Temperature and pH Effects on the Stability and Rheological Behavior of the Aqueous Suspensions of Smart Polymers Based on *N*-Isopropylacrylamide, Chitosan, and Acrylic Acid

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ABSTRACT: This study describes the stability and rheological behavior of suspensions of poly(*N*-isopropylacrylamide) (PNIPAM), poly(*N*-isopropylacrylamide)-chitosan (PNIPAM-CS), and poly(*N*-isopropylacrylamide)-chitosan-poly(acrylic acid) (PNIPAM-CS-PAA) crosslinked particles sensitive to pH and temperature. These dual-sensitive materials were simply obtained by one-pot method, via free-radical precipitation copolymerization with potassium persulfate, using *N,N*′-methylenebisacrylamide as a crosslinking agent. Incorporation of the precursor materials into the chemical networks was confirmed by elementary analysis and infrared spectroscopy. The influence of external stimuli such as pH and temperature, or both, on particle behavior was investigated through rheological measurements, visual stability tests, and analytical centrifugation. The PNIPAM-CS particles showed higher stability in acid and neutral media, whereas PNIPAM-CS-PAA particles were more stable in neutral and alkaline media, both below and above the lower critical solution temperature of PNIPAM (stability data). This is due to different interparticle interactions as well as those between the particles and the medium (also evidenced by rheological data), which were also influenced by the pH and temperature of the medium. Based on the results obtained, we found that the introduction of pH-sensitive polymers to crosslinked PNIPAM particles not only produced dual-sensitive materials but also allowed particle stability to be adjusted, making phase separation faster or slower, depending on the desired application. Thus, it is possible to adapt the material to different media. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 334–345, 2013

INTRODUCTION

The development of stimuli-responsive polymer particles has gained considerable notoriety in recent years,[1–4] due to their capacity to abruptly alter their structure/properties in response to slight changes in the surrounding environment, such as variations in pH,[1–6] temperature,[7–11] ionic strength,[12–15] and magnetic and/or electrical fields.[16–18] Temperature- and pH-sensitive polymer particles, in particular, have been widely investigated in light of their considerable importance in typical physiological and chemical systems.[19–21] The most extensively studied smart particles are those based on the thermosensitive poly(*N*-isopropylacrylamide) (PNIPAM).[22] Several studies exploit the hydrophobicity of PNIPAM above its lower critical solution temperature (LCST) of approximately 32°C in water, to encapsulate poorly soluble drugs.[23] When chemically crosslinked, these particles are not strictly soluble, even below LCST, but rather form colloidal dispersions.[24]

Chitosan (CS) and poly(acrylic acid) (PAA) are commonly pH-responsive polymers applied to modify PNIPAM particles[8,25–32] and produce dual-sensitive materials. PAA becomes negatively charged when the pH of the solvent is above its pK_a (approximately 4) due to the ionization of its COOH groups. CS contains —NH$_2$ groups and becomes positively charged in acid medium (pK_a around 6).[33] In addition, CS is a biofriendly material, which is important in a number of applications from biomedical to technological.[34] Suspensions of particles composed of these dual-sensitive polymers have proved useful in several areas, including the removal of contaminants from water, surface functionalization, and controlled release processes.[32,33,35–43]

Recently, novel dual-stimuli responsive copolymers, which are responsive to pH and temperature and referred to as schizophrenic copolymers, were prepared through copolymerization of N-isopropylacrylamide (NIPAM) and acrylic acid (AA) on CS chains, using an initiating system of tert-butyl hydroperoxide/amino group (from CS). In dilute aqueous media, two types of reversible nanoparticles were obtained by changing pH: positively charged micelles with CS-shell in acidic solutions above 33°C and negatively charged micelles with PNIPAM-co-PAA shell in alkaline solutions.[44] Particles with a PNIPAM-co-PAA core and a CS shell structure have also recently been produced.[35,36,45] However, the synthesis process consisted of two stages: first, PNIPAM-co-PAA was produced, crosslinked with N,N′-methylenebisacrylamide (MBA)[45] or without adding the crosslinking agent;[35,36] next, the CS shell was formed by a catalyzed conjugation reaction.

Particle preparation in only one-pot, where all reagents are placed in a single reaction system, simplifies the synthesis process. This can be accomplished via simple radical initiation, in which reactive groups of CS actively participate in copolymerization by hydrogen abstraction of amine or hydroxyl groups, for example.[46–48] Furthermore, PNIPAM-CS particles exhibiting homogeneous morphology (obtained using persulfate as an initiator) have a higher swelling capacity and greater volume variation than those with the same composition displaying core-shell morphology.[49,50] Likewise, homogeneous distribution of CS in the particles would be ideal for biomedical applications, where degradation of the material is essential.

Many works have used the study of parameters, such as size, morphology, degree of swelling, loading and delivery ability of substances to characterize their smart particles.[49–58] However, the literature still requires more detailed information about how modifications to crosslinked PNIPAM particles alter the speed and intensity with which these materials respond to environmental changes. As such, the knowledge of the physical–chemical stability of these polymer suspension systems is a key parameter to their application. For example, during storage time, aggregation of particles in the aqueous media and subsequent formation of precipitates may occur, which can limit the industrial applicability of these formulations.[26,59] Suspension stability is governed by a balance of interactions that occur among particles themselves and between the particles and the medium.[22,60] Long-term stability studies are carried out to provide this information but can take a significant amount of time, and accelerated testing can be undertaken while screening components. For suspensions and emulsions, these accelerated stability tests can be accomplished through storage at adverse temperatures, centrifugation, or rheological assessment.[61]

Analytical centrifugation is a recently introduced method for investigating the sedimentation stability of several types of materials under accelerated conditions.[62,63] These include bentonite/xanthan gum mixtures,[63] Interpenetrating Polymer Network (IPN) nanogels composed of PAA and gelatin,[64] carbon nanotube materials,[65] hexosomes,[66] oil/water emulsions and particulate suspensions,[67] preformed polyelectrolyte complex particles and polyelectrolyte complexes in silica dispersions.[68]

Table I. Particle Abbreviations and Feed Reactant Concentrations

Particle code	Reactant concentration (mmol L^{-1})				
	NIPAM	CS[a]	AA	MBA	KPS
PNIPAM	200	–	–	5	10
PNIPAM-CS	200	50	–	5	10
PNIPAM-CS-PAA	200	50	100	5	10

[a]mmol of sugar unit.

However, very little has been reported in the literature regarding the stability behavior of smart particles.[69–71] To date, only one study has described the stability behavior above LCST of particles containing PNIPAM, PAA, and CS.[33] However, in this case, as CS chains were connected to the PNIPAM-co-PAA networks only through ionic interactions (polyelectrolyte complexes), PNIPAM-co-PAA particles remained in suspension at high pH, and the CS chains moved out of the network, because this polysaccharide has no longer —NH$_3^+$ groups to interact with the COO$^-$ groups from PAA.

