Journal of Applied Polymer Science

# Preparation and swelling properties of homopolymeric alginic acid fractions/poly(*N*-isopropyl acrylamide) graft copolymers

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**ABSTRACT:** Graft copolymers of crosslinked poly(*N*-isopropyl acrylamide) (PNIPAAm) and homopolyguluronic acid (GG) and homopolymannuronic acid (MM) fractions of alginic acid were synthesized. MM and GG block fractions were obtained by partial acid hydrolysis of the alkaline extract from the brown seaweed *Macrocystis pyrifera*. The conjugation of these block fractions with the synthetic polymer was achieved by amidation with crosslinked PNIPAAm functionalized with an amino group at the end of the polymer chain. The structure of conjugates was determined by Fourier transform infrared and NMR spectroscopy. Atomic force microscopy of the graft copolymer GG-*g*-PNIPAAm showed a regular porous pattern, whereas the MM-*g*-PNIPAAm graft copolymer showed a regular netlike structure. Aqueous solutions of the synthesized graft copolymers afforded hydrogels by stirring with 0.1*M* $CaCl_2$. The hydrogels showed a well-defined stimulus–response to temperature and pH. The swelling, thermal, and pH characterizations demonstrated the superior properties of the GG-*g*-PNIPAAm hydrogel over the MM-*g*-PNIPAAm hydrogel. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42398.

**KEYWORDS:** grafting; hydrophilic polymers; polysaccharides; swelling

Received 6 October 2014; accepted 21 April 2015
**DOI: 10.1002/app.42398**

## INTRODUCTION

In recent years, polymeric materials that change their chemical or physical properties in response to external stimuli have been a subject of increased interest. This is due to the wide range of applications of these materials. On the other hand, the response to external stimuli requires the appropriate control of the network copolymer structure and then an adequate molecular design.[1–3] Moreover, it has been proposed that the swelling of polyelectrolyte gels depends on network parameters, which are determined by the osmotic pressure of counterions and by the elasticity of the polymeric strands forming a gel.[4] Despite this importance of the structure of macromolecular materials, the structural properties have scarcely been considered in the synthesis and use of conjugates formed by a polysaccharide and a synthetic polymer.

Poly(*N*-isopropyl acrylamide) (PNIPAAm) has become one of the most interesting synthetic polymers for graft copolymerization because of its temperature stimulus–response. It is well known that PNIPAAm is a thermosensitive polymer that presents a phase transition from hydrophilic to hydrophobic macromolecules when the temperature is raised above its lower critical solution temperature (LCST; 32–35°C).[5,6] This polymer has been grafted to polysaccharides, among these, alginic acid obtained by the extraction of brown seaweeds. This polysaccharide is a natural, biocompatible, and biodegradable polymer with a well-defined response to pH and with many applications in biomedicine, biotechnology, and pharmacology.[7–9]

Alginic acid consists of 1→4 linked β-D-mannuronic acid (M) and (1→4)-α-L-guluronic acid (G), which occurs mainly in brown seaweeds; both uronic acids are arranged in homopolymeric [homopolymannuronic acid (MM) and homopolyguluronic acid (GG)] and heteropolymeric (MG) blocks.[10–13] GG chains in alginate are responsible for gel formation with calcium ions.[14,15]

The conjugation of polysaccharides with synthetic polymers greatly improves their physicochemical and biological properties. In recent decades, much attention has been paid to the synthesis and characterization of graft copolymers of polysaccharides with vinyl polymers. Li *et al.*[16] prepared thermoresponsive guar gum/PNIPAAm hydrogels, and Guo and Gao[17] obtained a pH- and temperature-responsive hydrogel through the conjugation of carboxymethyl chitosan with PNIPAAm.[17] Studies of the graft copolymerization of sodium alginate with PNIPAAm have been published.[18–24] Commercial sodium alginate was used in these studies, but in a few cases, the M/G ratio, block composition, and chain lengths have been reported. Only recently have studies of the grafting of homopolymeric fractions of alginic acids to vinyl polymers been reported. The

group of Albertin used macroradicals from oligomers of homopolymeric blocks for the reversible addition–fragmentation chain-transfer polymerization of -[(2-hydroxyethyl)methacrylamide]. According to the authors, copolymers containing 10–20 residues of G formed gels in the presence of $Ca^{2+}$ ions.[2,25] In a previous study, a different approach was used for the preparation of graft copolymers, which covalently linked GG (molecular weight = 24,000) to the terminal amino group of PNIPAAm–$NH_2$; the hydrogel obtained in the presence of $Ca^{+2}$ showed interesting swelling properties.[26]

The main goal of this study was to synthesize well-defined graft copolymers (in terms of composition, molar mass, and functionality) by the covalent linkage of MM and GG from alginic acid with crosslinked PNIPAAm–$NH_2$; the response to the pH and external stimuli of the hydrogels obtained by the interaction of graft copolymers with $Ca^{+2}$ ions was studied. MM and GG block fractions were prepared by the partial acid hydrolysis of alginic acid from the brown seaweed *Macrocystis pyrifera*.

## EXPERIMENTAL

### Materials and Methods

2-Aminoethanethiol (AESH), 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), *N*-isopropyl acrylamide (NIPAAm), *N,N′*-methylene bisacrylamide (BIS), and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich Co. (St. Louis, MO). NIPAAm was recrystallized from *n*-hexane/toluene, and AIBN was obtained from methanol. Calcium chloride was purchased from Merck (Darmstadt, Germany). Distilled water was purified with a Milli-Q Barnstead Easypure II system (Thermo Scientific, Dubuque, IA). Spectroscopic-grade tetrahydrofuran (THF) and acetonitrile (ACN; Merck, Darmstadt, Germany) were used without any further purification. The brown seaweed *M. pyrifera* was collected in Río Seco (53°0.5′75″ S, 70°53′63″ W), southern Chile, in the summer. Microanalysis was performed at Facultad de Química, Pontificia Universidad Católica de Chile, Chile. The p$K_a$ values of the polymers were determined by potentiometric titration, and the pH values were measured with a pH meter (Corning 430). The LCST transition temperature was obtained in the range 26–36°C at a heating rate of 2°C/min, and we recorded the ultraviolet–visible absorbance of the polymer solutions with a Hewlett-Packard diode array spectrophotometer.

