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Thermoresponsive hydrogels based on alginate-*g*-poly (N-isopropylacrylamide) copolymers obtained by low doses of gamma radiation

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ABSTRACT

Low doses of gamma radiation from ^{60}Co have been used in the synthesis of water soluble alginate-*g*-poly(N-isopropylacrylamide) (PNIPAAm) copolymers. Irradiation was carried out using glass vials containing aqueous solutions of alginate and NIPAAm monomers. The resulting copolymers have been characterized by different characterization techniques such as Fourier transform infrared spectra, ^{1}H Nuclear Magnetic Resonance, elemental analysis and thermogravimetric analysis. NIPAAm concentration in the initial reaction mixture and irradiation dose evidenced a direct effect on the grafting percentage; an increasing in NIPAAm concentration in the initial reaction mixture always led to a higher content of grafted PNIPAAm in the copolymers. On the other hand, for a fixed NIPAAm concentration in the reaction mixture, the amount of PNIPAAm in the copolymer increased with the dose. Copolymers were used in hydrogels formation by cross-linking alginate carboxylic groups with $Ca^{2+}$and swelling properties of the new materials were studied at 24 °C and 37 °C. Hydrogels presented thermo-sensitivity, showing lower swelling ratios at 37 °C. Hydrogel water uptake was hindered as a consequence of the collapsed state of PNIPAAm moieties in the copolymers.

1. Introduction

Processes that involve electromagnetic radiation are an important tool for polymer modification; thus, they have become a huge area of interest in the last decades due to their industrial applicability. Nowadays, gamma radiation is being considered as a green approach to fabricate new materials by means of producing different chemical reactions in polymers, specially chain crosslinking and graft copolymerization, without chemical reagents, at low temperature, under mild conditions [1].

As a consequence of the emergence of novel biomedical technologies such as, tissue engineering, regenerative medicine, gene therapy, controlled drug delivery and bionanotechnology, all of which require biodegradable platform materials, the use of biopolymers has increased in the last decades. In this field, polysaccharides present greater advantages against synthetic materials, such as biocompatibility, biodegradability, and non-toxicity. Additionally, the fact that are obtained from renewable sources offers an alternative to maintaining sustainable development of an economically and ecologically attractive technology [2,3].

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It is known that polysaccharides and their derivatives undergo chain scission reactions when exposed to gamma radiation [4]. However, it has also been demonstrated that under controlled conditions some polysaccharides can be crosslinked or furthermore they can been copolymerized with other monomers in order to obtain copolymers or hydrogels [5–7]. Moreover, radiation modification of natural polymers also solves the problem of product sterilization, offering the possibility of fabricate a pure product non contaminated with residual toxic initiators, leading to biodegradable and biocompatible materials [8–10].

Hydrogels are characterized by their extraordinary ability of swelling in water or physiological fluids; presenting an important similarity with body tissues due to the high water content [11]. On the other hand, some hydrogels may also show a swelling behaviour depending on the external environment, called stimuli-responsive hydrogels or stimuli-sensitive systems [12,13]. Thermosensitive hydrogels that can respond to temperature variations have been developed and widely explored in recent years because of the importance of this property in the field of biomedicine regarding that human body is thermo-regulated [14].

When polysaccharides are grafted with a thermosensitive polymer, the polysaccharide backbone can supply biodegradability, non-toxicity and good mechanical properties to the matrix while the synthetic polymer provides the thermal sensitivity. Thus, mechanically stable hydrogels presenting thermo-response can be obtained using alginate-*g*-PNIPAAm copolymers [15–17].

Alginates are the salts of alginic acid, a lineal polysaccharide obtained from brown algae constituted by two uronic acids as repetitive units, 1,4 β-D-mannuronic acid (M) and 1,4 α-L-guluronic acid (G), in the form of homopolymeric (MM- or GG-blocks) and heteropolymeric sequences (MG- or GM-blocks) [18]. Due to the 1,4 α-linkages, G-block segments are able to stablish electrostatic interactions between carboxylate groups and bivalent cations such as Ca^{2+} leading to the formation of mechanically stable networks [19]. Alginate has been regarded as an excellent polysaccharide for gel systems because of its unique features such as biocompatibility, biodegradability, immunogenicity, and non-toxicity. Even more, they have interesting properties such as emulsifier, thickener, stabilizer, gelling and film forming, resulting in several applications for the food and pharmaceutical industries [16,20,21].