Due to the lack of more detailed information regarding stability of these kind of chemically crosslinked particles in the literature, this study aimed to investigate how modifying crosslinked PNIPAM particles by copolymerization of NIPAM with pH-sensitive polymers of opposite charges affects the stability and thermosensitivity of PNIPAM particles in suspension, as well as the reversibility of the transition using heating and freezing cycles. We sought to examine PNIPAM, PNIPAM-CS, and PNIPAM-CS-PAA crosslinked particles, obtained by one-pot free-radical polymerization, observing how their properties vary as a function of pH and temperature. To that end, the particles were analyzed using accelerated stability and rheological measurements.

EXPERIMENTAL

Materials

Low-molecular weight CS was purchased from Polymar S.A. (Brazil). Its viscosity-average molecular weight was 3.7 × 10^4 g/mol, estimated at 25°C in 0.3M acetic acid/0.2M sodium acetate. Degree of deacetylation (82%) was determined by ^{1}H NMR, according to procedures described in the literature.[72] NIPAM and MBA were purchased from Sigma-Aldrich (Brazil), potassium persulfate (KPS) was obtained from Vetec (Brazil), and AA was kindly supplied by Oswaldo Cruz Química (Brazil). NaOH, KCl, NaCl, KH$_2$PO$_4$, and Na$_2$HPO$_4$ were purchased from Synth (Brazil). Acetic acid (HAc) was obtained from Cromoline (Brazil). NIPAM was purified twice by recrystallization in hexane,[73] and the other reagents were used as received.

Precipitation Polymerization

Particles containing different amounts of CS, NIPAM, AA, and MBA were simply prepared in one pot by free-radical precipitation polymerization,[49] under the conditions listed in Table I. The system consisted of a 250-mL three-necked flask coupled to a reflux condenser, thermometer, and N$_{2(g)}$ inlet and outlet, in oil bath. Polymerization was carried out at 50°C, using acetic acid (0.5 M) as a solvent and KPS as an initiator, under

constant magnetic stirring. The acetic acid promotes the ionization of the amino groups from CS to form the —NH$_3^+$ groups, so the CS can be easily dissolved in the media. Therefore, in this reaction system, CS, NIPAM, AA, MBA, and KPS are soluble in the solvent. As a result, the reaction mixture was initially homogeneous but became heterogeneous when the initiator was added. This was evident from the color change (viscous milky aspect) and led to the production of precipitate. After 3 h of polymerization, the system was opened to allow oxygen to enter, and the mixture was filtered to separate unreacted CS. To remove the remaining monomers and initiator, the product was washed with hot distilled water several times and then dried in a vacuum oven at 40°C.

Infrared Spectroscopy

Infrared spectra were obtained in KBr pellets and scanned from 400 to 4000 cm^{-1} using a ThermoNicolet model Nexus 470 FTIR Spectrometer.

Elementary Analysis

Elementary analysis (EA) was performed using a Perkin Elmer CHNS/O 2400 Series II elemental analyzer, with a combustion column temperature of 925°C and separation column temperature of 640°C. The sample (about 2.7 mg) was wrapped in tin foil and examined in synthetic air using oxygen as a combustion gas and helium as a carrier gas.

Aqueous Suspensions Characterization

Polymer suspensions were prepared at a concentration of 2% (w/v), in different aqueous media: (i) 0.5 M acetic acid (pH 3), (ii) phosphate buffered saline (PBS) (pH 7), and (iii) PBS containing 0.25% NaOH (pH 10). Suspensions were submitted to constant stirring for 24 h at room temperature and subsequently used in all suspension analyses.

Visual stability. The previously prepared suspensions were placed in test tubes to rest, and the time dependence of sedimentation for suspensions was visually monitored and photographed with an Olympus C170 digital camera.

Optical centrifugation. To evaluate the stability of stimuli-responsive materials, sedimentation measurements of the polymer suspensions were performed with a stability analyzer LUMIFuge 116, at an acceleration velocity of 2400 rpm and temperatures of 25°C and 35°C. This equipment is an analytical centrifuge with an optic-electronic sensor system, which continuously measures near infrared (NIR) transmission profiles along horizontally inserted sample tubes during centrifugation. Thus, separation kinetics can be studied under accelerated conditions.[63] Data obtained were integrated by the computer program, and the resulting graph shows the percentage of light transmittance, over the entire sample length, as a function of time.[74] Local variations in particle concentration are identifiable due to changes in light transmission. Regions of well-mixed dispersions will scatter and absorb the light, making transmission low. By contrast, any local clarification will raise the transmission.[66]

Rheological measurements. Rheological properties of polymer suspensions were measured in a RheoStrees RS 150 rheometer from Haake, equipped with concentric cylinder geometry (DG41). The samples were submitted to three consecutive runs, the first of which consisted of a 1–100 s^{-1} forward sweep and a return sweep of 100–1 s^{-1}, at 25°C. Next, the sensor containing the sample was heated to 35°C, and shear stress sweeps were repeated. The sensor was then cooled to 25°C, and a final back and forth sweep was performed. Temperature was controlled by a thermostatic bath coupled to the equipment.

Zeta potential measurements. Aiming to investigate the occurrence of negative charges onto PNIPAM-CS surface in alkaline media, the zeta potential measurement was obtained using Zetasizer Nanoseries equipment (Malvern Instruments), at 25°C.

RESULTS AND DISCUSSION

Infrared spectroscopy was used to detect functional groups of CS, PNIPAM, and PAA in the particles. CS, PNIPAM-CS, and PNIPAM-CS-PAA spectra (Figure 1) displayed a broad band around 3400 cm^{-1}, attributed to overlapped N—H and O—H group stretching vibrations; peaks at approximately 1645 and 1535 cm^{-1} corresponding to absorptions of C=O stretching (primary amide) and N—H bending, respectively; CH$_3$ antisymmetric bending, CH$_2$ scissoring, and C—N stretching of amide groups at about 1455 cm^{-1}, and C—N stretching of amine groups between 1308 and 1380 cm^{-1}. In addition, bands of polysaccharide structures are observed in the region between 890 and 1156 cm^{-1}.[75] Both PNIPAM-CS and PNIPAM-CS-PAA smart particles exhibited two peaks representing the symmetric bend (or umbrella bend) of isopropyl groups of NIPAM, at 1365 and 1385 cm^{-1} (gem-dimethyl),[76,77] suggesting the incorporation of PNIPAM chains onto particles. Moreover, the introduction of PAA in PNIPAM-CS-PAA was identified by the additional presence of a peak at approximately 1720 cm^{-1} [Figure 1(c)], corresponding to C=O axial stretching of carboxylic acids.[78]

EA data indicated that precursors were incorporated into the particles obtained during experimental part (Table II). Theoretical compositions, acquired from the amount of reactants in the feed, are close to experimental ones for all syntheses.