### Preparation of the Homopolymeric Block Fractions from Alkaline Extract

The ground blades of *M. pyrifera* were extracted with *n*-hexane and soaked afterward in formaldehyde/ethanol as previously described.[12] The treated algae were extracted with a 3% sodium carbonate solution, and the extract was purified by precipitation cycles in HCl, and $CaCl_2$.[27] The M/G ratio was determined by the total hydrolysis of the extract and high performance liquid chromatography (HPLC) analysis of the monosaccharides according to Gacesa *et al.*[28] The homopolymeric fractions were obtained by the partial acid hydrolysis of sodium alginate according to Haug *et al.*[29] Briefly, to a 1% aqueous solution of sodium alginate, 3*M* HCl was added to obtain a 0.3*M* concentration in HCl, and this mixture was heated at 100°C for 30 min under $N_2$ gas, cooled, and centrifuged. The supernatant was neutralized with 1.0*M* NaOH, poured into five volumes of ethanol (this afforded a solid that was dissolved in distilled water), and freeze-dried (fraction $F_1$). The precipitate was suspended in 0.3*M* HCl, heated for 2 h at 100°C, and cooled, and the mixture was separated by centrifugation. The pellet was dissolved in water by neutralization, adjusted to pH 2.85 by the addition of 1*M* HCl, and centrifuged. The supernatant was dialyzed against distilled water with a Spectra/Por membrane (molecular weight cutoff = 3500, Spectrum Laboratories, Rancho Domínguez, CA) and freeze-dried (fraction $F_2$). The precipitate was solubilized by neutralization, dialyzed, and freeze-dried (fraction $F_3$).

The average molecular weight of the fractions was determined by the Park and Johnson method, as previously described.[30]

### Preparation of the Linear and Crosslinked PNIPAAm–$NH_2$

The free-radical photopolymerization of NIPAAm (0.6*M*) was carried out in ACN under a $N_2$ gas atmosphere at 25°C with AIBN (0.01*M*) as a photoinitiator and AESH (0–20 m*M*) as a chain-transfer agent. The solutions were irradiated in a Rayonet photochemical reactor with a 360-nm irradiation source for fixed periods of time. The irradiated solutions were dialyzed against distilled water for 48 h, with the distilled water changed every 2 h during the day, and freeze-dried. The number-average molecular weights ($M_n$s) of the synthesized polymers were determined by gel permeation chromatography (GPC) at 30°C with an HP 1100 liquid chromatograph (Hewlett-Packard) equipped with three series of Styragel HR 1–2-4 columns (Waters). THF at 1 mL/min was used as the mobile phase, and differential refractometry was used for detection. Poly(methyl methacrylate) standards with $M_n$s from 1580 to 52,500 g/mol (Waters Co., Framingham, MA) were used for calibration.

Crosslinked PNIPAAm was synthesized under the same conditions with the addition of 2% BIS. The crosslinked polymer was dialyzed against distilled water for 48 h, with the distilled water changed every 2 h during the day; precipitated into THF–hexane (4 : 1); and dried *in vacuo*.

### Graft Copolymerization

An aqueous solution (2%) containing the sodium salt of homopolymeric MM or GG fractions of alginic acid and crosslinked PNIPAAm–$NH_2$ at a ratio of 1 : 1 were mixed with EDC and NHS. Polysaccharide/EDC/NHS with a molar ratio of 2 : 2 : 1 was used. The resulting solution was stirred at room temperature for 16 h, dialyzed against distilled water for 48 h, and extracted with a solution of THF–*n*-hexane (4 : 1, 25 mL). The aqueous layer was concentrated *in vacuo* and freeze-dried.

### IR and NMR Spectroscopy Measurements

Fourier transform infrared (FTIR) spectra of the samples in KBr were registered in the 4000–400-$cm^{-1}$region on a Bruker IFS 66v instrument (Bruker, Billerica, MA). Second-derivative IR spectra were obtained with the OPUS/IR version 1.44 software incorporated into the hardware of the instrument.[12] One-dimensional (1D) and two-dimensional (2D) NMR spectra were recorded on a Bruker Avance 400 spectrometer (Bellerica, MA) operating at 400.13 MHz ($^1$H) and 100.62 MHz ($^{13}$C) at 25°C, after isotopic exchange with $D_2O$ (3 × 0.75 mL) with $D_2O$ as the solvent and the sodium salt of 3-(trimethylsilyl)propionic

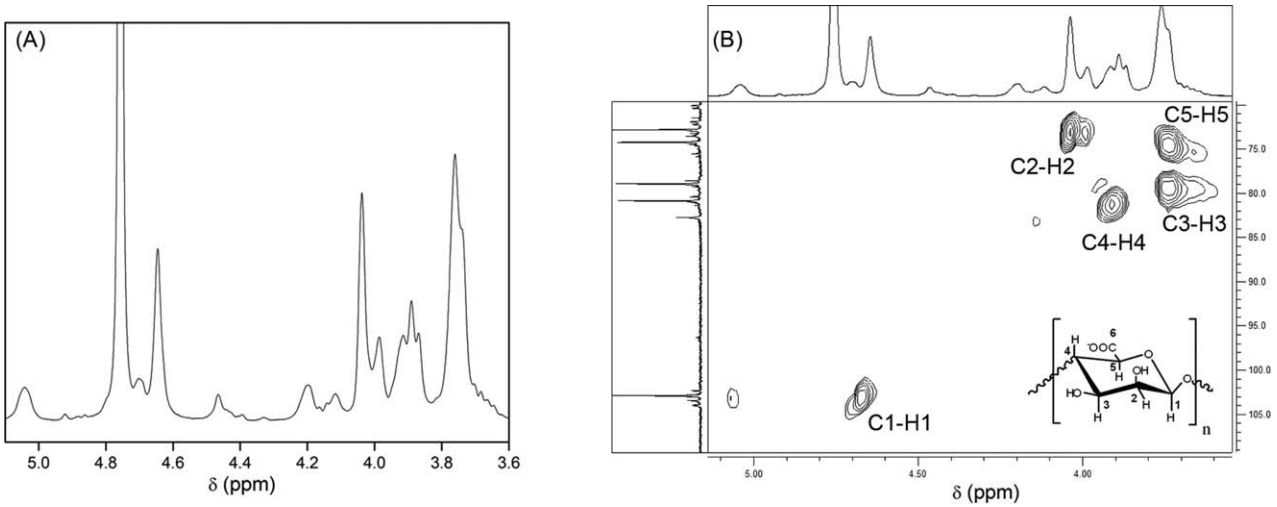



**Figure 1.** (A) $^{1}$H-NMR, (B) $^{13}$C-NMR, (C) 2D $^{1}$H/$^{1}$H COSY NMR, and (D) 2D $^{13}$C/$^{1}$H HSQC NMR spectra in $D_2O$ of the $F_2$ fraction obtained by the partial acid hydrolysis of sodium alginate from *M. pyrifera*.

2,2,3,3-$d_4$ acid as the internal standard. Two-dimensional homonuclear correlation (COSY) $^{1}$H/$^{1}$H spectra were acquired with 128 × 2048 data points with a spectral width of 1200 Hz and processed in a 1024 × 1024 matrix to give a final resolution close to 2.3 Hz/point in two dimensions. The 2D $^{13}$C/$^{1}$H heteronuclear single quantum coherence correlation (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectra were registered with 128 × 1024 data points and processed in 512 × 512 and 1024 × 1024 matrices, respectively, to give a final resolution close to 2.3 Hz/point in $^{1}$H and close to 2.4 Hz/point in $^{13}$C. The number of scans in each experiment was dependent on the sample concentrations.

