentration. The other point to consider is that PNIPAM-A10 grafts have no ionic end groups and are more inclined to form hydrogen bonds at pH 3 than telomers. The formation of such hydrogen bonds between NIPAM and AA units

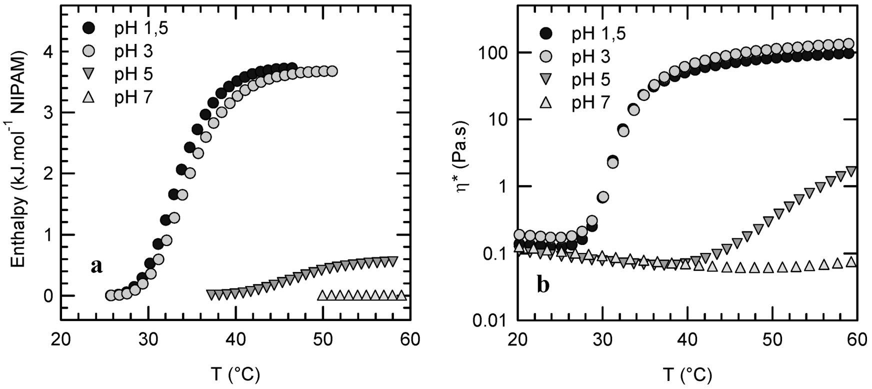

Figure 10. Variation of the transition enthalpy (a) and complex viscosity (b) with temperature and pH of PAM-*g*-PNIPAM-A10 solutions ($C_p = 8$ wt %).

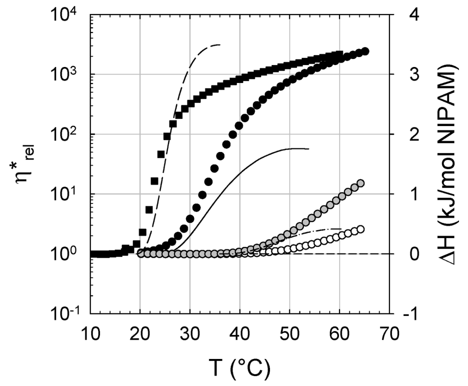

Figure 11. Variation of the relative viscosity (symbols) and transition enthalpy (lines) with temperature for aqueous solutions of PAM-*g*-PNIPAM-A10 (C_p = 8 wt %) for different pH and ionic strengths: pH = 7/water (○; dashed line) pH = 7; [NaCl] = 0.1 mol/L (gray ○; dash-dotted line) pH = 7; [NaCl] = 1 mol/L (●; solid line) pH = 3; [NaCl] = 0.5 mol/L (■; long dashed line).

is obviously the main reason for the low T_{as} value of the graft copolymer. By comparison, in the same conditions of pH, a PAM grafted with homoPNIPAM side chains will display only a very weak concentration dependence of the phase transition, with T_{as} values located around 36 °C, i.e., close to the T_d of telomers. As shown in Figure 10b, the thermothickening behavior remains unmodified at low pH, between 1.5 and 3.5, but dramatically changed as soon as the side chains become charged. At pH 5, the phase transition determined either by rheology or DSC starts at about 40 °C, and the amplitude of the thermothickening is not very high. At 60 °C for instance, the complex viscosity is about 100 times lower at pH 5 compared to pH 3. The low associating efficiency observed at pH 5 can be correlated to the very low transition enthalpy determined in the same conditions. With a value as low as 0.6 kJ/mol, one can expect that only a very low fraction of PNIPAM-A10 really participate to the formation of associating clusters. By increasing the ionization of side chains, going up to pH 7, the transition enthalpy given is no longer observable in Figure 10a, suggesting that PNIPAM-A10 side chains cannot phase separate in these conditions. The conclusion is almost the same if we take into account the temperature dependence of η^*, but here the very weak upturn observed above 50 °C is a typical signature highlighting that some intermolecular associations take place in this range.

From this first set of experiments we can readily conclude that the use of ionizable PNIPAM side chains is a very convenient way to drive responsive assemblies as a function of environmental conditions—here pH and temperature. At pH 7, when acrylic acid units are ionized, the copolymer is almost unable to self-associate, but one can imagine several ways, playing with electrostatic interactions, to tune the assembling properties.

