

Thermoresponsive Sodium Alginate-*g*-Poly(*N*-Isopropylacrylamide) Copolymers III. Solution Properties

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ABSTRACT: Stimuli-responsive biocompatible and biodegradable materials can be obtained by combining polysaccharides with polymers exhibiting lower critical solution temperature (LCST) phase behavior, such as poly(*N*-isopropylacrylamide) (PNIPAAm). The behavior of aqueous solutions of sodium alginate (NaAl) grafted with PNIPAAm (NaAl-*g*-PNIPAAm) copolymers as a function of composition and temperature is presented. The products obtained exhibit a remarkable thermothickening behavior in aqueous solutions if the degree of grafting, the concentration, and the temperature are higher than some critical values. The sol–gel-phase transition temperatures have been determined. It was found that at temperatures below LCST the systems behave like a solution, whereas at temperatures above LCST, the solutions behave like a stiff gel, because of PNIPAAm segregation. This behavior is reversible and could find applications in tissue engineering and drug delivery systems. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 000: 000–000, 2012

KEYWORDS: thermothickening; sodium alginate; poly(*N*-isopropylacrylamide); graft copolymers

Received 29 November 2010; accepted 23 March 2012; published online

DOI: 10.1002/app.37789

INTRODUCTION

Thermoresponsive aqueous formulations, that is systems exhibiting viscosity increase by increasing temperature, contrary to the usual Arrhenius thermothinning behavior, have attracted a lot of interest during the last two decades. Most of these systems are based on graft copolymers, consisting in a hydrophilic backbone and side chains phase separating by heating.^{1–5}

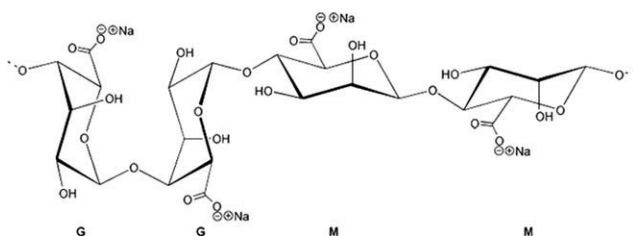
Most synthetic polymers in solution precipitate by cooling, but some water-soluble polymers phase separate upon heating, showing a lower critical solution temperature (LCST) behavior.^{6,7} Exactly, this property is exploited in the synthesis of the above thermoresponsive graft copolymers. LCST polymers used for this purpose, as side chains, include poly(ethylene oxide),^{1,3,4} ethylene oxide–propylene oxide random copolymers^{2,4} and poly(*N*-isopropylacrylamide) (PNIPAAm).⁵

The hydrophilic backbone of the thermoresponsive graft copolymer consists in a water-soluble polymer of a relatively high molecular weight, like poly(acrylic acid).^{1,5,8} Besides, polysaccharides, which combine large water solubility with biocompatibility and/or biodegradability characteristics and other specific properties, are

attractive materials for the synthesis of such thermoresponsive graft copolymers. Between them, carboxymethylcellulose,^{4,9,10} carboxymethylguar,¹¹ chitosan,^{12–14} carboxymethylchitosan,¹⁵ hyaluronan,¹⁶ dextran,^{17,18} and sodium alginate (NaAl)^{10,19} have been successfully used to obtain graft copolymers with thermoassociative properties. Although all these graft copolymers have different chemical compositions, their associating behavior follows the same general scheme. As temperature increases, a self-assembling of the side chains is induced, so that they function as responsive stickers which interconnect the hydrophilic counterpart (graft copolymer backbone), and a physical network can be formed, provided that the concentration is high enough to percolate through the whole volume. Such systems provide technological solutions when improved rheological properties above a given temperature are required, in a wide range of bioengineering²⁰ and biomedical²¹ applications, as drug release,¹² and tissue engineering.²²

In our previous study, multi stimuli-responsive polymeric materials have been obtained by combining the properties of alginate-chitosan with PNIPAAm in various architectures such as interpolymeric complexes, graft copolymers, or hydrogels.^{23,24}

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Scheme 1. The NaAl structure.

Alginic acid is a biopolymer extracted from different brown seaweeds, with many applications in food, pharmaceutical, and medical industries.²⁵ It attracts a broad research interest, owing to its biocompatibility and biodegradability. Moreover, as its repeating unit brings one carboxylic acid unit, it is accessible to chemical modifications which could enrich it with new properties and uses in new applications. It is an anionic copolymer, comprising of mannuronic acid (M block) and guluronic acid (G block) units arranged in an irregular blockwise pattern of varying proportion of GG, MM, and alternating or randomly organized blocks (Scheme 1). The relative amount of each block type varies with the origin of the alginate. Alternating blocks form the most flexible chains and are more soluble at lower pH than the other blocks. The number and sequence of the mannuronate and guluronate residues vary in the naturally occurring alginate.

Grafting of NaAl with PNIPAAm chains in dimethylformamide was proposed by Kim et al.²⁶ for the preparation of alginate-*g*-PNIPAAm beads with Ca^{2+} in aqueous solution, bearing pores which are contracted and expanded as a function of temperature.

In this study, we report on the solution behavior of graft copolymers of NaAl with PNIPAAm, envisaging to the development of thermoresponsive agents with potential applications in various fields as *in situ* thermo-gelling drug vehicles.