Poly(N-isopropylacrylamide), PNIPAAm, based hydrogels have, in aqueous media, a volume phase transition when heated, due to the lower critical solution temperature (LCST) of the PNIPAAm. At low temperatures, intermolecular hydrogen bonds between water and polar groups of PNIPAAm solubilise the polymer. Above LCST hydrogen bonds break and hydrophobic associations between polymer chains take place resulting in a collapsed state. The value of LCST for high molar mass PNIPAAm is around 32 °C but this critical temperature transition is a function of molar mass and polymer concentration and it can also be changed by the incorporation of comonomers. In this way, the LCST can be tuned to a desire temperature range by copolymerization with a more hydrophobic comonomer which displace the LCST to lower values, or with a more hydrophilic comonomer producing the opposite effect [22–25].

The synthesis of alginate-*g*-PNIPAAm copolymers has been previously studied by chemical reactions [13,26–31], and also an approach in the use of electromagnetic radiation for hydrogel formation was done [7,32,33]. The novelty of this work is the use of low doses of gamma radiation from a ^{60}Co source in the synthesis of thermosensitive water soluble copolymers of alginate with different concentrations of grafted PNIPAAm. Polymer characterization was made using several techniques such as elemental analysis, Fourier transform infrared (FTIR), thermogravimetric analysis (TGA) and proton nuclear magnetic resonance (^1H NMR). Also, alginate-*g*-PNIPAAm copolymers were used in hydrogels formation by cross-linking carboxylic groups of alginate with Ca^{2+} ions and the swelling properties of the new materials were studied as a function of temperature.

2. Experimental section

2.1. Materials

N-isopropylacrylamide (NIPAAm) monomer provided by ALDRICH was recrystallized in hot hexane. Sodium alginate was obtained from Fluka (Switzerland, N°. 71238), Mw = 231,500 g/mol) and a mannuronic/guluronic ratio (M/G) estimated in 0.79 by ^1H NMR according to the literature [18,34].

2.2. Synthesis of graft copolymers

2.2.1. Samples preparation

A 5 wt% aqueous solution of NIPAAm was prepared, fractionated and later added to 1 g of alginate; water was used to reach 50 mL as the final volume. Different volumes of NIPAAm solution were used, in order to have alginate/NIPAAm proportions of 20/80; 33/67 and 50/50 (moles of repetitive units).

Composition of the reaction mixtures prepared and doses applied in copolymers synthesis are resumed in the nomenclature used. Samples are named with the capital letters COPI – which means that are copolymers produced by irradiation, then followed by a number indicating the dose used for the synthesis, 03 for 0.3 kGy, 05 for 0.5 kGy and 1 for 1 kGy. The nomenclature ends with two numbers (50, 67, or 80) followed by the letter N, associated to the molar percentage of NIPAAm in the aqueous solutions prepared for irradiation; for instance, 80N implies 80% of NIPAAm in the total amount of moles of repetitive units.

2.2.2. Samples irradiation

Irradiation of polysaccharides in presence of oxygen can lead to oxidative degradation reactions [8]. Thus, in order to avoid alginate degradation, oxygen was removed by vacuum from aqueous solutions and they were sealed into glass vials before irradiation. Samples irradiation was done, using ^{60}Co γ-ray, at the Atomic Centre of Ezeiza (CNEA, Argentina). The dose rate was selected at 1 kGy/h

and doses of 0.3 kGy, 0.5 kGy, and 1 kGy were studied. Doses were calibrated using Perspex dosimeters.

After irradiation, samples were Soxhlet extracted with methanol in order to remove the unreacted NIPAAm monomer and the free PNIPAAm homopolymer. This step ensured that remaining PNIPAAm in the material obtained corresponds to graft molecules into alginate backbone. Finally, samples were dried until constant weight.

2.3. Characterization

2.3.1. ^1H NMR

^1H NMR characterization of synthesized copolymers was performed in deuterated water, using a BRUKER AVANCE DPX 400 MHz spectrometer. Measurements were performed at room temperature.

2.3.2. FTIR

Infrared transmission spectra (FTIR) were recorded in a Nicolet 520 spectrophotometer by accumulation of 30 scans, with a resolution of 4 cm^{-1}. Some samples were studied as films obtained from aqueous solution by solvent casting and others were recorded in solid state using KBr pellet (1 wt%).

2.3.3. Thermogravimetric analysis

Thermogravimetric analysis (TGA), using a Discovery TGA™ equipment, was carried out under nitrogen atmosphere, with a flow rate of 40 mL/min. The heating rate used was 10 °C/min, in the range from 30 to 800 °C. Each sample were analysed by triplicate and the reported composition corresponds to the average of the three obtained values with their associated standard deviation.