As PNIPAM-A10 side chains cannot readily self-associate due to the electrostatic contribution of AA units, the simplest way to recover the phase transition is to screen this contribution by increasing the ionic strength. Such treatment is reported in Figure 11 where various amount of sodium chloride have been added to the graft copolymer solution. In order to make a direct comparison between the different systems, we have normalized the complex viscosity of the polymer solution ($\eta^*(T)$) by the viscosity of the same solution without interaction ($\eta^*_{dis}(T)$), this function being extrapolated from the Arrhenian behavior at low temperature ($T < T_{as}$). By removing the contribution of the water-soluble copolymer, the relative viscosity ($\eta^*_{rel}(T)$) allows to specifically focus on the associating behavior even when this one is very weak. This is the case in Figure 11 for the copolymer solution prepared at pH 7 without added salt which points out that some interactions effectively occur at high temperature ($\eta^*_{rel}(60) \cong 2$) in spite of electrostatic repulsions between the side chains. While the impact of added salt remains rather weak at 0.1 mol/L ($\eta^*_{rel}(60) \cong 10$), a true thermothickening behavior is really recovered with 1 mol/L of NaCl ($\eta^*_{rel}(60) \cong 2000$). In this case, the solution starts to thicken well below 30 °C and reaches high viscosity values at high temperatures even if the transition occurs on a broader range. By comparison, the transition of the solution prepared at pH 3 and high ionic strength ([NaCl] = 0.5 mol/L) is more abrupt, pointing out a higher cooperativity in the presence of hydrogen bonding with acrylic acid units.

The same conclusion can be made from DSC experiments that have been superimposed in Figure 11. The transition enthalpy, nonobservable in water at pH 7, increases progressively with added salt. Nevertheless, at pH 7 and [NaCl] = 1 mol/L, the transition enthalpy is still much lower than at pH 3, with or without added salt: ΔH_T = 1.8 against 3.5 kJ/mol, respectively. This difference should normally impact the structure and dynamics of the transient network (number of side chains involved in connecting clusters and/or lifetime of the associations) and influence the viscoelastic properties of the solution. If this is not really observed in Figure 11 at high temperature, where the levels of relative viscosity are very similar at pH 3 and 7 for high salt concentrations, this is because in this high segregation regime the dynamic of the network is mainly controlled by the experimental conditions (f = 1 Hz). From a thermodynamic point of view, the addition of salt has two main effects on the copolymer solution. It decreases the electrostatic contribution of AA units and increases the hydrophobic interactions between NIPAM monomers by salting out.[10,11] The consequence is that associations are favored and the transition takes place at lower temperatures. At pH 3, for example, where electrostatic interactions do not interfere, the association temperature is shifted from 27 down to 17 °C by adding NaCl at 0.5 mol/L.

The impact of salt on self-assembling properties is nonspecific, and different salts will provide qualitatively similar effects with quantitative differences mainly due to their impact on the salting out of PNIPAM chains. The other way to tune the association behavior of PAM-*g*-PNIPAM-A10 which is negatively charged at pH 7 is to play with oppositely charged species like surfactants or more specifically with PNIPAM-M10. In order to investigate this possibility, we have mixed the grafted copolymer (C_p = 8 wt %) and the PNIPAM-M10 telomer (C_p = 2.7 wt %) in order to work with the same amount of grafts and telomers, i.e., close to the stoichiometry of oppositely charged ionizable units. As we can see in Figure 12, the addition of PNIPAM-M10 into the graft copolymer solution triggers at pH 7 the formation of a responsive network above 40 °C. As expected from the thermodynamic behavior of the precursors (PNIPAM-M10 and PNIPAM-A10), the oppositely charged PNIPAM chains are able to form an interpolyelectrolyte complex under temperature control. The thermogram of the mixture given in Figure 12 is indeed very similar from all points of view (T_d and ΔH_T) to the one given in Figure 7 for the mixture of telomers. This means that the responsive precursors that have been tailored previously work in a very systematic way and can be readily used to design complex macromolecular architectures and to promote sophisticated self-assemblies