EXPERIMENTAL

Materials

Alginic acid was a product of Fluka, France, with a relative molecular weight, $M_r = 48,000\text{--}186,000$ g/mol. NaAl was prepared after neutralization of alginic acid with NaOH in aqueous solution. It is an anionic linear copolymer with homopolymeric blocks of (1-4)-linked β -D-mannuronate (M) and its C-5 epimer 72 α -L-guluronate (G) residues, respectively, covalently linked together in different sequences of blocks or randomly organized blocks (Scheme 1). The ratio of mannuronic/guluronic was around 1.3. It was purified with ultrafiltration with a tangential flow filtration Pellicon System (Millipore), equipped with an ultrafiltration membrane of molecular weight cutoff 10 kDa and finally obtained by freeze drying. Its viscosity average molecular weight, \bar{M}_V , was determined in 0.1M NaCl at 25°C by the equation:

$$[\eta] = 6.9 \times 10^{-4} \bar{M}_V^{1.13} \quad (1)$$

given by Martinsen et al.²⁷ for *Laminaria hyperborea* origin NaAls, and found equal to 1.4×10^5 g/mol.

N-isopropylacrylamide (NIPAAm) from Aldrich, USA, 2-aminoethanethiol hydrochloride (AET) from Aldrich, Karlsruhe, Ger-

many, potassium persulfate (KPS) from Merck, Darmstadt, Germany, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) from Aldrich, Karlsruhe, Germany, *N*-hydroxybenzotriazole (HoBt) from Alfa Aesar, Karlsruhe, Germany, were used as received. Water was purified by means of a Seralpur PRO 90C apparatus combined with a USF Elga laboratory unit. Acetone used was for analysis, Carlo ERBA, Milano, Italy.

Synthesis of Amino-Functionalized PNIPAAm

The monomer, NIPAAm, was dissolved in water (100 mmol in 80 mL of water), and the solution was deaerated with nitrogen bubbling. The temperature was adjusted at 30°C by using a water bath. The initiators (1.5 mmol AET and 1.5 mmol KPS) were dissolved separately in 5 mL of water, deaerated in a super sounds water bath, and added to the monomer solution after 30 min. The reaction time was 4 h. The product was purified by dialysis against water through a membrane (cutoff = 12 kDa, Sigma) and freeze dried. Its number average molecular weight, \bar{M}_n , was found equal to 6.7×10^3 g/mol by end group potentiometric titration with NaOH 0.1M using the 751 GDP Titrino of Metrohm.

Its viscosity average molecular weight was determined in LiNO_3 0.5M at 25°C by the equation:²⁸

$$[\eta] = 0.047 \times M_V^{0.61}$$

and was found equal to 1.1×10^4 g/mol.

Preparation and Characterization of Graft Copolymers, Alginate-*g*-PNIPAAm

Grafting of PNIPAAm chains onto NaAl backbone was realized by using EDC as a condensing agent in the presence of HoBt as a coupling agent, and hence the amide group between the carboxylate groups of NaAl and the amine group of the amine-terminated PNIPAAm was formed. The use of HoBt was imposed after many unsuccessful efforts to make the grafting reaction by using only EDC as is the case when polyacrylic acid²⁸ or carboxymethylcellulose⁹ had been used. HoBt has been suggested as a means to improve the efficiency of this reaction.²⁹ Indeed, after using it, we observed an almost quantitative grafting reaction.

In brief, 5% (w/v) solutions of NaAl and aminofunctionalized PNIPAAm in water were mixed at different ratios. HoBt in a stoichiometry and EDC in a tenfold quantity, relatively to the aminofunctionalized PNIPAAm, were added. In 24 h, we repeated the addition of EDC in a fivefold quantity, and, after another 24 h, the reaction mixture was fully neutralized with NaOH and poured into a tenfold volume of acetone. The precipitated graft copolymers were redissolved in water and freeze dried.

The graft copolymers obtained were characterized by elemental analysis carried out on a Carlo-Erba CHNS-O elemental analyzer EA1108. From the determination of the *N/C* ratio (where *N* is the nitrogen percentage and *C* the carbon percentage found in the experiment) the w/w % composition of the graft copolymers in PNIPAAm was determined.

¹H-NMR spectra were recorded, by using a BRUKER AVANCE DRX 400 MHz apparatus in D₂O. Figure 1 shows the spectra obtained for PNIPAAm, NaAl, and the graft copolymer G51. In