2.3.4. Elemental analysis

The CHN elemental analysis was performed with a Carlo Erba C–H–N–S EA1108 instrument. C, H, and N content were determined and N content was used to find NIPAAm composition in the copolymers. This characterization was carried out by triplicate for each sample, the resumed NIPAAm content is the average of the three estimated compositions with their corresponding standard deviation.

2.3.5. Critical transition temperature

2 wt% solutions of the synthesized copolymers were prepared using distilled water without salt addition. pH measurements indicated that all samples presented values in the range of 5.2–5.5. Solutions were poured in colourless glass vials and they were immersed in a thermo-controlled water bath. Water temperature was initially fixed at 26 ± 0.4 °C and it was step increased until 37 °C. One degree step was used waiting at least half an hour for thermal stabilization. After that, pictures of the solutions were taken at each temperature.

2.4. Hydrogel formation and swelling experiments

Synthesized copolymers were used to obtain hydrogels by cross-linking carboxylic groups of alginate with Ca^{2+}. Cross-linked beads were obtained by external gelation method, where an 8 wt% aqueous solution of the copolymer was dropped into a calcium solution (2% $CaCl_2$). Beads were washed in distilled water and left to dry until constant weight. Hydrogels obtained were named prefixing H – to the nomenclature previously used for the copolymers.

Swelling of hydrogels can be characterized by measuring their equilibrium degree of swelling [7,35]. Cross-linked beads were immersed in a 0.9% aqueous solution of NaCl at 24 °C ± 1 °C, below LCST of PNIPAAm, and at 37 °C, above this transition temperature. Experiments were done in a temperature controlled water bath. Weight of swollen hydrogels was measured for each sample, at different times, after surface water was wiped off carefully with an absorbent paper. All experiments were performed in triplicate, and the average of the three measurements is reported. The equilibrium swelling ratio SR was calculated according to Eq. (1).

$$SR = \frac{W_s - W_d}{W_d} \quad (1)$$

where W_s is the weight of gels in the swollen state, and W_d is the weight of gels in the dry state.

3. Results and discussion

Different radiation doses were applied to aqueous solutions of different contents on NIPAAm and alginate. Copolymerization reaction is based on the fact that radiation promotes free radicals on the alginate backbone and then grafting of NIPAAm onto the alginate reactive sites and the consecutive PNIPAAm homopolymerization at these sites takes place [36]. Other undesired radioinduced reactions could be produced, such as unattached PNIPAAm chains [37], depolymerisation of alginate backbone [4,38] and crosslinking of pendant PNIPAAm chains [39]. Taking into account that reaction mixture was purified by removal of unreacted NIPAAm as well as unattached PNIPAAm, only the radioinduced depolymerisation of alginate backbone and crosslinking of PNIPAAm pendant chains will affect the final product.

3.1. Characterization of the graft copolymers

3.1.1. FTIR

Fig. 1 shows FTIR spectra of alginate, PNIPAAm homopolymer, and one of the synthesized graft copolymer, COPI-1-50N. Similar spectra were obtained for all the graft copolymers. In order to show the common absorption bands between homopolymers and the copolymer, spectra in Fig. 1 were shifted in the transmitance axis.

Alginate characteristics bands have been widely described in the literature; the principal signals have been lighted out in the spectrum of Fig. 1 [37,40,41]. At 3400 cm^{-1} a broad band corresponding to stretch vibration of hydroxyl groups (ν_{O-H}) is observed. The vibration of Csp^3–H bond appears at 2928 cm^{-1}. Two strong peaks at 1613 and 1413 cm^{-1} are attributed to the asymmetric and symmetric stretching of carboxyl groups (ν^{as}_{COO-}; ν^{s}_{COO-}), respectively. The signals observed in the range of