### Atomic Force Microscopy (AFM)

The morphology of the adsorbed graft copolymers was studied by AFM with a Nanoscope IIIa extended multimode AFM with an E scanner (Digital Instruments, Santa Barbara, CA). The surfaces were scanned in the intermittent contact mode (tapping mode) with a scan rate of 0.4 Hz with commercial AC200TS three-sided silicon probes (Olympus). Height and phase AFM images were collected in air at room temperature (24°C). Samples of the homopolymeric MM and GG fractions of sodium alginate and the synthesized graft copolymers were dissolved in distilled water (0.1 mg/mL), filtered through 0.45 Millipore filters, and deposited onto freshly cleaved mica surfaces. The samples were placed in desiccators for 12 h as the incubation and drying time, and then, the AFM measurements were done immediately.

### Preparation of the Hydrogels

The synthesized graft copolymers (50 mg) were stirred with distilled water (500 μL), and the solution was added drop by drop to 10 mL of a 0.1*M* $CaCl_2$ cold solution. The resulting mixture was maintained at 4°C for 24 h, and the hydrogels, separated in the form of small spheres, were filtered through a sintered glass funnel and dried for 24 h at 40°C.

### Swelling Properties and Stimulus–Response of the Hydrogels.

Weighed dry samples of the hydrogels were immersed in a 0.1*M* $CaCl_2$ solution at 25°C. The weight of the swollen sample at different times was measured, the excess of solution was removed on the sample surface with a Whatmann number 5 filter article (Whatmann International, Ltd., Maidstone, England). The swelling ratio was monitored until there was no change in the weight of the sample. Also, the swelling ratios of the hydrogels were measured in the temperature range 20–45°C (at pH 4.5) and in the pH range 2.0–7.0 (at 25°C). The swelling ratio was calculated according to the following equation:

$$\text{Swelling ratio} = \frac{w_s - w_d}{w_d}$$

where $w_s$ and $w_d$ are the weights of the swollen and dry hydrogels, respectively.

## RESULTS AND DISCUSSION

### Preparation of the Homopolymeric MM and GG Block Fractions from Sodium Alginate

MG and homopolymeric block fractions were obtained by the partial hydrolysis of the alkaline extract (M/G 1.5) from *M. pyrifera*. According to Haug *et al.*,[29] the soluble fraction obtained in the first hydrolysis step was mainly composed of the MG fraction ($F_1$), the soluble fraction at pH 2.85 was enriched in MM ($F_2$), and the insoluble fraction was mainly composed of GG ($F_3$). The fractions were analyzed by FTIR spectroscopy in the solid state. The FTIR spectrum of fraction $F_2$ presented characteristic bands of MM at 940, 885, and 820 $cm^{-1}$, whereas the FTIR spectrum of fraction $F_3$ showed bands at 947, 903, 811, and 781 $cm^{-1}$; these were assigned to GG. FTIR spectra were very similar to those previously reported for homopolymeric fractions of sodium alginate from *M. pyrifera* and also for fractions of sodium alginates from different species of algae.[12,31–33] Structures deduced by FTIR spectroscopy were confirmed by NMR spectroscopy. Figure 1(A) presents the $^{1}$H-NMR spectrum of fraction $F_2$; it showed a strong signal centered at 4.65 ppm, which was assigned to the anomeric proton (H-1) of M units.[12,32] In addition, the spectrum showed small signals at about 5.07 and 4.70 ppm assigned to H-1 and H-5, respectively, of the G in MG blocks. An approximate integration

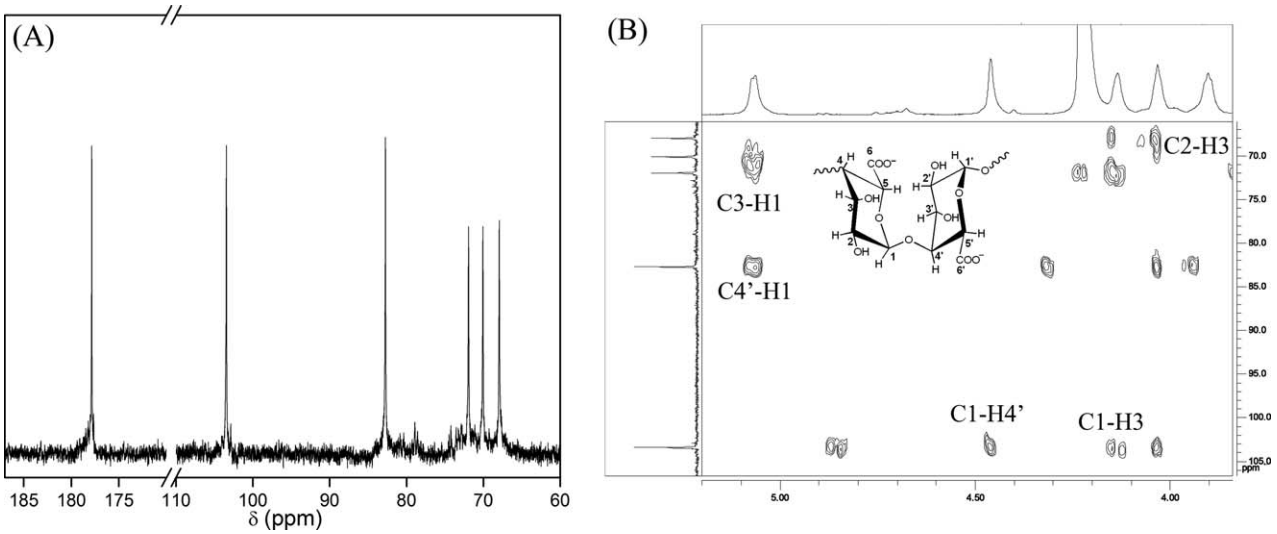



**Figure 2.** (A) $^{13}$C-NMR and (B) 2D $^{13}$C/$^{1}$H HMBC NMR spectra of fraction $F_3$ obtained by the partial acid hydrolysis of sodium alginate from *M. pyrifera*.

of signals in the $^{1}$H-NMR spectrum indicated that fraction $F_2$ from *M. pyrifera* contained around 20% MG blocks. These results were similar to those published for fraction $F_2$ of sodium alginate from *Desmarestia distans*.[12] The assignment of the major signals in the $^{1}$H-NMR spectrum was accomplished with the aid of the 2D $^{1}$H/$^{1}$H COSY NMR spectrum, as illustrated in Figure 1(B). Correlations between H-1 and H-2, H2 and H3, H3 and H4, and H4 and H5 are shown. On the other hand, the 2D $^{13}$C/$^{1}$H HSQC NMR spectrum [Figure 1(C)] shows all the correlations between the $^{13}$C and $^{1}$H systems in MM; furthermore, the 2D $^{13}$C/$^{1}$H HMBC spectrum (figure not shown) confirmed the presence of $\beta$-1→4 glycosidic linkages. Figure 2(A) depicts the $^{13}$C-NMR spectrum of fraction $F_3$; it shows only six intense signals assigned to G residues in a regular GG. Furthermore, the 2D HMBC $^{13}$C/$^{1}$H-NMR spectrum [Figure 2(B)] presented the connectivity between the G residues and indicated the presence of α-1→4 glycosidic linkages. The assignments of chemical shifts in the $^{1}$H-NMR and $^{13}$C-NMR spectra of the $F_2$ and $F_3$ fractions are presented in Table I; the values were in good agreement with those published in the literature and confirmed that the $F_2$ fraction was mainly composed of MM and the $F_3$ fraction was pure GG.[12,31–37] Molecular weight determinations by the reducing end method indicated $M_n$ values of 32,100 and 31,500 for the $F_2$ and $F_3$ fractions, respectively.