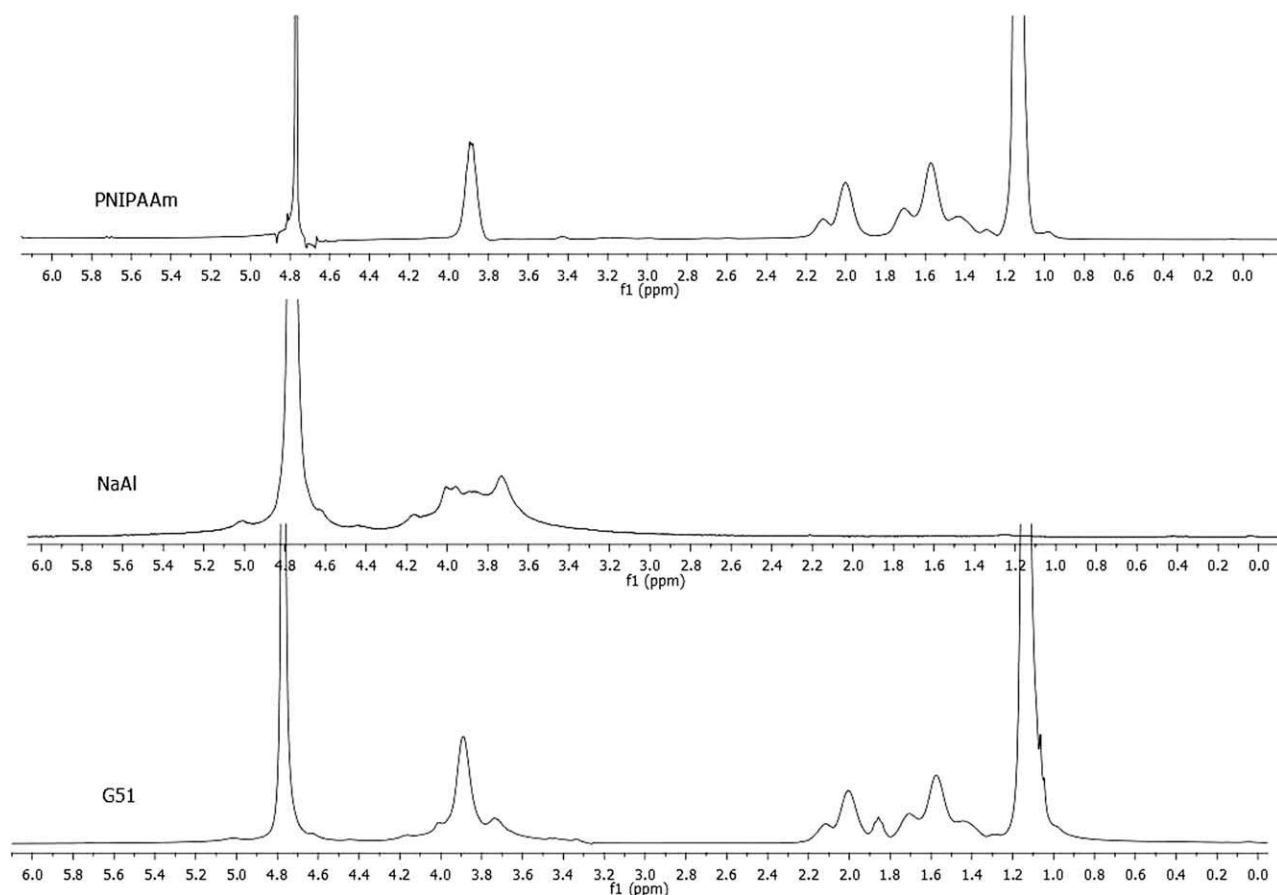


Figure 1. $^1\text{H-NMR}$ spectra of PNIPAAm and NaAl, and of a graft copolymer, NaAl-g-PNIPAAm, G51.

the PNIPAAm spectrum, the signal around 0.9–1.2 ppm is assigned to $-\text{CH}_3$ protons, around 1.2–2.2 ppm is assigned to the $-\text{CH}_2-\text{CH}-$ polymer skeleton protons, and around 3.8–4.0 ppm to the $-\text{CH}$ proton. In the spectrum of NaAl, we estimate that three protons of the alginate ring are assigned in the 3.4–4.3 ppm region.³⁰ In the G51 spectrum, around 3.4–4.3 ppm appear in the signals of the three alginate protons together with that of the $-\text{CH}$ proton of PNIPAAm, whereas in the 0.9–2.2 ppm region appear the $-\text{CH}_3$ and $-\text{CH}_2-\text{CH}-$ protons of PNIPAAm. The signal around 4.6–4.9 ppm is ascribed to D_2O . Accordingly, it is reasonable that alginate and PNIPAAm have been covalently bonded.

The composition results obtained by both methods are summarized in Table I. The graft copolymers are designated as G_x , where x is their weight percentage composition in PNIPAAm, which is the mean value of elemental analysis and $^1\text{H-NMR}$ determinations. Other characteristics are given in the previously published article.³¹

Even if the actual distribution of the side chains on the NaAl backbones remains unknown, each NaAl chain should bear more than one PNIPAAm chains, as, following the composition results summarized in Table I, each graft copolymer chain should on average contain 5–36 PNIPAAm side chains, by taking into account the molecular weights of the NaAl backbone and of the PNIPAAm side chains.

Rheological Measurements

The rheological investigations were performed on salted (NaCl, 1M) aqueous solutions of concentration of 10 g/dL by means of an Anton Paar 301 Rheometer device (USA) using a cone-and-plate geometry measuring system with a cone angle of 1° and a diameter of 50 mm at different shear rates and angular frequencies. The samples were placed onto the plate for 5 min to eliminate residual shear history, and then experiments were carried out immediately. The measuring device was equipped with a temperature unit that gave good temperature control ($\pm 0.05^\circ\text{C}$).

Table I. Composition in PNIPAAm of the Graft Copolymers as Determined by Elemental Analysis and $^1\text{H-NMR}$

Sample	PNIPAAm in copolymer (wt %)	
	Elemental analysis	$^1\text{H-NMR}$
G27	25	29
G33	32	34
G35	35	35
G38	32.4	43
G51	49	53
G61	54	68
G65	62	68
G78	76	80

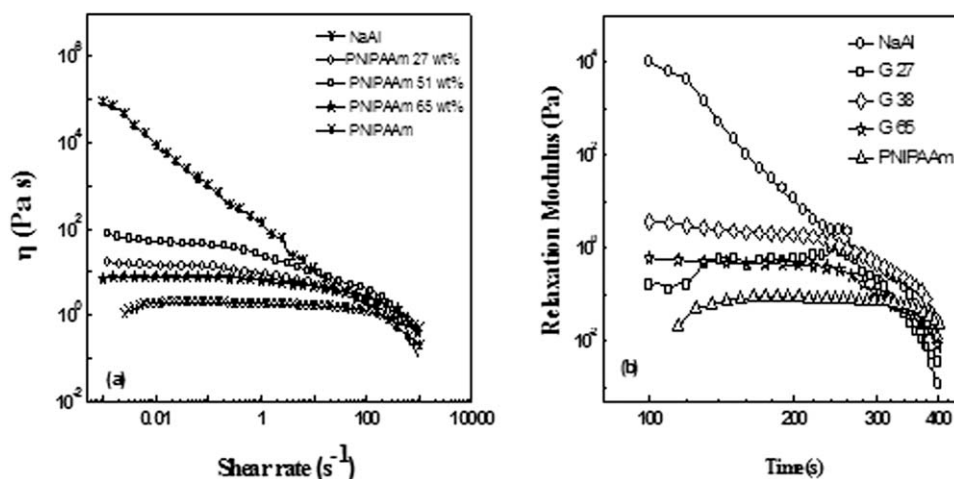


Figure 2. (a) Variation of the dynamic viscosity versus shear rate, and (b) variation of the relaxation modulus versus time, for NaAl, PNIPAAm, G27, G51, and G65.