Investigating the dispersed state of suspensions is fundamental for application in diverse fields such as nanomaterials, coatings, ceramics, cosmetics, water treatment, and controlled release processes, among others.[70,71,79–83] To evaluate the effect of pH on particle stability, suspensions were prepared at pH 3, 7, and 10. These were allowed to rest and photographed at four time intervals. The images in Figure 2 show the visual aspects of suspensions obtained from PNIPAM, PNIPAM-CS, and PNIPAM-CS-PAA particles as a function of the type of solvent used, at room temperature. In all cases, suspensions were formed owing to the reticulation of polymer chains with MBA during the synthesis process. Immediately after the preparation, PNIPAM particle suspensions were less opaque, whereas PNIPAM-CS and PNIPAM-CS-PAA particle suspensions exhibited more opacity in acid, neutral, and alkaline media. Although PNIPAM particle suspensions are less opaque than those obtained with the other particles, suggesting greater interaction with the medium, at 30 min sedimentation occurred at pH 10. After 1 day, PNIPAM
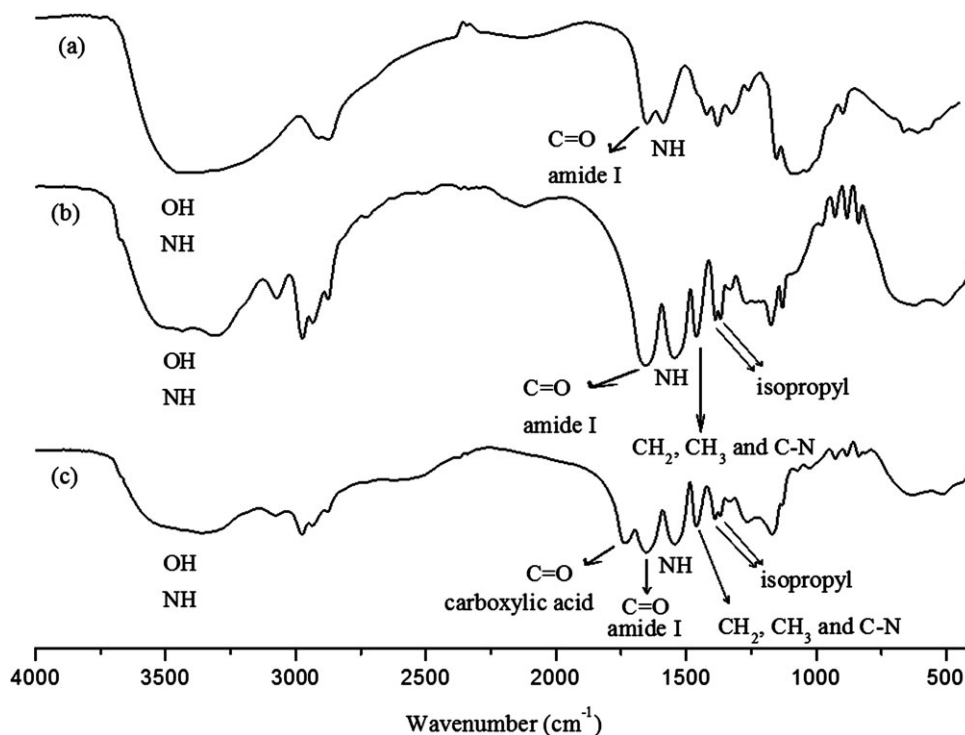
Figure 1. FTIR spectra of (a) CS; (b) PNIPAM-CS, and (c) PNIPAM-CS-PAA.

showed sedimentation in the three media, and following 96 h of rest, particles further sediment in all media.

Interestingly, although CS chain solubility is restricted only to acid media, PNIPAM-CS particles maintained stability in all pH range (3–10) for 24 h of rest. This result indicates that CS chemically linked into PNIPAM networks created modified particles with improved stability in acid, neutral, and alkaline aqueous solution, during this time, when compared to precursor materials. PNIPAM-CS instability increases along with pH. After 96 h of rest, these particles exhibit a concentration gradient at pH 7 and clear phase separation in alkaline medium.

Conversely, suspensions prepared from PNIPAM-CS-PAA particles showed contrasting behavior, with visible stability improvement as pH increases. Particles visibly sediment only after 30 min of rest, in acid medium, likely because the AA pK_a value (around 4.25) is above the pH of the medium. As such, AA groups are mostly unionized at this pH, which facilitates contraction of crosslinked particles and consequently, their sedimentation. Additionally, as previously mentioned in the literature,[84–86] PAA-g-PNIPAM graft copolymers are insoluble at room temperature for pH values lower than 4, as hydrogen bond interactions occur among PNIPAM and PAA chains at low pH.

However, at pH 7 and 10 the suspension showed stability, with the alkaline medium slightly less opaque when compared to the suspension in neutral medium. These results indicate that PAA was incorporated into polymeric networks during the reaction. After 96 h of rest, stability of PNIPAM-CS-PAA was still better than PNIPAM-CS in neutral and alkaline aqueous media, suggesting that AA enhanced particle stability behavior at higher

pH values. Electrostatic repulsion among negative charges of carboxylate groups promoted greater swelling behavior, preventing sedimentation.

Accelerated stability tests confirmed the observations that PNIPAM and PNIPAM-CS particles are more stable in acid and neutral media and less so in basic medium, and that PNIPAM-CS-PAA particles exhibit greater stability in neutral and alkaline media and lower stability in acid medium, as per Figure 3. Particles' stability at temperatures below and above the LCST of PNIPAM was analyzed in a centrifuge equipped with an optical transmittance meter in the NIR region, showing far lower absorbance than in the IR region. This lower absorbance allows NIR incident radiation to penetrate even opaque samples, such as suspensions.[87] During testing, cells containing the samples were placed horizontally and positioned radially in relation to the rotor axis. Transmittance along the length of the cells was

Table II. Molar Percentages of C, H, and N Atoms, Calculated Based on the Amount of Reactants in the Feed and Elementary Analysis Results

Particle code	C (%)		H (%)		N (%)	
	Calcul.[a]	Exper.[b]	Calcul.	Exper.	Calcul.	Exper.
PNIPAM	63.4	56.6	9.6	9.9	12.5	11.3
PNIPAM-CS	59.7	55.4	9.1	10.0	11.8	11.5
PNIPAM-CS-PAA	57.0	55.3	8.1	9.2	8.5	9.5