**Synthesis of PNIPAAm**

Linear PNIPAAm with an amino group at the end of the polymer chain was obtained by the photoinitiated free-radical polymerization of NIPAAm at room temperature with AIBN as a radical source and AESH as a chain-transfer agent. This method prevented polymerization at high temperature, which could result in a poorly controlled polymer network structure. Also, it allowed the introduction of terminal amino groups and the control of the polymer molecular weight. The addition of AESH in the concentration range 0–17 m*M* did not change the polymerization rate, but it decreased the apparent polymer molecular weight in the range of 50.000–10.000 g/mol, which was inversely related to the concentration of the chain-transfer agent, as shown in Table II. The values presented in Table II corresponded to the apparent molecular weights because they were determined with poly(methyl methacrylate) standards, although the use of THF as solvent for the standards and the synthesized polymers. It can be pointed out that GPC separates by hydrodynamic volume not by molecular weight.[38]

A chain-transfer constant of 0.17 was obtained by Mayo's equation; this value was lower than that obtained for the same chain-transfer agent in acrylamide polymerization (0.47).[39] The steric hindrance of the *N*-isopropyl group may explain this result.

**Table I.** $^{1}$H-NMR and $^{13}$C-NMR Chemical Shifts for the Polymannuronate (MM) and Polyguluronate (GG) Block Fractions Obtained by the Partial Acid Hydrolysis of Sodium Alginate

| | Chemical shift (ppm) | | | | | |
|---|---|---|---|---|---|---|
| Fraction | H-1/C-1 | H-2/C-2 | H-3/C-3 | H-4/C-4 | H-5/C-5 | C-6 |
| $F_2$ | 4.65/102.34 | 4.04/72.80 | 3.74/74.23 | 3.89/80.85 | 3.76/78.93 | 177.64 |
| $F_3$ | 5.06/103.45 | 3.90/67.93 | 4.03/71.93 | 4.14/82.75 | 4.46/70.06 | 177.86 |

1D and 2D NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400.13 MHz ($^{1}$H) and 100.62 MHz ($^{13}$C) at 25°C, using $D_2O$ as solvent and the sodium salt of 3-(trimethylsilyl)propionic 2,2,3,3-$d_4$ acid as internal standard

**Table II.** Polymerization Conditions and $M_n$ Values of PNIPAAm–$NH_2$

| Polymer[a] | [AESH] (m*M*) | $M_n$ (g/mol)[b] |
|---|---|---|
| P-1 | 0 | 52,000 |
| P-2 | 2 | 46,100 |
| P-3 | 5 | 38,500 |
| P-4 | 7 | 34,000 |
| P-5 | 9 | 29,300 |
| P-6 | 12 | 24,600 |
| P-7 | 15 | 15,700 |
| P-8 | 17 | 12,200 |

Free-radical photopolymerization of NIPAAm (0.6*M*) was carried out in ACN under $N_2$ gas atmosphere at 25°C, using AIBN (0.01*M*) as photoinitiator and AESH (0–17 mM) as chain-transfer agent. The solutions were irradiated in a Rayonet photochemical reactor with 360 nm irradiation source.

[a] PNIPAAm-$NH_2$.

[b] Average molecular weight was determined by GPC using monodisperse poly(methyl methacrylate) with $M_n$ from 1580 to 52,500 g/mol

Figure 3 presents the FTIR spectra of the synthetic polymer P-5; we observed that in the second-derivative spectrum [Figure 3(B)], signals assigned to the amide groups (1654.6, 1548.8, and 1367.3 $cm^{-1}$) for the amide I, II, and III bands, respectively, were well resolved. Also, this spectrum showed that at 3077.8 $cm^{-1}$, the band corresponding to the —$NH_2$ group introduced at the end of the polymer chain. Experimental conditions used for the synthesis of the linear polymer with an $M_n$ of 29,300 (P-5) were applied for the preparation of the crosslinked PNIPAAm polymer.

### Synthesis and Characterization of the MM-*g*-PNIPAAm and GG-*g*-PNIPAAm Graft Copolymers

The graft copolymers of MM and GG, obtained by the partial hydrolysis of alginic acid extracted from *M. pyrifera*, were prepared by conjugation with crosslinked PNIPAAm–$NH_2$. The synthesis was performed by the amidation of the carboxyl group of the homopolymeric fraction of sodium alginate with amino-terminated PNIPAAm in the presence of EDC as an activator and NHS, as shown in Scheme 1 for the conjugation of the GG fraction.[26] Around 75% of the initial mass was recovered; the graft copolymers were characterized by spectroscopic methods. Table III presents the characteristics bands in the FTIR spectra of the graft copolymers of MM (MM-*g*-PNIPAAm) and GG (GG-*g*-PNIPAAm) along with those of the synthetic polymer (PNIPAAm). Characteristic IR absorption bands for the MM and GG homopolymeric fractions in the graft copolymers (Figure 4) were assigned according to previously published data.[12,31,32,34] The presence of vibrations of amide groups in the IR spectra of the graft copolymers was indicative of the conjugation of the homopolymeric fraction with the synthetic polymer. It is noteworthy that in the IR spectra of the MM and GG graft copolymers, the presence of the characteristic bands were attributed to the anomeric $\beta$- and $\alpha$-C—H vibrations of the M and G residues, respectively. The nitrogen contents in the graft copolymers, determined by elemental analysis, were 7.65 and 7.43% for the MM-*g*-PNIPAAm and GG-*g*-PNIPAAm copolymers, respectively; this indicated the incorporation of the PNIPAAm synthetic polymer in the backbone of the MM and GG chains. These results were in agreement with those reported by Dumitriu *et al.*[19] for the copolymer of sodium alginate and PNIPAAm.