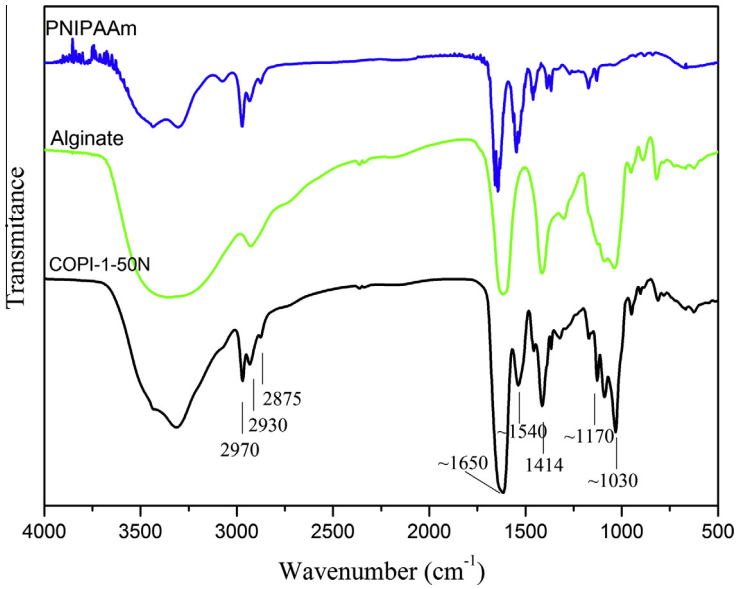

Fig. 1. FTIR spectra of PNIPAAm (blue, up), alginate (green, middle) and the graft copolymer COPI-1-50N (black, down). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

~1170–1030 cm^{-1} correspond to symmetric and asymmetric vibration bands of C—O—C bonds (ν^{s}_{C-O-C}; ν^{as}_{C-O-C}) typical of polysaccharide rings. At 1320 cm^{-1} a weak band appears representing the vibration of C—O bond (ν_{C-O}). PNIPAAm spectrum displays absorption bands at 2973 cm^{-1} and 2876 cm^{-1} corresponding to symmetric and asymmetric vibration bands of methyl groups (ν^{s}_{CH3}; ν^{as}_{CH3}), respectively. As in the spectrum of alginate a band due to Csp^3—H bond vibration appears at 2925 cm^{-1}. Typical amide I band consisting of C=O stretching ($\nu_{C=O}$) at1650 cm^{-1} and amide II band representing the N—H vibration bond (δ_{N-H}) at 1547 cm^{-1}, are clearly shown. Asymmetric and symmetric vibration bands of geminal methyl groups are observed as a weak signal at 1457 cm^{-1} (δ^{as}_{CH3}) and as a double peak band at 1388 and 1368 cm^{-1} (δ^{s}_{CH3}) [32,35].

COPI-1-50N spectra presents some common characteristic absorption bands, such as the symmetric and asymmetric vibrations of methyl groups (ν^{s}_{CH3}; ν^{as}_{CH3}) of PNIPAAm at 2970 cm^{-1} and 2875 cm^{-1}, and the vibration of C—H bonds (ν_{C-H}) at 2933 cm^{-1}observed in both PNIPAAm and alginate molecules. Signal at 1651 cm^{-1} corresponds to the vibrations of C=O bonds ($\nu_{C=O}$) from carbonyl groups in alginate and PNIPAAm; while at 1537 cm^{-1} appears the signal of amide II (δ_{N-H}) due to the presence of PNIPAAm in the copolymer. Furthermore, signals of symmetric and asymmetric vibrations of the C—O—C bonds (ν^{s}_{C-O-C}; ν^{as}_{C-O-C}) typical from alginate rings are shown in the range of wavelength between ~1030 and ~1170 cm^{-1}, also an asymmetric stretching is detected at 1413 cm^{-1}, associated with carboxylic groups (ν^{s}_{COO-}) of alginate.

FTIR spectra of all graft copolymers (figures not shown) presented the same characteristics bands previously described for COPI-1-50N. Although it was not possible to associate a specific band to the bond between alginate and PNIPAAm pendant chains, the presence of common absorption bands of both homopolymers corroborate the grafting. This conclusion is based on the fact that an extensive washing of unattached PNIPAAm and non-reacted monomer was performed after irradiation by soxhlet extraction, thus, PNIPAAm bands detected in the final material must correspond to PNIPAAm grafts.

3.1.2. NMR

^{1}H NMR spectrum of each graft copolymer showed the presence of the signals at 1.14 ppm (—CH_3), 1.57 ppm (—CH—) and 2.2 ppm (—CH_2—) attributable to the PNIPAAm chains and the signal at 5.01 ppm due to the anomeric hydrogen of the guluronic units of alginate. The rest of hydrogen signals of the polysaccharide and the isopropyl group of NIPAAm (—CH—) were overlapped in the range of 3.9–4.2 ppm. The composition of the copolymers were obtained using the areas corresponding to PNIPAAm in the range of 1.14–2.2 ppm (9H) and the area of alginate at 5.01 ppm (1H).