Two types of tests have been performed namely at constant temperature and with variable temperature. The flow behavior of copolymers was tested by rotational controlled shear rate condition at 10^{-3} – 10^5 rot/s. The viscoelastic behavior was isothermally tested by oscillatory conditions of amplitude and frequency sweep at 25°C within a frequency range of 0.01–100 rad/s. The rheological behavior of copolymers with increasing temperature (heating rate of $2^\circ\text{C}/\text{min}$ from 25 to 60°C) was monitored at a constant shear rate of 50 rot/s (temperature sweep tests). Plateau modulus values, G_{NO} , were determined from frequency sweep measurements based on complex modulus (G^*) by using a mathematical model (Plateau modulus G_{NO} SN21003887) available within the Rheoplus software. To prevent dehydration, a thin layer of low-viscosity silicone oil was laid on the air/sample interface.

Fluorescence

Fluorescence measurements were conducted by a luminescence spectrometer (Perkin–Elmer, model LS50B, USA), equipped with a circulating water bath to control the temperature of the measuring cell. Pyrene was used as a micropolarity sensitive probe. The excitation wavelength was 334 nm. The intensity ratio of the first ($[I_1]$ at 373 nm) over the third ($[I_3]$ at 384 nm) vibronic peak of the pyrene spectrum, I_1/I_3 , was used to detect hydrophobic microdomains.³² Pyrene concentration was 10^{-6}M , taken from a stock solution in ethanol 10^{-3}M .

RESULTS AND DISCUSSION

Discussion of the Rheological Behavior

Influence of Content in PNIPAAm. Strain sweep tests were performed at 25°C at 1 Hz (6.28 rad/s) over the strain range of 0.01–100% to determine the linear viscoelastic region (LVE), in which storage, G' , and loss, G'' , moduli are practically constant and independent of the strain amplitude; the results showed a LVE regime up to a strain value of 10%. The results obtained from oscillatory dynamic measurements carried out in the angular frequency range of 0.01–100 rad/s at a constant strain of 0.01% are shown in Figure 2.

The flow behavior of NaAl-g-PNIPAAm copolymers was determined within a shear rate range of 0.0001 – 1000 s^{-1} .

Figure 2(a) shows the variation of the apparent viscosity, η , as a function of the shear rate, of 10 wt % solutions in NaCl 1M of the parent polymers, NaAl and PNIPAAm, and the graft copolymers G27, G51, G65 at 25°C . The curves are rather typical of shear thinning fluids. As shear rate increases, the apparent viscosity decreases. Moreover, there is no apparent trend of the flow curves to a Newtonian plateau at low shear rate, where viscosity should be independent of the shear rate, as is expected for polymer solutions. In this region, the viscosity decreases with increasing PNIPAAm content which induces a liquid-like character to the copolymer at 25°C . The intrinsic viscosity decreases linearly with increasing PNIPAAm content. It is probable that a much packed conformation is formed owing to the much smaller molecular weight of the PNIPAAm side chains. The decrease in viscosity with increasing PNIPAAm content is expected because the contour length of the alginate backbone per total polymer unit mass will decrease with PNIPAAm content, as the structure becomes more branched.

At very low shear rate, the graft copolymer samples exhibit a slight shear thickening effect for shear rates up to 0.001 s^{-1} , followed by a constantly falling dynamic viscosity from low to high shear rates at least in the low shear rate range up to 1 s^{-1} . This behavior, observed mainly in the case of copolymers containing a lower amount of PNIPAAm, corresponds to a gel-like structure. As the composition in PNIPAAm increases, the flow curve shows a Newtonian behavior. At high shear rate a shear thinning behavior is observed for all the samples, indicating an orientation of the macromolecules in the shear direction or their disentanglement, owing to the high shear load which lowers their flow resistance.

At higher shear rates, the viscosity decreases for all copolymers reaching approximately the same value of 0.1 Pa s at a shear rate of 1000 s^{-1} .

The variation of the relaxation modulus versus time, shown in Figure 2(b), shows a similar trend with viscosity, Figure 2(a).