Figure 5(A) depicts the $^{13}$C-NMR spectrum of the graft copolymer GG-*g*-PNIPAAM. Present in the 170–180-ppm region were three carbon peaks: one at 178.13 ppm, a band assigned to the carbonyl of the amide linkage between the carboxyl acid of G and the terminal amino group of PNIPAAm–$NH_2$, and two bands that were assigned to the carbonyl group of G (178.30 ppm) and the amide group of PNIPAAm (178.0 ppm).[26] The $^1$H-NMR and $^{13}$C-NMR spectra of the graft copolymers were rather complex, but the 2D $^{13}$C/$^1$H HMBC NMR spectrum afforded useful information about the linkage of the carbonyl carbon of the polysaccharides to the synthetic polymer. Figure 5(B) shows the connectivity between the two $^{13}$C/$^1$H systems in the carbonyl carbon region. The signal at 178.30 ppm assigned to the carbonyl carbon resonance of G residue in GG-*g*-PNIPAAm showed a two-bond correlation with the proton resonance at 4.70 ppm, which was assigned to $H_5$. Furthermore, a correlation was observed between the carbonyl carbon resonance and the resonance at 3.89 ppm, assigned to the methylene protons of the PNIPAAm residues. These results altogether with the nitrogen contents and FTIR assignments indicate the conjugation of the GG and MM blocks of sodium alginate with PNIPAAm.

On the other hand, the graft copolymers were analyzed with respect to the pH and thermal properties. Values of p$K_a$ of 3.30 and 3.75 were found for the GG and MM blocks, respectively. These values were very similar to those for the respective graft copolymers and indicated that the presence of the NIPAAm chains in the polysaccharide did not affect the ionization of the carboxylic acid group.

Experiments of the LCST measurements gave a value of 32.1°C (pH = 5.8) for linear PNIPAAm and crosslinked PNIPAAm. A decreased phase-transition temperature was found with the grafting to the polysaccharide, and values of 31.0 and 30.1°C were obtained for the graft copolymers containing MM and GG blocks, respectively. The lower value of the phase-transition

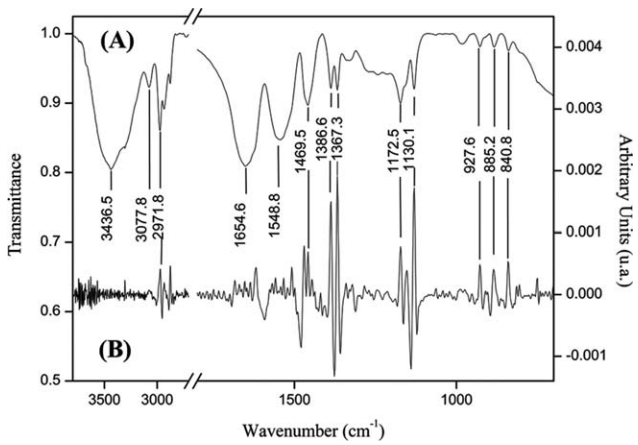
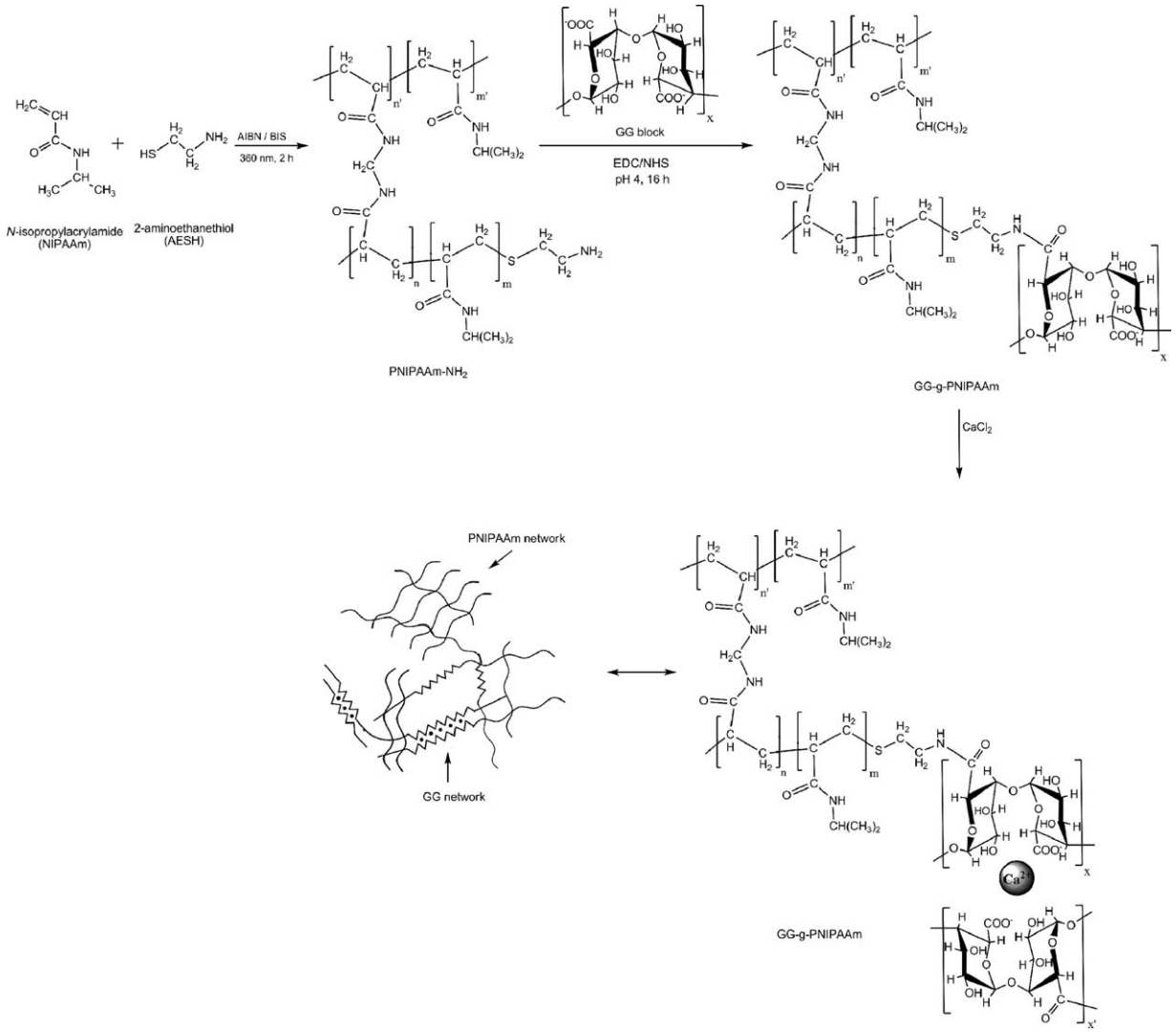



**Figure 3.** Normal and second-derivative FTIR spectra of the PNIPAAm–$NH_2$ synthesized with a 9 m*M* concentration of AESH (P-5).




**Scheme 1.** Formation of the graft copolymer of the GG fraction from alginic acid and crosslinked PNIPAAm with a terminal amino group.

temperature for the copolymer containing the homopolymeric GG block was in agreement with its compact structure, which facilitated polymer–polymer interactions.