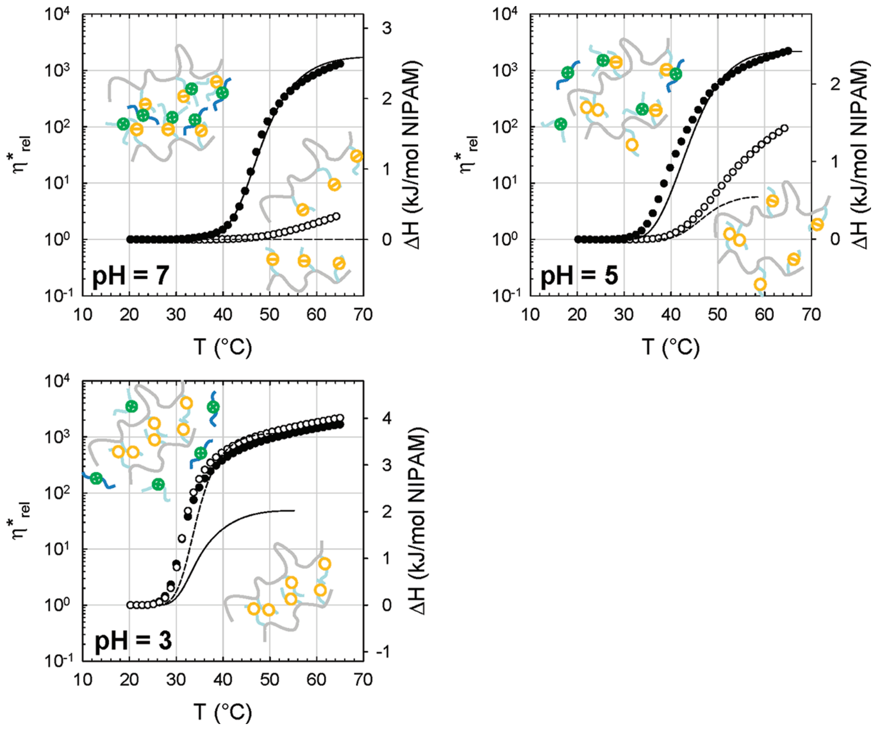

Figure 12. Variation of the relative viscosity (symbols) and transition enthalpy (lines) with temperature of PAM-g-PNIPAM-A10 solutions (C_p = 8 wt %) at different pH without (○ and dashed line) and with (● and solid line) added PNIPAM-M10 (C_p = 2.7 wt %). For each system a molecular scenario of self-assembling is sketched on the figure: down for the copolymer solution alone and top for the mixture.

through the formation of mixed microdomains as sketched in Figure 12. While the thermothickening behavior described at pH 7 with the polymer mixture relies on complementary interactions between PNIPAM-M10 and PNIPAM-A10, the association process involved at pH 3 with the same mixture is totally different as only PNIPAM-A10 grafts are able to self-associate upon heating.

As previously discussed, the charged telomer PNIPAM-M10 is totally soluble in these conditions and does not participate in the aggregation process. The resulting properties of the mixture are consequently the same than those obtained with the copolymer solution itself, and the relative viscosities are totally superimposable in that case. The same holds for the thermograms if we take into account that only PNIPAM-A10 grafts are able to phase separate. Finally at pH 5, where different types of interactions are expected (PNIPAM-A10/PNIPAM-A10 and PNIPAM-A10/PNIPAM-M10 as sketched in Figure 12), the addition of PNIPAM-M10 greatly favors the phase transition and improves the network properties with a high relative viscosity at high temperature. Even if we cannot quantitatively compare the network properties at different pH, we can notice from Figure 12 that in all the cases the transition enthalpy and the relative viscosity are very close at high temperature. In these experimental conditions, the gain of viscosity is ~3 decades for all the systems, underlining that a high level of association is reached irrespective of the association mechanism.

4. CONCLUSION

The main goal of this work was to investigate different ways to couple electrostatic interactions with the LCST behavior of PNIPAM in order to tailor multiresponsive polymers that could be used afterward to design more sophisticated macromolecular assemblies. For that purpose, two oppositely charged ionizable comonomers (AA and MADAP) have been copolymerized with NIPAM, and their solubility has been studied as a function of pH and temperature. In the case of un-ionized PNIPAM—MADAP copolymers studied at pH 12, we show that the hydrophilic character progressively increases with the MADAP content and that the LCST phase transition disappears for 50 mol % of MADAP in the copolymer chain. Conversely, all the PNIPAM samples copolymerized with acrylic acid (up to 50 mol %) phase separate above 34—37 °C at pH 3 due to the formation of hydrogen bonds between AA and NIPAM units. When the copolymer chains are progressively charged by tuning the pH, the phase transition of PNIPAM-AA and PNIPAM-MADAP is shifted at higher temperature and is no longer observable below 60 °C when the ionization of the chain exceeds 10 mol %. By comparison with these systems, where the association properties are mainly controlled by hydrophobic attractions taking place at high temperature between NIPAM units and electrostatic repulsions between AA (or MADAP) monomers, we also investigate the possibility to promote electrostatic attractions by mixing oppositely charged copolymers. Here, the main challenge was to find the right pair of copolymers in order to get the best equilibrium between the LCST phase transition of homoPNIPAM on one hand and the formation of a strong and irreversible interpolyelectrolyte complex on the other hand. This was achieved with the copolymer pair containing 10 mol % of ionizable groups which was the most adaptable one from the point of view of responsivity. Indeed, at pH 7 when AA and MADAP units are totally ionized, the two copolymers which are unable to self-associate separately in these conditions clearly display a LCST phase transition when they are mixed together. The originality of this associating behavior, which has not been reported yet to the best of our knowledge, is that the phase transition proceeds through specific interactions between complementary chains (formation of a reversible interpolyelectrolyte complex) that can be switched either with the pH or the temperature. In the present case, starting at room temperature with the copolymer mixture PNIPAM-A10/PNIPAM-M10 which remains soluble at all pH, we show that the association process can be released by increasing the temperature above 30—40 °C and that the pH can be tuned to specifically address the associations: from A10/A10 at pH 3 to A10/M10 at pH 7 up to M10/M10 at pH 12.