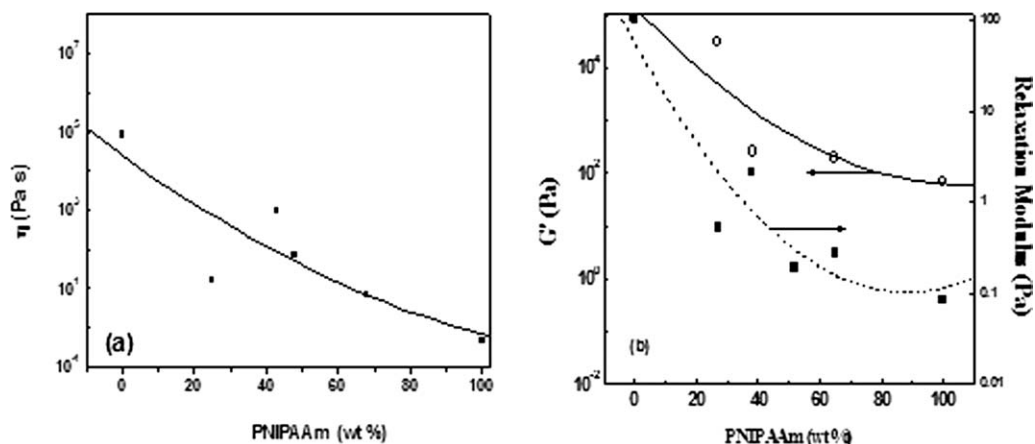


Figure 3. (a) Variation of the viscosity, at a shear rate of 0.001 s^{-1} , and (b) of the storage modulus (G') and of the relaxation modulus (G_r) at 120 min, on the graft copolymer composition, at 25°C .

The plateau relaxation modulus is time-independent till 250 s, decreasing with increasing PNIPAAm content of the copolymers, whereas at longer time a sudden decrease is observed to very low values of the relaxation modulus. Hence, the graft copolymers are not stable at high shear rates and long time under stress. To analyze the mechanical (relaxation) spectra of alginate/PNIPAAm graft copolymers, obtained by means of small deformation oscillatory tests, we use the generalized Maxwell model,^{33,34} which gives more details about the intermolecular interactions and copolymer's texture.

The generalized Maxwell model analyzes the interdependence between the structure and the ratio of the two components in copolymer and describes a homogeneous gel system in which the thermodynamic compatibility of the solvent and the polymer is observed. The model can be extrapolated toward very low frequencies, showing the flow curves in the terminal region based on which the constants characterizing the entangled gel network can be calculated: zero shear viscosity (η_0); plateau modulus (G_{NO}); zero-relaxation time (t_0). Applying the generalized Maxwell model to alginate gels, they can be defined as homogeneous

entangled networks and those of alginate/PNIPAAm as heterogeneous strong physical gels. The higher plateau modulus obtained with the variation of composition defines a stronger network structure.

Figure 3(a) shows the dependence of η , at the low shear rate of 0.001 s^{-1} , and Figure 3(b) shows the dependence of the relaxation and G' moduli, at 120 s, on the graft copolymer composition, at 25°C .

The decrease of the viscosity of the graft copolymers with increasing PNIPAAm content is attributed to their branched structure and the lower molecular weight of the side chains. As the copolymers are synthesized on the basis of the same alginate backbone, their hydrodynamic volume remains substantially the same, even if their composition in PNIPAAm increases, and their structure becomes more compact, resulting in lower viscosity values.

Temperature Influence

The rheological behavior of NaAl-g-PNIPAAm solutions depends on temperature and composition. Figure 4 shows the plots of the storage, G' , and loss, G'' , moduli as functions of the angular frequency for the pure components, whereas Figure 5 shows the same graphs for copolymers of various compositions, Figure 5(a): G27, (b): G38, and (c): G65.

Pure polysaccharide, NaAl, (Figure 4) showed higher values of G' in comparison with G'' for the entire frequency range in all temperatures studied, being in these conditions, under a gel state. The effect of temperature is not significant and only a small variation of the moduli is observed. In the case of PNIPAAm, G' is higher than G'' at low frequencies, up to an angular frequency of 1 s^{-1} , whereas at higher angular frequencies G' takes slightly lower values, as compared with G'' . At a temperature close to LCST, for example, 27°C , the two moduli trend lines are a bit different than at lower temperature, indicating the beginning of a sol-gel transition and collapse of the PNIPAAm chains.

As Figure 5(a–c) shows, the behavior of the graft copolymers is different, the influence of temperature, composition, and

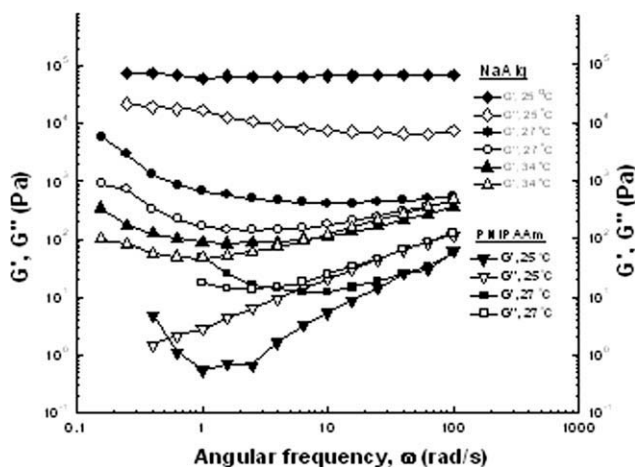


Figure 4. The storage, G' , and loss modulus, G'' , versus the angular frequency, for NaAl and PNIPAAm at different temperatures.