### AFM Studies

Figure 6 shows the results of AFM study of the morphology of both the homopolymeric GG and MM blocks and graft copolymers. A noticeable and significant difference among the morphology of the adsorbed polymer films was observed. The film from the GG chains [Figure 6(A)] showed a closed laminar–globular mixed structure, whereas the film from the MM blocks [Figure 6(B)] showed an opened and clear ribbonlike structure. The differences observed between both polymeric chains were related to their tertiary structures. The structure of GG chains was like pleated sheets because of their $\alpha$-1→4 linkages, whereas for the MM chains, the tertiary structure was an extended sheet with the M units in $\beta$-1→4 linkages. Thus, the polymeric MM chain had an extended ribbon structure, whereas the polymeric GG chain had a folded ribbon structure; these results were in accordance with those previously found by morphological studies conducted by SEM.[26] Figure 6(C,D) shows the topographic and phase image for the GG-*g*-PNIPAAm and MM-*g*-PNIPAAm graft copolymers, respectively. By comparing the surface morphology of the MM and GG blocks with the graft copolymers, we found that the graft process drastically changed the morphology of the polymer films. The GG-*g*-PNIPAAm graft copolymer [Figure 6(C)] showed a regular and homogeneous pattern of a surface constituted by open and segregated uniform structures. There was no evidence of microphase separation; this was related to the good compatibility between the PNIPAAm polymer and GG blocks. The homogeneous feature of the film surface was related to the complete interpenetration on the network of the graft copolymer. The lower sections on the AFM image correspond to the pores of the interpenetrating polymer structure with 100–300-nm lengths. The phase image of the MM-*g*-PNIPAAm graft copolymer [Figure 6(D)] showed two structural domains, a regular netlike structure as a base frame and over this some round and spherical polymeric aggregates.

**Table III.** Wave Number Assignments (in $cm^{-1}$) for FTIR Vibrational Bands of PNIPAAm–$NH_2$, and the Graft Copolymers MM-*g*-PNIPAAm and GG-*g*-PNIPAAm

| Wave number ($cm^{-1}$) | | | |
|---|---|---|---|
| PNIPAAm-$NH_2$ | MM-*g*-PNIPAAm | GG-*g*-PNIPAAm | Assignment |
| 3436.5 | 3429.2 | 3436.0 | $\upsilon$ NH— $\upsilon$ O—H |
| 3077.8 | | | $\upsilon$ $NH_2$ |
| 1654.6 | 1664.4 | 1658.7 | $\upsilon$ C=O (Amide I) |
| | 1641.3 | 1647.1 | $\upsilon$ C=O (Amide I) |
| 1548.8 | 1544.9 | 1544.9 | $\upsilon_{Asym}$ $COO^-$ $\delta$ N—H (Amide II) |
| | 1461.9 | 1461.7 | $\upsilon_{Sym}$ $COO^-$ |
| 1367.3 | 1367.7 | 1367.4 | $\upsilon$ C—N (Amide III) |
| | | 948.9 | $\upsilon$ C-O-C ($\alpha$-glycosidic linkage) |
| | | | $\upsilon$ C-O-C ($\beta$-glycosidic linkage) |
| | | 902.6 | $\delta$ C—H ($\alpha$-anomer) |
| | 883.3 | | $\delta$ C—H ($\beta$-anomer) |
| | 821.6 | | $\delta$ C-O-C ($\beta$-glycosidic linkage) |
| | | 812.0 | $\delta$ C—O—H, $\upsilon$ O—C—H, $\delta$ C—C—H |
| | | | Ring deformation ($\alpha$-L-gulopyranuronate residues) |

This netlike structure resembled homopolymeric MM blocks and indicated a poor interaction between the MM fraction and PNIPAAm chains. The surface morphology of the graft copolymers could have been related to their water-uptake capacity. Large regular surfaces lead to a homogeneous surface contact with water molecules; at the same time, a regular surface leads to a uniform and easy water diffusion to the inner part of the hydrogel as was the case for the GG-*g*-PNIPAAm graft copolymer.[26] On the other hand, the more collapsing surface of MM-*g*-PNIPAAm graft copolymer could have been related to the minor water-uptake capacity and restricted diffusion of water molecules.

**Swelling Properties and Stimulus–Response of the Hydrogels**

Hydrogels from the synthesized graft copolymers were prepared by immersion in an aqueous $CaCl_2$ solution, and their water absorption was analyzed over a controlled period of time. Figure 7(A) shows the swelling ratio of the GG-*g*-PNIPAAm and MM-*g*-PNIPAAm hydrogels. The rate of swelling was high during the first 3 h in both cases because of the lower osmotic pressure inside the hydrogels in relation to bulk water. The water molecules diffused into the hydrogels until a plateau was reached in accordance with the Flory–Rehner swelling theory.[39] As the swelling process took place, the osmotic pressure increased because of the elastic force of the copolymer backbone; this caused a drop off in the swelling rate. Figure 7(A) shows that the swelling ratio of the GG-*g*-PNIPAAm hydrogel was almost twice that of the MM-*g*-PNIPAAm hydrogel. The higher water retention capacity observed in the former might have been due to the nature of the hydrogel by interaction of the GG chains with $Ca^{+2}$, as shown in Scheme 1. On the contrary, in the MM-*g*-PNIPAAm hydrogel, the tertiary structure

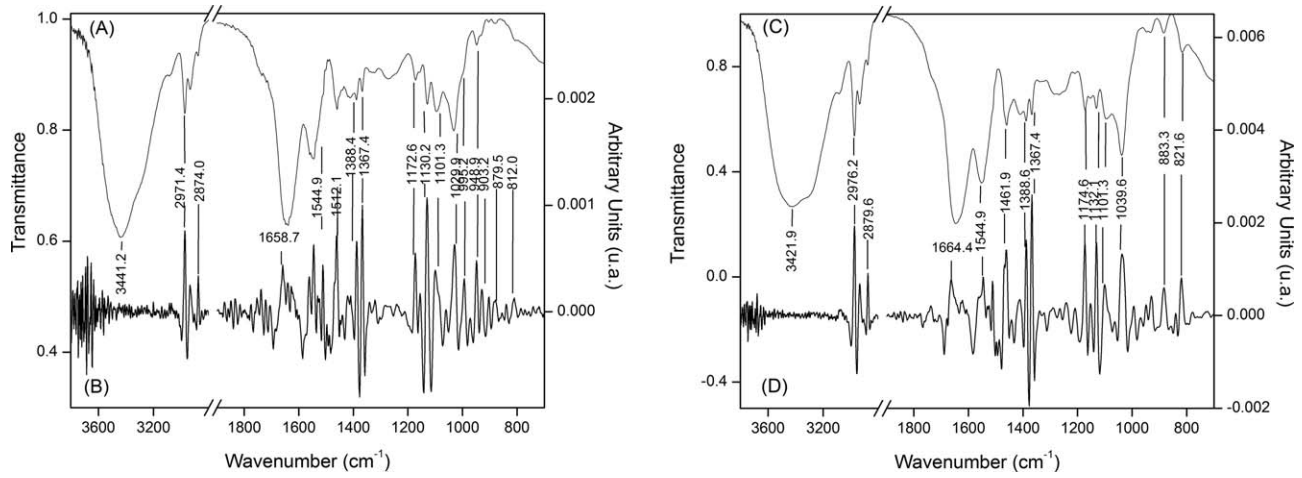



**Figure 4.** (A) FTIR and (B) second-derivative FTIR spectra of the graft copolymer (GG-*g*-PNIPAAm). (C) FTIR and (D) second-derivative FTIR spectra of the graft copolymer (MM-*g*-PNIPAAm).