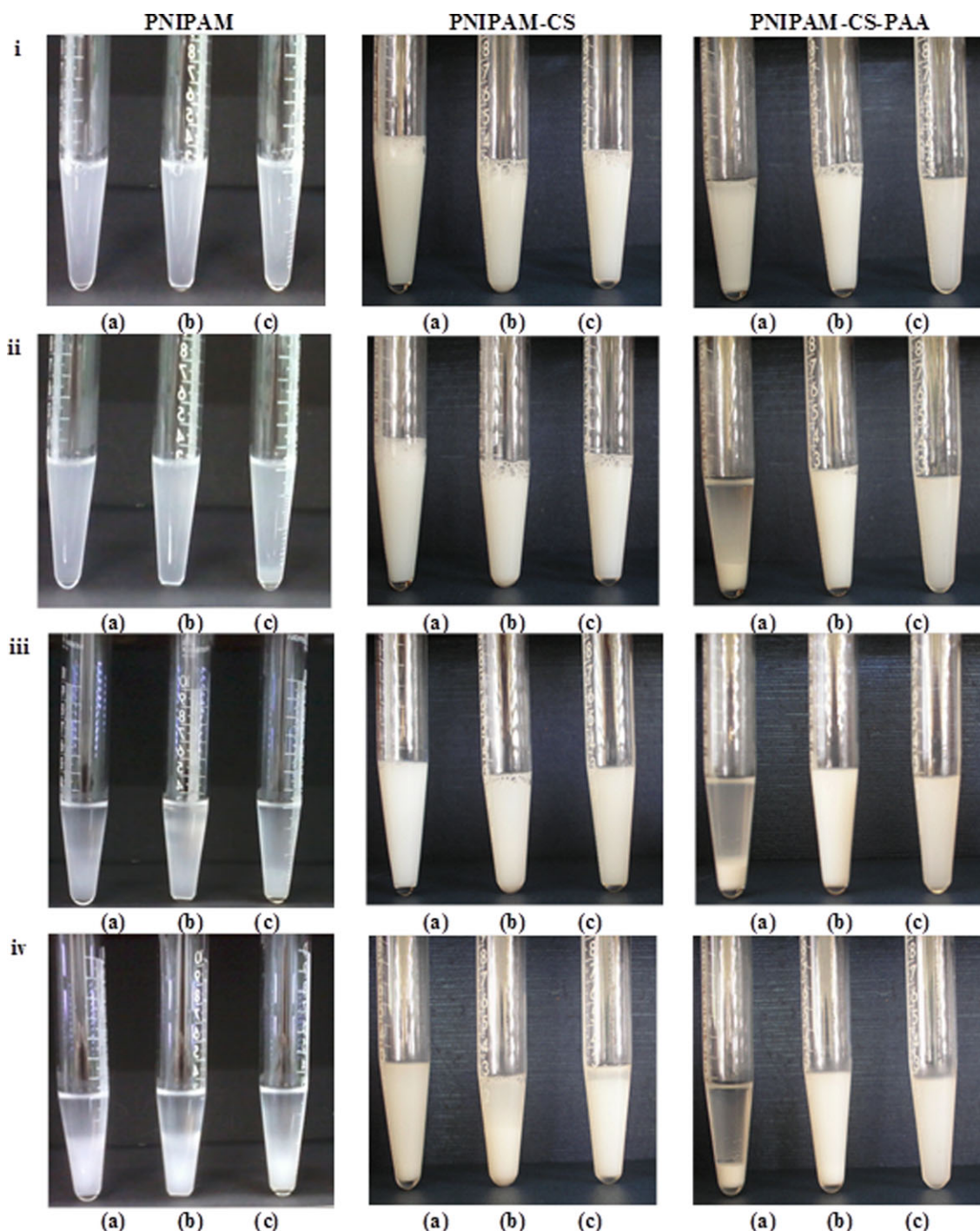[a]Calcul.: calculated values.
[b]Exper.: experimental values.

Figure 2. Images of suspensions obtained from PNIPAM, PNIPAM-CS, and PNIPAM-CS-PAA particles in: (a) pH 3; (b) pH 7, and (c) pH 10, (i) immediately after preparation (stirring for 24 h) and following (ii) 30 min, (iii) 24 h, and (iv) 96 h of rest. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

measured as a function of time. Upon centrifugation, the suspended material moves to the bottom of the cell, indicating which suspensions sediment more rapidly, showing greater instability. Test results were obtained using a LUMiFuge 116 separation analyzer, in acid, neutral, and basic media, below and above the LCST of PNIPAM. The sedimentation process was followed by a change in optical transmittance with centrifu-

gation time, at a constant rotation speed. This allows a greater understanding of the behavior of these particles in different media.