Copolymer compositions obtained by NMR, expressed as molar % of NIPAAm grafted to alginate, are shown in Table 1.

3.1.3. TGA

Thermo-gravimetric analysis (TGA) was used to determine the thermal stability of the copolymers as well as the composition, based on the degradation of pure alginate and PNIPAAm homopolymers. Copolymers composition was estimated from the weight loss associated to each component. The first derivative of TGA curves was used in order to obtain the mass associated to weight loss of both components. In the plots two marked peaks were observed, the area value under the curve of the first peak

Table 1
Graft copolymer composition obtained by ^{1}H NMR, elemental analysis, and TGA.

	% molar NIPAAm		
	^{1}H NMR	Elemental analysis	TGA
COPI-03-80N	38.0	39.2 ± 1.4	48.2 ± 0.3
COPI-03-67N	34.7	33.1 ± 1.3	31.7 ± 0.7
COPI-03-50N	22.3	13.1 ± 0.6	20.4 ± 0.2
COPI-05-80N	59.0	58.0 ± 2.5	69.7 ± 0.5
COPI-05-67N	58.1	49.6 ± 2.0	55.3 ± 0.4
COPI-05-50N	54.4	49.1 ± 1.9	54.8 ± 0.4
COPI-1-80N	80.5	79.2 ± 1.7	80.6 ± 0.6
COPI-1-67N	46.0	46.7 ± 1.2	57.7 ± 1.0
COPI-1-50N	40.3	39.7 ± 1.1	47.7 ± 0.7

(m_1) in addition with the corresponding residual mass at ~600 °C (m_r) were attributed to alginate mass content (m_{alg}) into the copolymer; in the same way, the area of the second peak (m_2) was considered as the loss of mass associated to PNIPAAm content. From these estimations, the total mass of the graft-copolymer represented the summation of both values ($m_{alg} + m_2$), thus, NIPAAm molar % composition was determined using Eq. (2).

$$\text{NIPAAm } \% = \frac{\frac{m_2}{113{,}16}}{\left(\frac{m_{alg}}{198} + \frac{m_2}{113.16}\right)} 100 \quad (2)$$

Fig. 2a shows mass percentage for alginate and PNIPAAm as a function of temperature. Both homopolymers presented an initial weight loss until 150 °C, corresponding to the dehydration of the samples. Alginate thermogram shows an important mass decrease, approximately 45%, in the temperature range of 200–300 °C (ca. 249 °C), as a first step of thermal degradation. Then, a second weight loss at 550 °C (~15%) is observed, obtaining a residue of about 20% of the initial mass. In the case of PNIPAAm, only one step of degradation at 408 °C is observed, which involves almost the total mass, remaining less than 2.5% of the initial mass as residue.

Accordingly to previous discussion, thermograms obtained for all copolymers showed three major weight loss zones. The first zone represents the dehydration that begins immediately after the temperature is increased and finishes at around 150 °C. The percentage weight loss in this zone depends on the moisture content of the samples. The second weight loss zone corresponds to the thermal degradation between 200 and 300 °C due to the alginate content in the material; finally, the last degradation zone, in the temperature range of 300 and 400 °C, can be associated to the PNIPAAm contribution to the total mass of the copolymers. Table 2 summarize the estimated composition according the weight loss calculated in each degradation step.

Thermograms of graft-copolymers obtained with 0.3 kGy dose and different initial composition in the mixture of reaction are shown in Fig. 2b, similar curves were obtained for the rest of the synthesized graft-copolymers (doses of 0.5 and 1 kGy). From the plot it can be noticed that for higher PNIPAAm contents, more pronounced the weight loss for the second degradation step.

Results reveal that regardless of radiation dose, increasing NIPAAm content in the initial mixture reaction, the amount of PNIPAAm grafted to alginate increased (the weight loss at the second degradation step increases). On the other hand, an increase in the radiation dose also increase the amount of PNIPAAm grafted to alginate as expected due to a higher amount of free radicals produced.

3.1.4. Elemental analysis

The composition of graft copolymers, pure alginate, and PNIPAAm, were determined by the proportion of C, H, and N obtained by elemental analysis. Calculations were based on the fact that nitrogen is present only in PNIPAAm molecules.

Compositions of the copolymers are presented in Table 1, compared with values obtained by other techniques. Copolymer compositions estimated by different techniques showed a good agreement.