These highly specific interactions have been used in the last part of this work to design grafted copolymers and to demonstrate that the same set of interactions could promote responsive associations in more sophisticated macromolecular assemblies. By grafting PNIPAM-A10 onto a PAM backbone, we show that physical gels could be readily formed reversibly under cross controls of temperature, pH, and ionic strength. Moreover, the addition of complementary telomers (PNIPAM-M10) into the grafted copolymer solution also demonstrates that the same selectivity can take place under temperature control. In that case the phase separation induced by the complexation between oppositely charged PNIPAM moieties at high temperature remains localized at a nanoscopic scale with the formation of a transparent transient network connected through mixed microdomains.

While the viscoelastic properties are unequivocal to highlight qualitatively the interplay between hydrophobic and electrostatic interactions in the responsive formation of physical gels, quantitative informations concerning the structure of transient networks are missing. For instance, structural analyses by small-angle neutron scattering would provide some complementary information concerning the characteristics of microdomains (size and aggregation number) formed at different pH, with or without added PNIPAM-M10. Moreover, while this set of interactions has been mainly applied in solution in the framework of macro- and microphase separations, it will be interesting to transfer this responsive mechanism to other situations, like interfaces, where specific adsorption, binding or adhesion are an interesting challenge. Such works are currently undergoing in our laboratory and will be reported elsewhere.

■ ASSOCIATED CONTENT

Ⓢ **Supporting Information.** Details concerning the synthesis and characterization of copolymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Responsive Gels: Volume Transitions. *Adv. Polym. Sci.* **1993**, *109* and *110*.

(2) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163–249.

(3) Osada, Y.; Ross-Murphy, S. B. *Sci. Am.* **1993**, *268*, 82–87.

(4) Wang, X.; Wu, C. *Macromolecules* **1999**, *32*, 4299–4301.

(5) Pelton, R. *Adv. Colloid Interface Sci.* **2000**, *85*, 1–33.

(6) Zhang, X. Z.; Wu, D. Q.; Chu, C. C. *Biomaterials* **2004**, *25*, 3793–3805.

(7) Andriola Silva Brun-Graeppi, A. K.; Richard, C.; Bessodes, M.; Schermanc, D.; Merten, O.-W. *Prog. Polym. Sci.* **2010**, *35*, 1311–1324.

(8) Schild, H. G.; Tirrell, D. A. *Langmuir* **1991**, *7*, 665–671.

(9) Costa, R. O. R.; Freitas, R. F. S. *Polymer* **2002**, *43*, 5879–5885.

(10) Zhang, Y. Q.; Tanaka, T.; Shibayama, M. *Nature* **1992**, *360*, 142–144.

(11) Freitag, R.; Garret-Flaudy, F. *Langmuir* **2002**, *18*, 3434–3440.

(12) Durand, A.; Hourdet, D. *Polymer* **2000**, *41*, 545–557.

(13) Zhang, J.; Pelton, R. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 2137–2143.

(14) Durand, A.; Hourdet, D. *Macromol. Chem. Phys.* **2000**, *201*, 858–868.

(15) Maeda, T.; Yamamoto, K.; Aoyagi, T. *J. Colloid Interface Sci.* **2006**, *302*, 467–474.

(16) Brazel, C. S.; Peppas, N. A. *Macromolecules* **1995**, *28*, 8016–8020.

(17) Shibayama, M.; Mizutani, S.; Nomura, S. *Macromolecules* **1996**, *29*, 2019–2024.