Stability behavior of PNIPAM particle suspensions was evaluated below and above their LCST, at various pHs [Figure 3(a,b)]. Curve inclination in the first 200 s, shown in Figure 3(a), indicates that PNIPAM particle stability decreases when
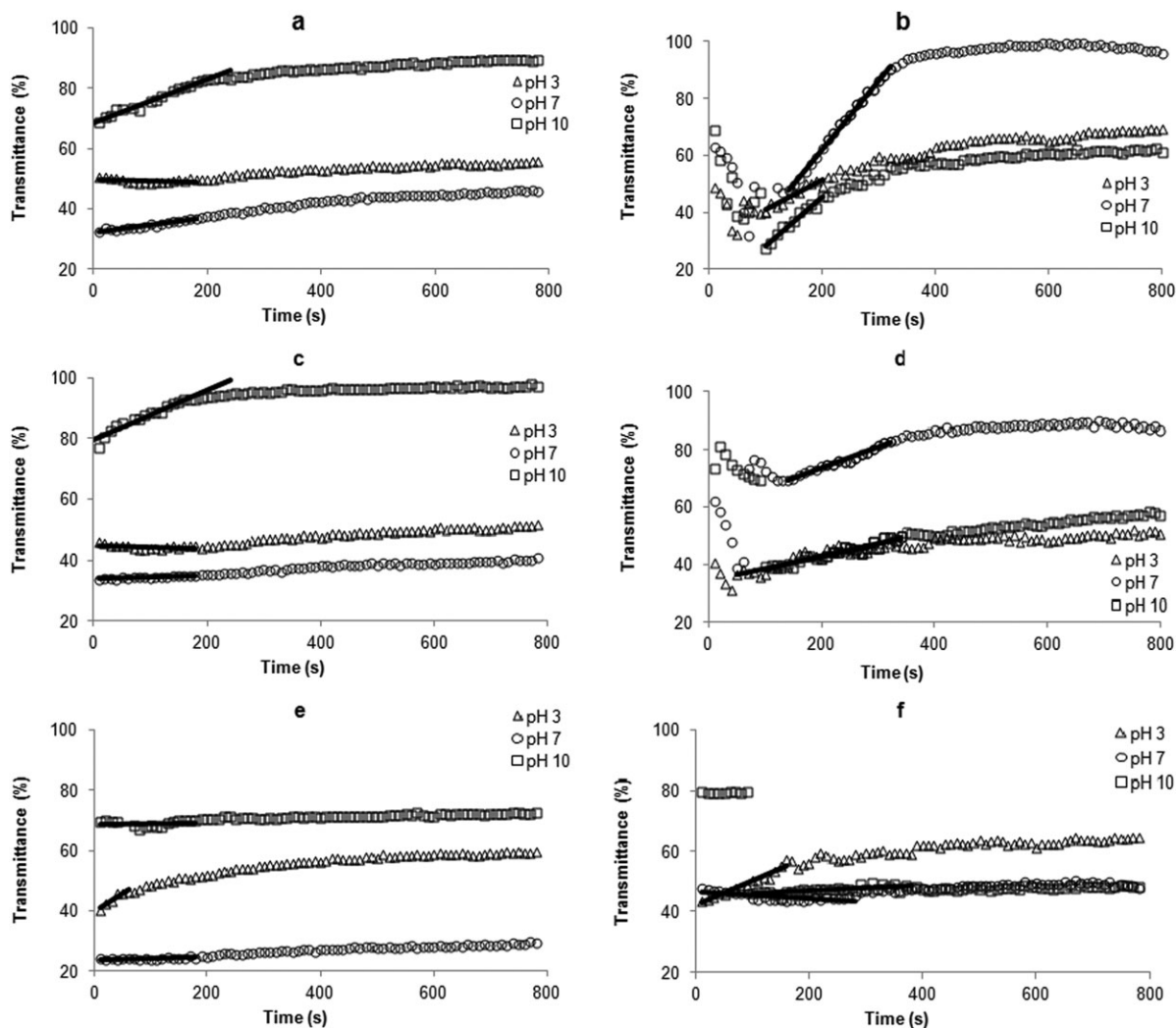
Figure 3. Dependence of transmittance on centrifugation time for: PNIPAM (a and b), PNIPAM-CS (c and d), and PNIPAM-CS-PAA (e and f). Left columns at 25°C (a, c, and e) and right columns at 35°C (b, d, and f).

pH increases, at 25°C. The introduction of CS onto the networks in PNIPAM-CS particles slightly increases the sedimentation rate in alkaline medium and increases stability in acid and neutral media below LCST [Figure 3(c)], when compared to PNIPAM particles.

Figure 3(b,d) show the results of particle activation by increasing the environmental temperature. As the equipment took around 40 s to reach the desired temperature (35°C), initial measurements reflect the phase transition of PNIPAM, with reduced transmittance due to greater turbidity of the medium. This is followed by particle precipitation, which is significantly faster for PNIPAM particles than at ambient temperature, as demonstrated by the greater inclination of the straight lines in Figure 3(b). Although PNIPAM-CS particles sediment more rapidly with heating at pH 3 and pH 7, the speed with which this occurs is slower than that of pure PNIPAM particles. At pH 10, sedimentation of PNIPAM-CS particles above the LCST is slower than at ambient temperature. Furthermore, PNIPAM-CS particles are more stable than PNIPAM particles at this pH, above the LCST. These results demonstrate that the presence of

CS chains in PNIPAM-CS particles increases their stability, as previously shown in Figure 2. The presence of rigid polysaccharide chains probably hinders full particle contraction above the LCST in all pH ranges evaluated, lowering their sedimentation. As expected, introducing PAA into PNIPAM-CS-PAA particles increases their stability in alkaline and neutral media and decreases it in acid medium, both above and below the LCST of PNIPAM. The decline in stability with the introduction of PAA to particles may be related to greater contraction in acid pH. This leads to the formation of denser particles, due to hydrogen bonds between hydrophilic groups of PNIPAM and PAA, as visually observed [Figure 2(a)]. As pH increases, the number of negative charges in the particles increases as they expand. This expansion allows greater penetration of the solvent, bringing particle density closer to that of the medium. Furthermore, the higher number of charges of the same signal with increased pH prevents particle approximation, hindering clustering, which also contributes to greater stability.

In general, we found that CS helped to control the contraction process of PNIPAM-CS and PNIPAM-CS-PAA particles above
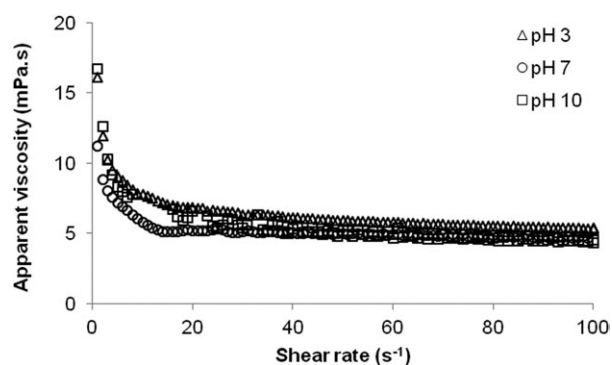
Figure 4. Apparent viscosity as a function of shear rate for suspensions prepared from PNIPAM particles in different solvents, at 25°C.

the LCST at all pHs. Particularly at high pH values, where the amino groups of CS are no longer protonated, the CS chains chemically bonded to the polymer network remained in the particles, and the rigidity of the polysaccharide chain contributes to the stability of the material [Figure 3(d,f)]. In contrast to findings reported in the literature, when CS chains interact only physically with PNIPAM-co-PAA particles,[33] precipitation of the excess CS occurs at high pH, clearing the suspension (CS is only soluble and protonated in acid media). This compromises properties such as biocompatibility, biodegradability, and low toxicity, affecting application in biomedical fields.

The rheology of smart polymers in aqueous media is another relevant aspect for investigation,[30,88,89] as it can help to elucidate association processes,[90] particularly their performance in response to shear rate applied at different pH and temperatures. When swollen smart particles are submitted to an environment above the LCST of PNIPAM, the particles shrink, owing to the disruption of hydrogen bonding with water molecules and the formation of hydrophobic associations among isopropyl moieties.[91–93] The formation of compacted particles can cause resistance to flow, increasing viscosity at low shear rates. When the shear rate applied is sufficiently high, associations between collapsed particles can be destroyed, resulting in shear thinning behavior.[94–97] However, as LCST depends on hydrogen bonding ability among hydrophilic segments of the polymer and water molecules, the phase transition is affected by the addition of hydrophilic comonomers such as acrylic acid, and also by different pH environments.[98,99] Moreover, reversibility can be observed by applying heating and cooling cycles.