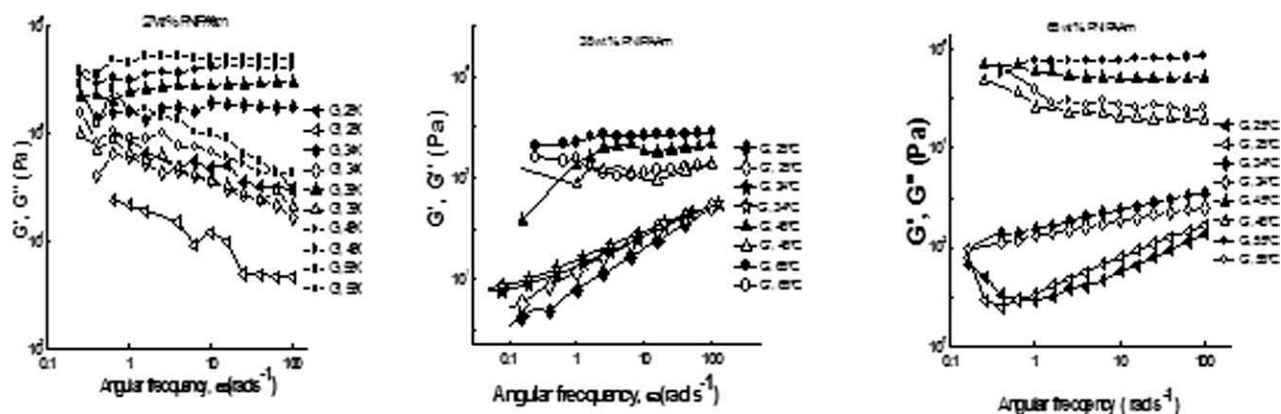


Figure 5. The storage, G' , and loss modulus, G'' , versus the angular frequency, for the graft copolymers (a) G27, (b) G38, (c) G65, at different temperatures.

frequency being evident. Regarding the curves of all three copolymers at 25°C, it can be observed that their shapes are totally different. By comparing the frequency sweep curves of the copolymers within the low-frequency range, that approximately means in a rest state, we observe that the copolymers with a higher composition in PNIPAAm (G38 and G65) have G' and G'' low values at low frequencies, but G'' is slightly higher than G' indicating a viscoelastic liquid at rest. The copolymer with lower composition in PNIPAAm, G27, has at 25°C a different trend line of frequency sweep curve, with higher values of moduli at low frequencies, while G' took higher values than G'' , indicating a “gel character” similar to pure NaAl, which is the prevalent component in the graft copolymer composition.

The effect of temperature is visible in the case of all three graft copolymers, but mostly in the case of the higher composition in PNIPAAm copolymers, G38 and G65. By increasing temperature, both viscoelastic moduli are significantly increased, and the values and the trend lines of the two moduli (G' , G'') are changing in a way that they become mostly parallel, indicating the formation of a gel at higher temperature. This can be explained by the shape of the curves of both G' and G'' , which

are changing slopes into the low-frequency range where G' took higher values than G'' , indicating the presence of a “gel-like” structure. Generally, the gel-like structure is indicated by the G' and G'' curves, which are in the form of almost parallel straight lines throughout the entire frequency range, showing a slight slope only.

The formation of a gel-like structure by increasing temperature should be associated with a thermo-thickening behavior owing to the PNIPAAm moieties within the graft copolymer structure, the values of G' being higher than 10 Pa, a value reported in the literature as being a condition for a gel stability.³⁵ This thermo-thickening effect is observed to be more pronounced in the case of copolymers with a higher PNIPAAm content, G38 and G65, especially at temperatures higher than 35°C, where both moduli have higher and mostly constant values.

Comparatively, the behavior of the copolymers G38 and G65 is a bit different, in the way the transition from solution to gel is observed starting with 43°C in the case of G38, whereas for the higher composition in PNIPAAm graft copolymer, G65, the increase of temperature determines a faster transition to the gel state starting even at 34°C, where both moduli have almost a

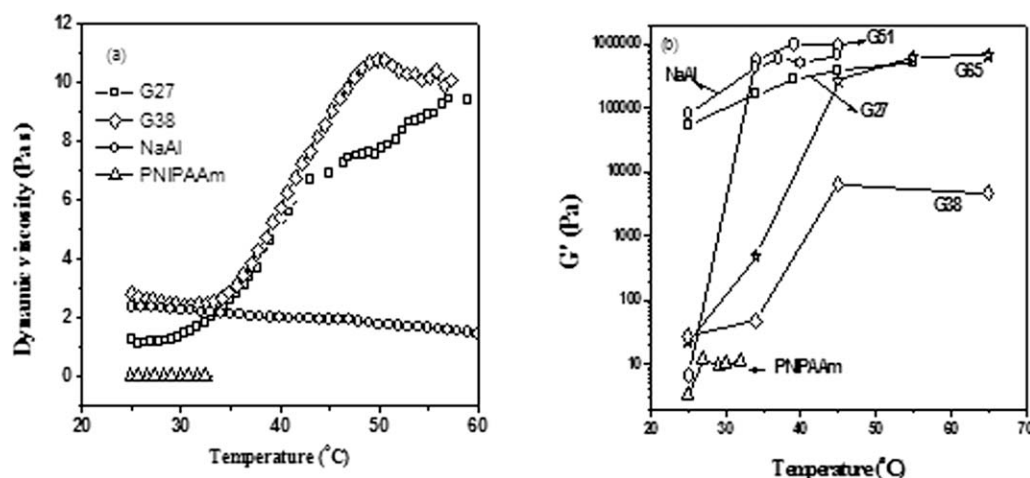


Figure 6. (a) Variation of the viscosity and (b) of the storage modulus G' corresponding to a frequency of 1 Hz, versus temperature for NaAl, PNIPAAm, and the graft copolymers G27, G38, G51, G65.