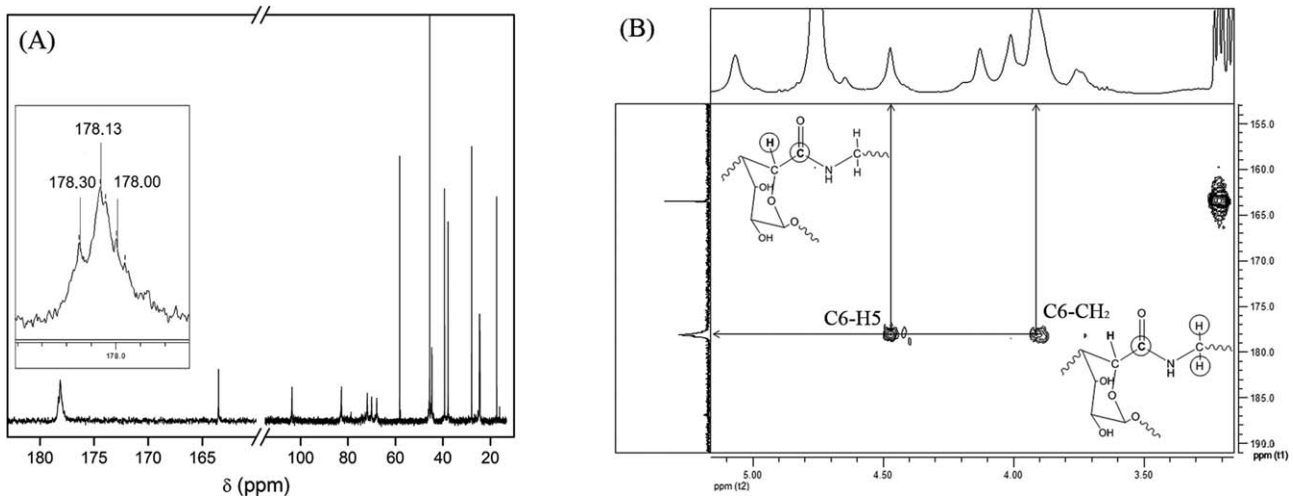



**Figure 5.** $^{13}C$-NMR and $^{13}C/^{1}H$ 2D HMBC NMR spectra of the graft copolymer (GG-*g*-PNIPAAm) obtained by the amidation of GG with terminal amino groups of the crosslinked PNIPAAm in $D_2O$.

adopted by the MM chains due to the $\beta$ configuration of the glycosidic linkage precluded the formation of an egg-box-type complex with calcium ions. Previously, researchers studied the water absorption capacity of the graft copolymer synthesized by the conjugation of polyguluronate (GG; $M_n = 24{,}000$) with linear PNIPAAm ($M_n = 9300$).[26] The swelling ratio for its hydrogel was 100% higher than the values found in this study for the GG-*g*-PNIPAAm hydrogel; the crosslinking of PNIPAAm chains, which restricted the polymeric network expansibility, could have explained the lower swelling capacity of the latter.

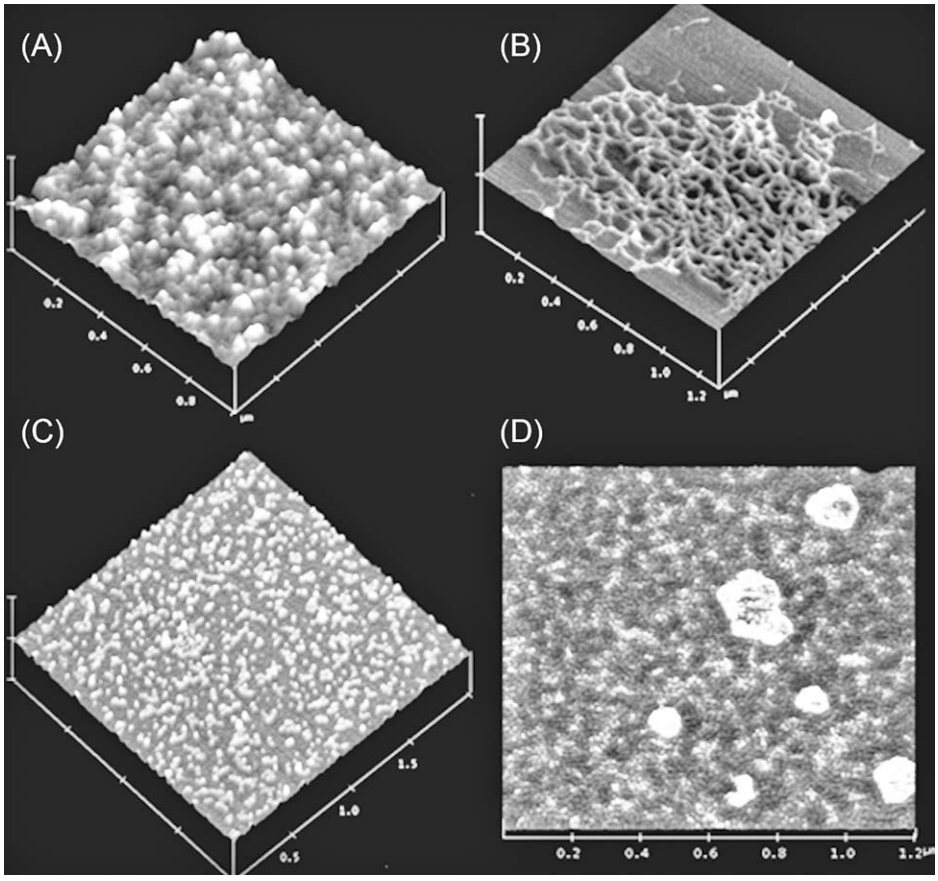



**Figure 6.** Topographical AFM images of the (A) GG and (B) MM homopolymeric block fractions and phase images of the (C) GG-*g*-PNIPAAm and (D) MM-*g*-PNIPAAm graft copolymers.