Figure 4 shows the apparent viscosity of suspensions prepared from PNIPAM particles at different pH values. All curves demonstrated pseudoplastic behavior at 25°C, lowering viscosity as shear rate increased. This may be attributed to the rearrangement of deformable particles under shearing, yielding a system with a lower degree of interparticle interaction. Above the LCST, PNIPAM precipitated from all aqueous media, with phase separation.

Analysis of PNIPAM-CS particle suspensions by rheology showed slightly lower viscosity than that of PNIPAM particles in acid medium, a significant increase in viscosity in neutral medium and a small increase in alkaline medium [Figure 5(a)].

Lower viscosity at pH 3 may be attributed to the formation of positive charges on the surface of the particle, hampering their approximation. Crosslinked PNIPAM-CS particles, obtained by polymerization with persulfate, exhibit a positive surface charge when the ratio between CS and PNIPAM is 3 : 10 (w/w),[49] the same ratio applied in this study. The presence of surface charges prevents approximation between particles and stabilizes the suspension[61] [Figure 6(a)]. This results in slower sedimentation and lower viscosity in the system, which corroborates with the results observed in visual stability, accelerated stability and rheology testing.

In neutral medium, the amino groups are no longer protonated, enabling the formation of loose clusters [Figure 6(b)]. These exhibit high hydrodynamic volume and are responsible for the elevated viscosity of the system at low shear rates. The increase in shear rate causes these structures to break, reducing the viscosity of the system, as shown in Figure 5(a). When pH rises to 10, the tendency to form clusters declines. The lower interparticle interaction may be attributed to the appearance of negative charges on particle surfaces, perhaps due to the ionization of some hydroxyl groups of CS [Figure 6(c)]. These charges reduce the number of interparticle interactions, lowering the viscosity of the medium. Zeta potential measurements confirmed the occurrence of negative charges onto PNIPAM-CS surface in alkaline media (−9.85 mV).

The introduction of PAA caused a significant change in particle behavior. Since the pK_a of PAA is approximately 4, PNIPAM-
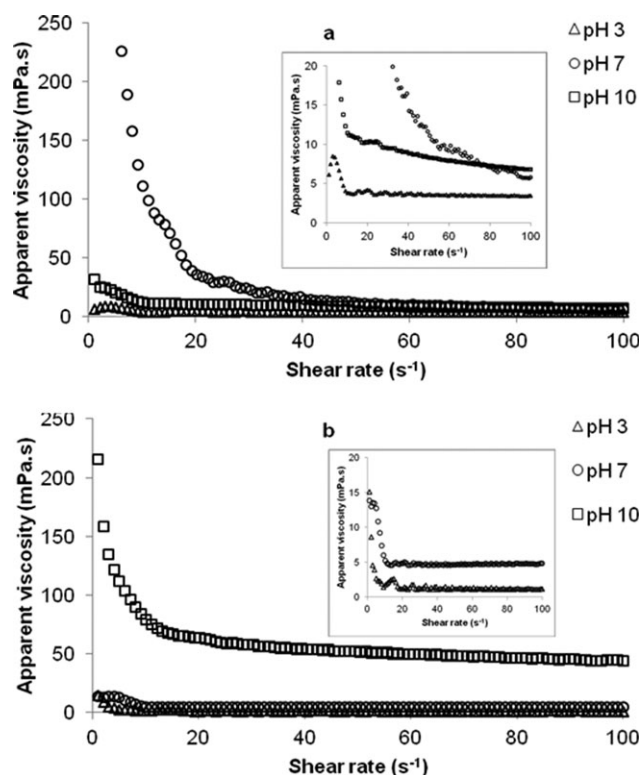

Figure 5. Apparent viscosity as a function of shear rate for suspensions prepared from: (a) PNIPAM-CS and (b) PNIPAM-CS-PAA, in different solvents, at 25°C.
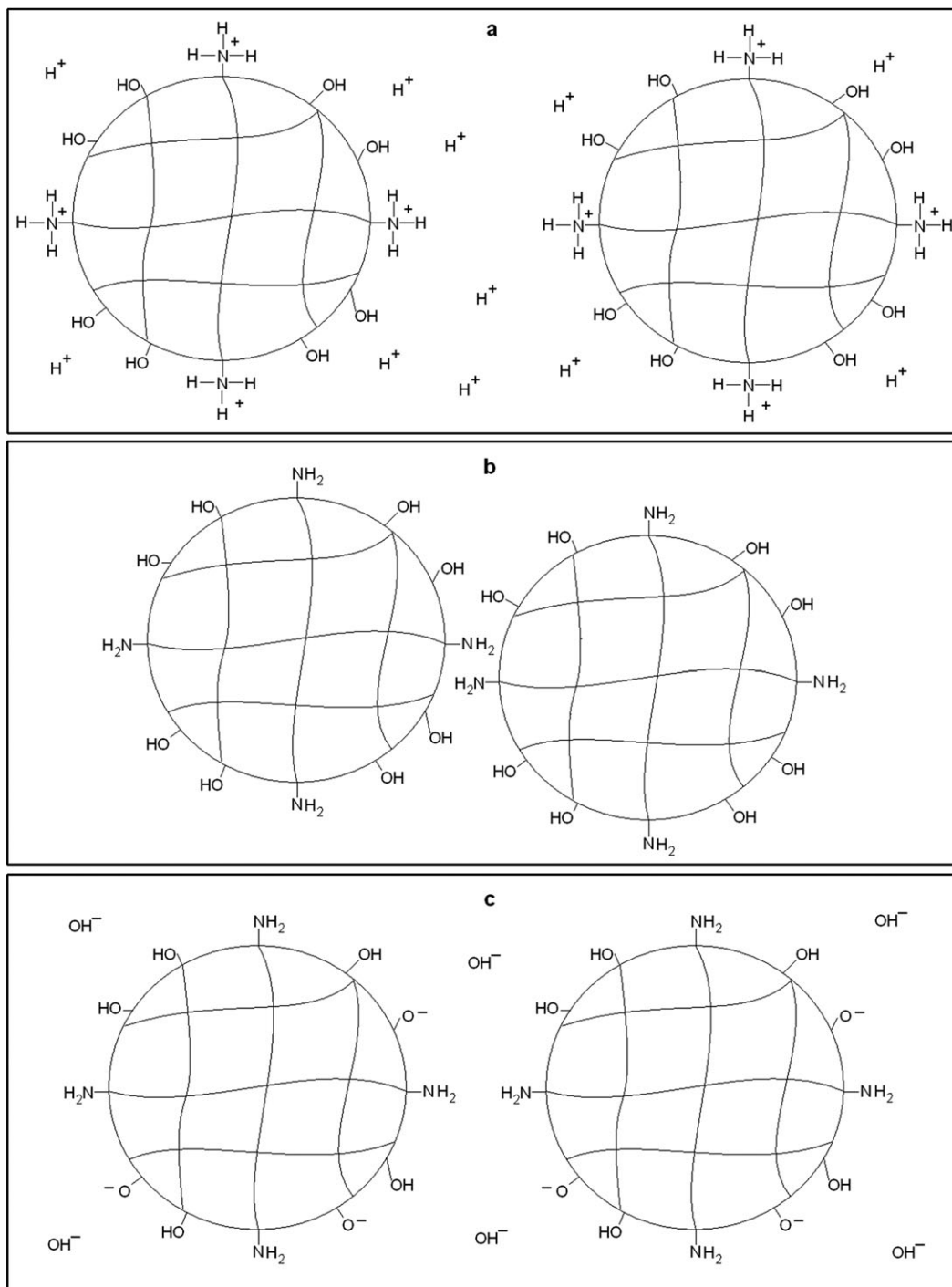
Figure 6. Illustration of the PNIPAM-CS particle in: (a) pH 3, (b) pH 7, and (c) pH 10, at 25°C.