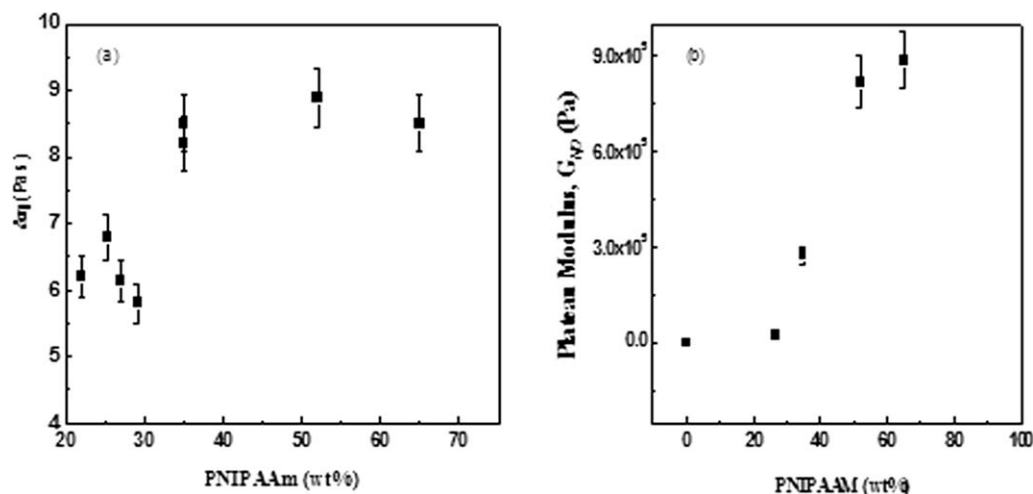


Figure 7. (a) Dependence of the viscosity increase from 25 to 55°C, and (b) of the plateau modulus, on the weight percentage graft copolymer composition in PNIPAAm.

parallel shape and G' took slightly higher values than G'' , indicating a thermo-associative behavior, owing to the PNIPAAm presence within the copolymer. A similar behavior was found by Seetapan for a copolymer of chitosan/PNIPAAm.¹⁴ At temperatures above of an association temperature, PNIPAAm side chains self-aggregate, forming hydrophobic microdomains which connect NaAl chains into a physical three-dimensional transient network.⁸ A rheological proof of the network formation is the shear-thickening behavior observed at $T > T_{\text{assoc}}$, whereas the behavior is essentially Newtonian at $T < T_{\text{assoc}}$.

The transition point, where the system behavior changes from solution to gel, the gel point, was calculated from the intersection of the two moduli, the so-called crossover point. Above the gel point, elastic property dominates owing to the network ability to store energy as can be seen from the increase in G' . This behavior is better evidenced in the variation of the viscosity with temperature, as shown in Figure 6. NaAl solution shows

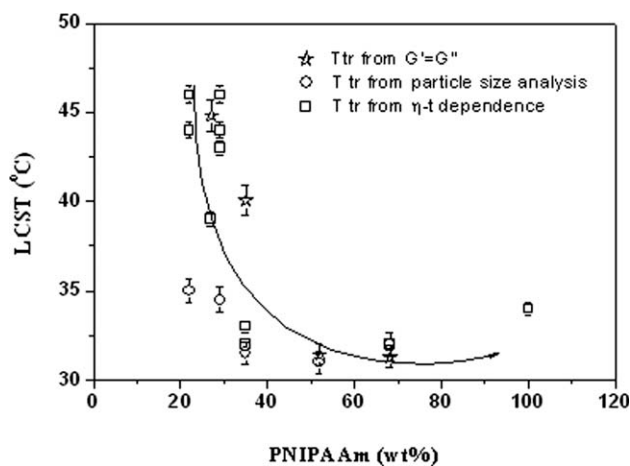


Figure 8. Dependence of the sol-gel transition temperature versus copolymers composition, as determined by different experiments from the crossover point of the dynamic moduli ($G' = G''$), from particle size analysis, and from $\eta - T$ variation (see legend).

thermothinning behavior, the viscosity decreasing with increasing temperature, Figure 6(a), whereas PNIPAAm phase separates at about 33°C. In the case of the graft copolymers, the viscosity, η , increases strongly with temperature, whereas no phase separation was observed, even if the solutions were opaque, with an opacity increasing with their composition in PNIPAAm. We observe an increase of the viscosity at 55°C about six to nine times in respect with the viscosity at 25°C, Figure 6(a), whereas the storage modulus, G'_0 , increases from 10 to 90,000 Pa [Figure 6(b)]. This increase is much clearly evidenced in Figure 7(a, b).

The increase of the number and/or strength of the intermolecular physical crosslinks could explain the increase of the plateau moduli as temperature increases. From a molecular point of view, an increase of the number and/or strength of crosslinks are owing to an increase of the number of the PNIPAAm side chains participating to hydrophobic clusters. This increase is not as big as in the case of other graft copolymers of polysaccharides previously reported.^{36,37} This should be attributed to the stiff conformation of the alginate backbone, which does not allow to the PNIPAAm side chains to come in very close vicinity and to form strong hydrophobic associations.

At high temperature, a strong segregation occurs by hydrophobic interactions, whereas at temperatures below LCST the solutions behave like a true solution, corresponding to a weak PNIPAAm segregation. This was confirmed by the linear shear relaxation data for other graft copolymers of PNIPAAm, which showed that the system relaxes according to a power-law function of time, indicating that the thermoassociative polymer solutions behave like soft critical gels.^{38,39} According to the Rouse-Zimm theory,⁴⁰ this dependence is characteristic for the viscoelastic gels, which are formed at temperatures higher than LCST. With increasing temperature, a sol-gel transition appears at a crossover point where $G' = G''$.