(18) Shibayama, M.; Ikkai, F.; Inamoto, S.; Nomura, S.; Han, C. C. *J. Chem. Phys.* **1996**, *105*, 4358–4366.

(19) Kawasaki, H.; Sasaki, S.; Maeda, H. *J. Phys. Chem. B* **1997**, *101*, 5089–5093.

(20) Bokias, G.; Vasilevskaya, V. V.; Iliopoulos, I.; Hourdet, D.; Khokhlov, A. R. *Macromolecules* **2000**, *33*, 9757–9763.

(21) Bokias, G.; Hourdet, D. *Polymer* **2001**, *42*, 6329–6337.

(22) Ikkai, F.; Susuki, T.; Karino, T.; Shibayama, M. *Macromolecules* **2007**, *40*, 1140–1146.

(23) Krzyminski, K. J.; Jasionowski, M.; Gutowska, A. *Polym. Int.* **2008**, *57*, 592–604.

(24) Cohen Stuart, M. A.; Huck, W. T. S.; Genzer, J.; Müller, M.; Ober, C.; Stamm, M.; Sukhorukov, G. B.; Szleifer, I.; Tsukruk, V. V.; Urban, M.; Winnik, F.; Zauscher, S.; Luzinov, I.; Minko, S. *Nature Mater.* **2010**, *9*, 101–113.

(25) Oya, T.; Enoki, T.; Grosberg, A. Y.; Masamune, S.; Sakiyama, T.; Takeoka, Y.; Tanaka, K.; Wang, G.; Yilmaz, Y.; Feld, M. S.; Dasari, R.; Tanaka, T. *Science* **1999**, *286*, 1543–1545.

(26) Kono, K.; Okabe, H.; Morimoto, K.; Takagishi, T. *J. Appl. Polym. Sci.* **2000**, *77*, 2703–2710.

(27) Dautzenberg, H.; Gao, Y. B.; Hahn, M. *Langmuir* **2000**, *16*, 9070–9081.

(28) Moselhy, J.; Vira, T.; Liu, F. F.; Wu, X. Y. *Int. J. Nanomed.* **2009**, *4*, 153–164.

(29) Durand, A.; Hourdet, D. *Polymer* **1999**, *40*, 4941–4951.

(30) Vasilieva, Y. A.; Thomas, D. B.; Scales, C. W.; McCormick, C. L. *Macromolecules* **2004**, *37*, 2728–2737.

(31) Schmidt, S.; Hellweg, T.; von Klitzing, R. *Langmuir* **2008**, *24*, 12595–12602.

(32) Sudor, J.; Barbier, V.; Thirot, S.; Godfrin, D.; Hourdet, D.; Millequant, M.; Blanchard, J.; Viovy, J.-L. *Electrophoresis* **2001**, *22*, 720–728.

(33) Petit, L.; Karakasyan, C.; Pantoustier, N.; Hourdet, D. *Polymer* **2007**, *48*, 7098–7112.

(34) Schild, H. G.; Tirell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352–4356.

(35) Petit, L.; Bouteiller, L.; Brûlet, A.; Lafuma, F.; Hourdet, D. *Langmuir* **2007**, *23*, 147–158.

(36) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283–289.

(37) Karakasyan, C.; Lack, S.; Brunel, F.; Maingault, P.; Hourdet, D. *Biomacromolecules* **2008**, *9*, 2419–2429.

(38) Shin, B. C.; Jhon, M. S.; Lee, H. B.; Yuk, S. H. *Eur. Polym. J.* **1998**, *34*, 1675–1681.

(39) Jones, M. S. *Eur. Polym. J.* **1999**, *35*, 795–801.

(40) Yoo, M. K.; Sung, Y. K.; Lee, Y. M.; Cho, C. S. *Polymer* **1998**, *39*, 3703–3708.

(41) Bokias, G. *Colloid Polym. Sci.* **2000**, *278*, 1109–1113.

(42) Tsolakis, P.; Bokias, G. *Macromolecules* **2006**, *39*, 393–398.

(43) Annaka, M.; Morishita, K.; Okabe, S. *J. Phys. Chem. B* **2007**, *111*, 11700–11707.

(44) Johansson, C.; Gernandt, J.; Bradley, M.; Vincent, B.; Hansson, P. *J. Colloid Interface Sci.* **2010**, *347*, 241–251.

(45) dos Santos, S.; Gustavsson, C.; Gudmundsson, C.; Linse, P.; Piculell, L. *Langmuir* **2011**, *27*, 592–603.