CS-PAA particles contract at pH 3 and their contribution to the viscosity of the suspension is small, as per the insert in Figure 5(b). These particles precipitate without flocculation, forming compact sediment (Figure 2, PNIPAM-CS-PAA). Several charges appear at pH 7, caused by the ionization of COOH groups, which stabilizes the material in the medium. Increasing the pH to 10 increases the degree of ionization of carboxylate groups, causing repulsion between them and the expansion of the three-dimensional network. This increase in particle hydrodynamic volume justifies the rise in viscosity of the suspension throughout the range of shear rate analyzed.

To examine the reversibility of particle contraction, particles were rheologically analyzed using heating and cooling cycles (25–35°C and 35–25°C). At each temperature, curves were produced for forward and return sweeps (i.e., of 1–100 s^{-1} followed by 100–1 s^{-1}), to determine whether shearing caused
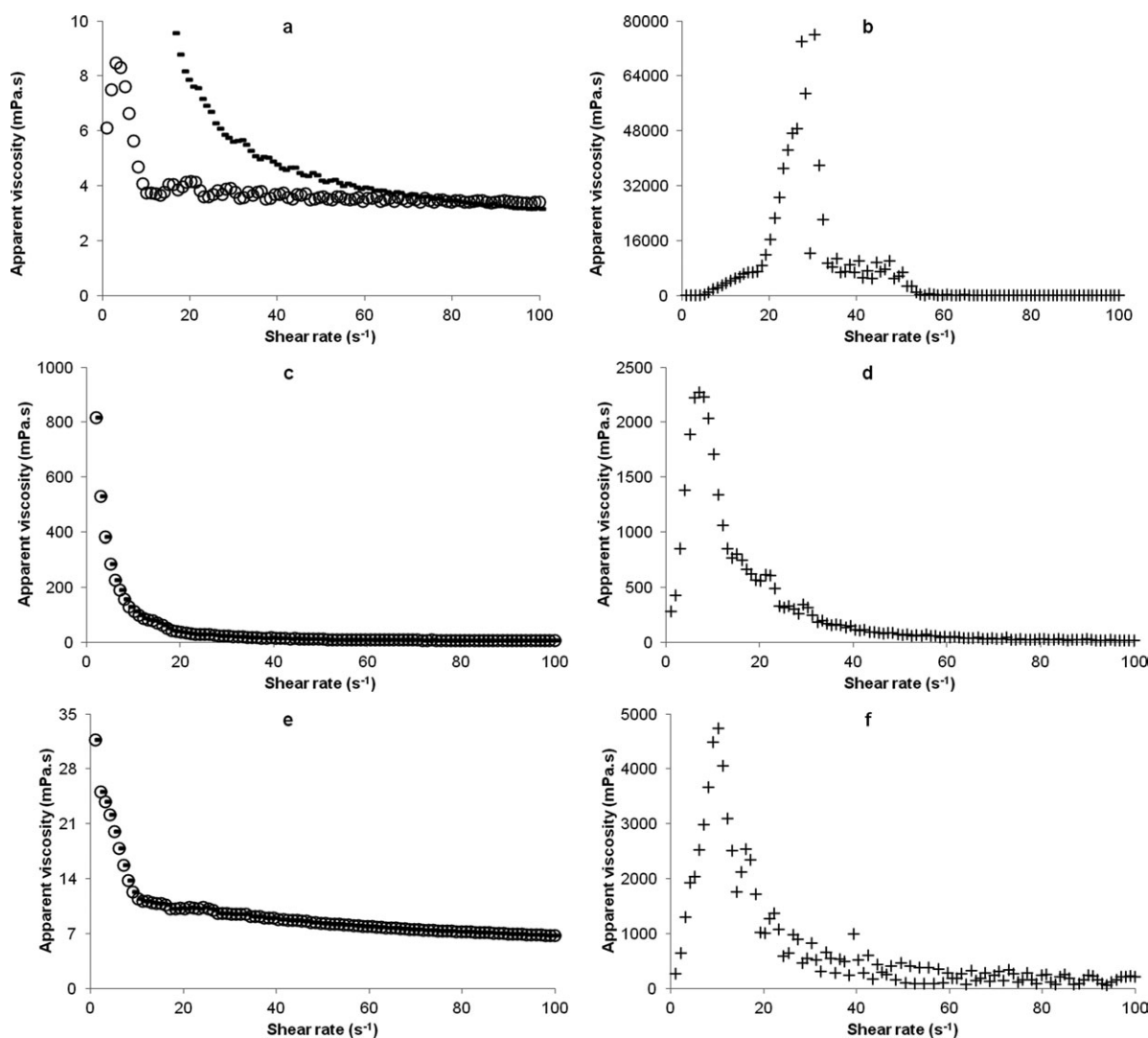
Figure 7. Apparent viscosity as a function of shear rate for PNIPAM-CS suspensions in (a) and (b) pH 3; (c) and (d) pH 7; and (e) and (f) pH 10. ○, measurements before heating; +, measurements with heating; −, measurements after heating.

irreversible changes in the structure of the suspensions. Results indicate that viscosity on the return sweep was identical to that of the forward sweep (data not shown).

At temperatures above the LCST of PNIPAM, PNIPAM-CS showed a rise in apparent viscosity at low shear rates in all the aqueous media, followed by a decline in viscosity at higher shear rates (Figure 7(b,d,f)]. This behavior was also observed for a carbon nanotube suspension at the same concentration.[94] The authors attributed the increase in the viscosity at low shear rates to a jammed state. It is important to note that polymer particles shrink above the LCST, hampering the flow. At higher shear rates, shear thinning behavior was observed, likely because clusters are destroyed. When the system was once again cooled to 25°C, collapsed PNIPAM-CS particles, at pH 7 and 10, likely immediately recovered their original shape, as evidenced by the reversible behavior shown in Figure 7(c,e). However, in acid medium [Figure 7(a)], the particles appeared to need more time to acquire the same shape and return to the same viscosity

values, which occurred at higher shear rates, probably because positive charges hinder particle approximation.