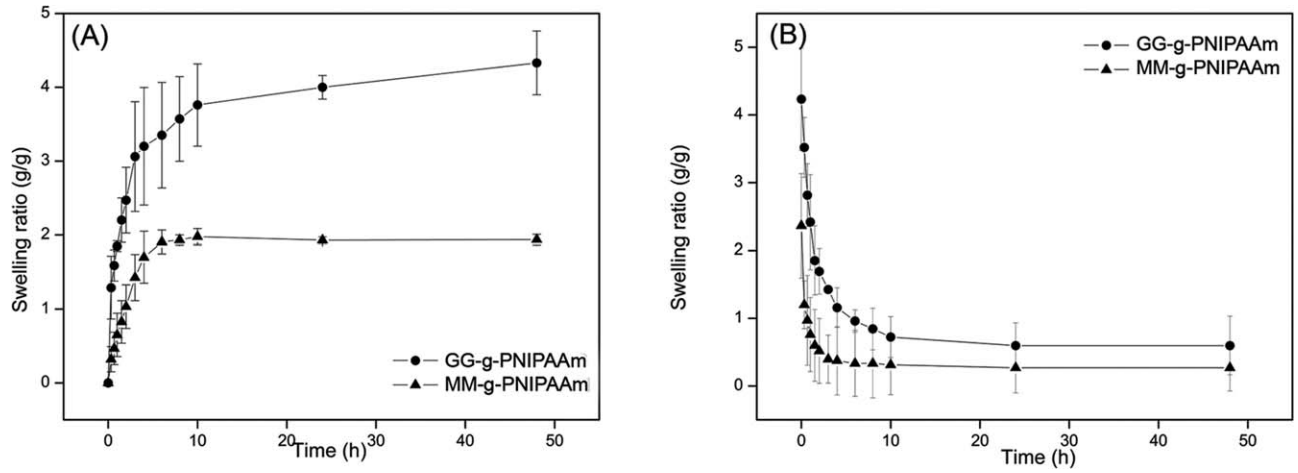



**Figure 7.** (A) Swelling kinetics of the GG-*g*-PNIPAAm and MM-*g*-PNIPAAm hydrogels in 0.1*M* $CaCl_2$ solutions at pH 5.4 and 25°C. (B) Deswelling kinetics of the GG-*g*-PNIPAAm and MM-*g*-PNIPAAm hydrogels in distilled water at 25°C.

As shown in Figure 7(B), the deswelling of the GG-*g*-PNIPAAm hydrogel in distilled water was slower than the deswelling of the MM-*g*-PNIPAAM hydrogel. The lower response rate of the GG-*g*-PNIPAAm hydrogel might have been due to its porous structure, which retarded the migration of water molecules out of the hydrogel.

The swelling behavior of both hydrogels as function of the pH is shown in Figure 8(A). At pH values lower than 3, a low swelling ratio was present because most of the carboxylic groups were protonated. At pH 4, the carboxylic groups became deprotonated; this increased the hydrophilicity of the network with a consequent increase in the swelling ratio. Also, the electrostatic repulsion between the polymer chains expanded the crosslinked network, and the exchange between the $Ca^{+2}$ and $Na^{+}$ ions decreased the swelling ratio by disruption of the network. Figure 8(B) presents the swelling ratio of both hydrogels as function of the temperature. The swelling ratio for both hydrogels, decreased with increasing temperature. A sharp decrease in the swelling ratio was found at higher temperature (ca. 35°C) compared to the critical solution temperature of PNIPAAm (32.1°C). These results were contrary to those determined for the graft copolymers where the grafting decreased the phase-transition temperature and indicated that the hydrogel formation increased the hydrophilic properties of the copolymer and increased the phase-transition temperature.

## CONCLUSIONS

The MM and GG block fractions of alginic acid gave, in a similar way, graft copolymers by amidation with the terminal amino group of the crosslinked PNIPAAm. However, AFM studies indicated that the surface morphology of the graft copolymers was quite different. The graft copolymer of the GG block fraction gave a more resistant hydrogel in the presence of $Ca^{2+}$ compared to the MM block fraction, which enabled it to form an egg-box structure. Swelling, thermal, and pH characterizations demonstrated the superior properties of the GG-*g*-PNIPAAm hydrogel over the MM-*g*-PNIPAAm hydrogel. The grafting of PNIPAAm onto the MM and GG blocks slowly reduced the phase-transition temperature, but a contrary effect was found for the hydrogels. We concluded that both hydrogels

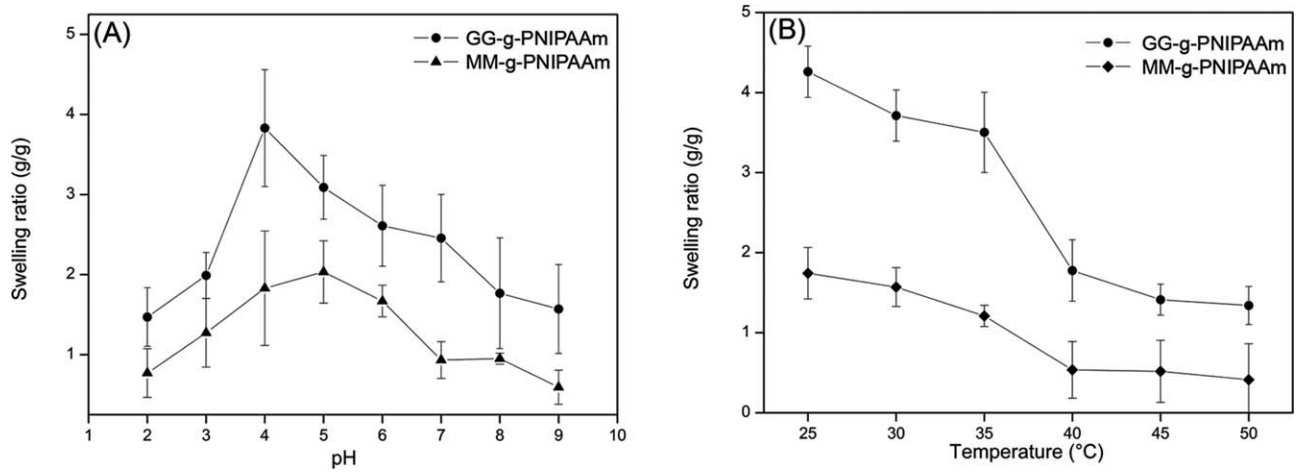



**Figure 8.** (A) Swelling ratio as a function of the pH for the GG-*g*-PNIPAAm and MM-*g*-PNIPAAm hydrogels in a 0.1*M* $CaCl_2$ solution. (B) Swelling ratio as a function of the temperature for the GG-*g*-PNIPAAm and MM-*g*-PNIPAAm hydrogels in a 0.1*M* $CaCl_2$ solution at pH 5.4.

constituted very interesting materials with potential applications in drug-delivery systems because of their pH and temperature-responsive properties.

## ACKNOWLEDGMENTS

Financial support from the Dirección de Investigación, Desarrollo e Innovación (DICYT) of the Universidad de Santiago de Chile and FONDECYT (contract grant numbers 1110536 and 1090627) is gratefully acknowledged.

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