The crossover point of the two moduli is indicative for gel formation in certain conditions. Plotting the values of this

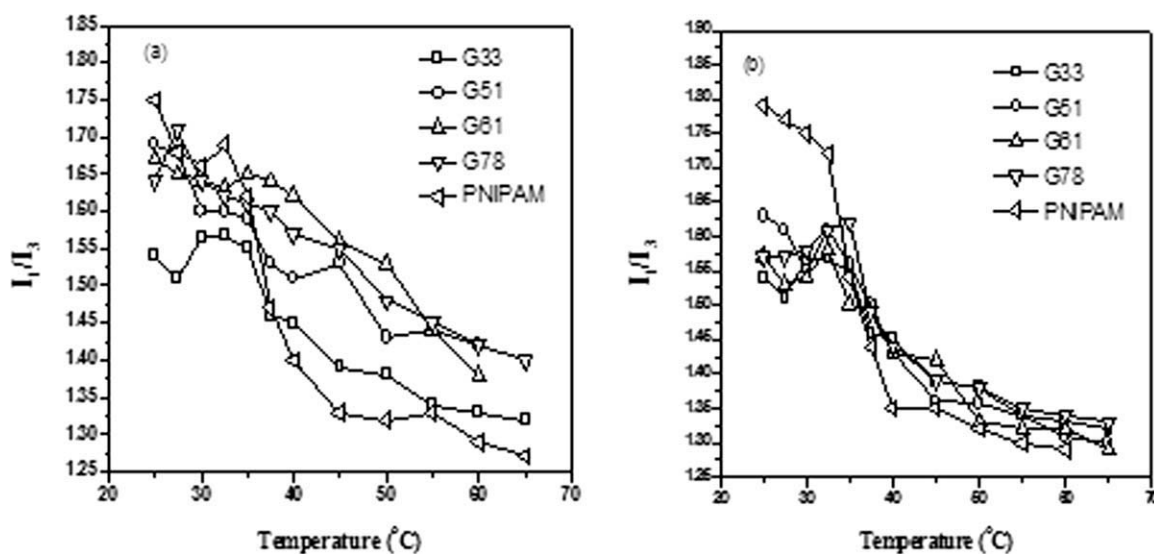


Figure 9. Variation of the pyrene fluorescence intensity ratio I_1/I_3 as a function of temperature for G33, G51, G61, G78, and PNIPAM in (a) 0.1 g/dL and (b) 1 g/dL solutions in water.

equilibrium modulus on temperature, transition sol–gel temperature is evaluated by a sigmoidal fitting.

The transition temperature depends on the copolymer composition, being shifted with the increase of the hydrophilic component (NaAl) content. At high PNIPAAm composition of the graft copolymer, it remains approximately constant, at around 33°C, close to that of PNIPAAm. Figure 8 shows that there is a good accordance between values obtained from $G' = G''$ from oscillatory tests, from the η dependence on temperature and from particle size analysis previously reported.²⁴

DISCUSSION OF THE FLUORESCENCE MEASUREMENTS

The formation of hydrophobic domains in the graft copolymers with increasing temperature was detected from the changes of the fluorescence emission spectrum of pyrene.

Figure 9(a) shows the variation of the I_1/I_3 ratio with temperature for 0.1 g/dL solutions of PNIPAAm and the graft copolymers, G33, G51, G61, and G78 in water. For PNIPAAm, we observe an abrupt decrease of I_1/I_3 from about 1.70 to about 1.30 as temperature increases over 32°C, indicating the transition from a hydrophilic to a hydrophobic environment, as temperature increases over the LCST of PNIPAAm, whereas for the four graft copolymers this transition seems to be much more gradual, and the I_1/I_3 values observed at higher temperatures are substantially higher. This result could be explained by the difficulty of the PNIPAAm side chains to form hydrophobic microdomains, owing to the rigidity of the NaAl backbone, revealed by the relatively high value of the Mark–Houwink exponent a in eq. (1), $a > 1$.

Figure 9(b) shows the variation of the I_1/I_3 ratio with temperature of PNIPAAm, and the four graft copolymers, G33, G51, G61, and G78, for 1 g/dL solutions in water. As temperature increases above 32°C, I_1/I_3 decreases abruptly for all four graft copolymers, as well as for PNIPAAm, from values of about 1.60 at 30°C at values of about 1.30 at 65°C. This behavior is indica-

tive of the formation of hydrophobic microdomains owing to PNIPAAm microphase separation above 32°C, and suggests the critical role of the concentration of the graft copolymers in the formation of these hydrophobic microdomains. A similar behavior was also reported by Gupta et al.¹¹ for carboxymethyl guar grafted with PNIPAAm.

CONCLUSIONS

Thermally responsive graft copolymers with a hydrophilic and biodegradable backbone of NaAl, and side chains of PNIPAAm, which is a polymer exhibiting a LCST phase behavior in aqueous solution, were synthesized.

The temperature effect on the rheological behavior of semidilute aqueous solutions of several thermoassociative graft copolymers of NaAl-g-PNIPAAm of various compositions has been studied. The samples were studied by steady shear, oscillatory shear, step-strain, and viscosity measurements, in the temperature range of 25–65°C, to study the thermothickening effect exhibited. It was established that these graft copolymers exhibit a thermothickening effect, dependent on copolymer composition and concentration.

These thermoresponsive copolymer solutions are related with a transient physical network, which is formed through weak reversible associations, owing to hydrophobic interactions, at least in the semi-dilute regime. The linear and nonlinear rheological data clearly show the existence of a transition temperature. Above transition temperature, the solutions behave like a stiff gel, corresponding to the PNIPAAm side chain segregation. The transition temperature has been determined and its dependence on the graft copolymer composition has been established.

The natural origin and the biocompatibility of the NaAl makes these graft copolymers attractive in pharmaceutical applications when thermally controlled formation of reversible gels is demanded.

ACKNOWLEDGMENTS

The authors thank CNCSIS through the IDEI project 17/2007, the European Community's Seventh Framework Programme [FP7/2007-2013] under grant agreement nr 218331 NaPolyNet.

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