When PAA was introduced into the particles, PNIPAM-CS-PAA particles no longer exhibited thermosensitivity in acid or basic media, as shown in Figure 8(a,b). In the alkaline medium, carboxyl moieties are probably fully ionized and as such, pH effect predominates the temperature effect, stabilizing the particles [Figure 8(b)], whereas in the acid medium chains are already completely contracted, as indicated by the very low viscosity [Figure 8(a)]. The PNIPAM-CS-PAA particles showed an LCST effect of PNIPAM only in PBS [Figure 8(d)]. However, when the particles containing PAA were cooled, they only returned to their original form once submitted to shearing, as per Figure 8(c). These results corroborate those obtained from sedimentation measurements, where introducing the pH-sensitive component into particles made them less thermosensitive. Indeed, AA is the most commonly used comonomer to produce pH and thermosensitive polymers. However, it restricts the resulting
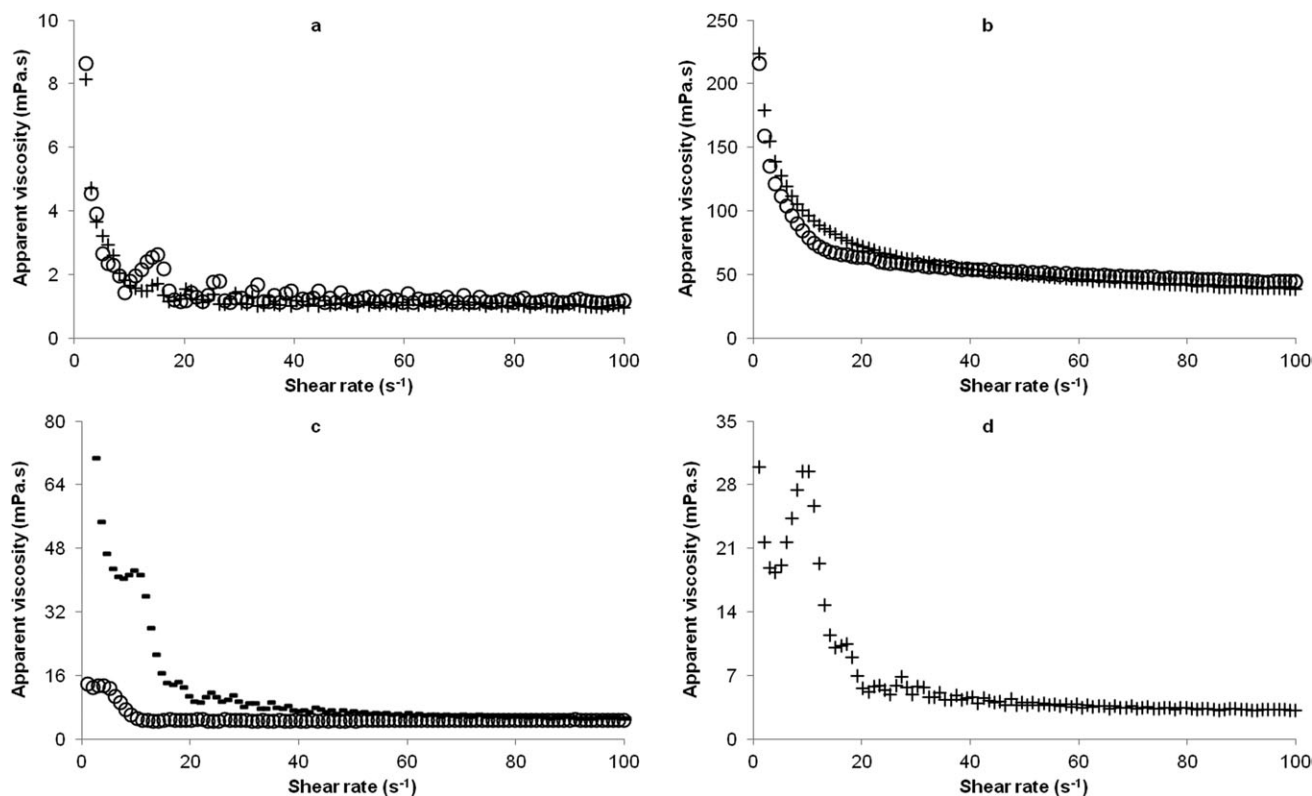
Figure 8. Apparent viscosity as a function of shear rate for PNIPAM-CS-PAA suspensions in (a) pH 3; (b) pH 10 and (c) and (d) pH 7. ○, measurements before heating; +, measurements with heating; –, measurements after heating.

thermosensitivity to a limited pH range as, at high pH, carboxylic groups are ionized and the polymer becomes more hydrophilic, thereby decreasing its thermosensitivity.[19]

A further relevant feature of PNIPAM-CS and PNIPAM-CS-PAA particles is that the amount of PAA in PNIPAM-CS-PAA is much greater than the one of CS in PNIPAM-CS. As a result, probably the great quantity of the hydrophilic component made the PNIPAM-CS-PAA particles to lose their thermosensitivity in high and low pH values, whereas the amount of CS in the PNIPAM-CS particles possibly was not enough to affect their peculiar performance at high temperatures.

CONCLUSIONS

The results obtained demonstrate that by introducing pH-sensitive polymers to crosslinked PNIPAM particles, it is not only possible to produce dual-sensitive materials but also to adjust the stability of these particles, quickening or slowing their phase separation, depending on the desired application. Thus, it is possible to adjust the material to different media. CS helped to control the contraction process of PNIPAM-CS and PNIPAM-CS-PAA particles above the LCST at all pHs. Particularly at high pH values, where the amino groups of CS are no longer protonated, the CS chains chemically bonded to the polymer network remained in the particles, and the rigidity of the polysaccharide chain contributes to the stability of the material. The PNIPAM-CS particles showed higher stability in acid and neutral media, whereas PNIPAM-CS-PAA particles were more stable in neutral and alkaline media, both below and above the LCST of PNI-

PAM. Another relevant aspect is that the amount of PAA in PNIPAM-CS-PAA is much greater than the one of CS in PNIPAM-CS. Then, the great amount of the hydrophilic component possibly made the PNIPAM-CS-PAA particles to lose their thermosensitivity in acid and alkaline media, whereas the amount of CS in the PNIPAM-CS particles was not enough to affect their response to change in the environmental temperature.

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