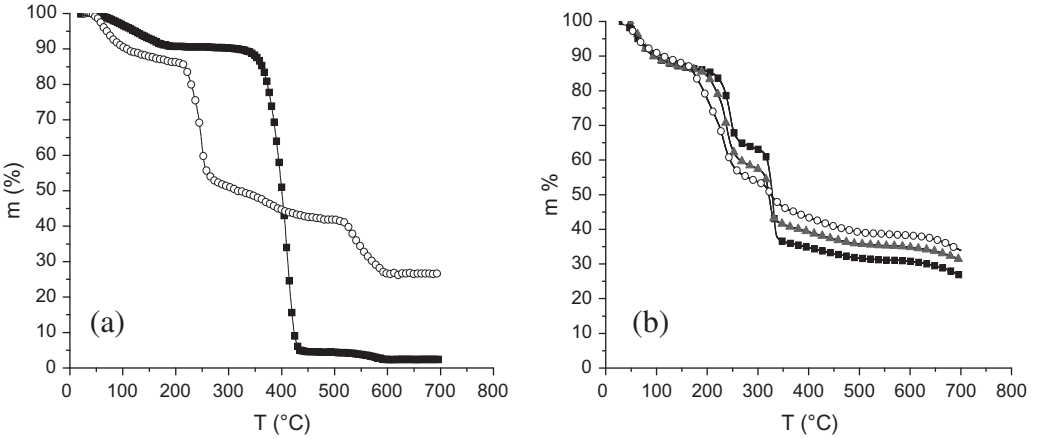

Fig. 2. Weight loss (%) as a function of temperature for: (a) (○) alginate and (■) PNIPAAm and (b) copolymers obtained by ^{60}Co γ irradiation at a dose of 0.3 kGy: (○) COPI-03-50N, (▲) COPI-03-67N, and (■) COPI-03-80N.

Table 2
Critical transition temperatures measured by turbidity, expressed in °C.

Copolymers	0.3 kGy	0.5 kGy	1 kGy
COPI-X-80N	31.5	32.0	32.5
COPI-X-67N	31.5	32.0	32.0
COPI-X-50N	32.0	32.0	32.0

3.1.5. Optical determination of the LCST

The behaviour of alginate-*g*-PNIPAAm copolymers in aqueous solutions was studied as a function of temperature. Synthesized copolymers are expected to present thermo-responsive behaviour, since intermolecular and intramolecular associations are favoured on warming. This particular characteristic is due to the presence of the PNIPAAm [17]. From a macroscopic point of view, below the LCST polymer chains dissolve or swell in water and above the critical temperature a collapsed state is achieved producing phase separation or a contraction of the system [23,42]. The change in the copolymer structure can be easily detected because cloudiness appeared in the solution, so that, the LCST can be estimated by a simple visual inspection. Digital photographs of 2 wt% salt free aqueous solutions (pH $\sim$ 5.4) of all the copolymers studied were taken at different temperatures and the critical transition temperature was estimated.

All the copolymers formed homogeneous translucent solutions at the initial temperature (26 °C), below the LCST. As a result of the increasing temperature samples went through a critical transition temperature due to the PNIPAAm content. Transparent solutions became opalescent, milkness and some of them achieved a white opaque state. Above LCST there is a reorganization of the macromolecules leading to aggregation among them. Thus, the appearance of the samples stop being transparent and light dispersion, due to molecules aggregation, produces milky and whitish solutions.

Estimated values of LCST for each copolymer are presented in Table 2. Critical transition temperature was taken as the temperature where the solution started to become turbid and whitish. All the materials showed a critical temperature in the range of 31.5–33 °C, being practically independent of copolymer composition. It is well known that PNIPAAm shows a LCST in the temperature range of 30–35 °C, being the value a function of the molar mass and concentration [43,44].

3.2. Swelling

After hydrogels formation, swelling depends on physical entanglements between polymeric molecules, chemical crosslinks, produced by gamma radiation, and Ca^{2+} crosslinking of alginate carboxylic groups. Cross-linkings induced by gamma radiation are permanent and the amount increase with an increase in the radiation dose. Ca^{2+} crosslinks of alginate are not permanent; the amount of this kind of crosslinks will depend on swelling media, due to ionic exchange.

Curves obtained from swelling experiments, reported as percentage of swelling ratio % SR (g/g) as function of time showed three steps. Initially, there is a linear increment of the swelling ratio (SR). As it was observed by Bajpai & Sharma [45] for calcium alginate beads, from the total calcium ions that are bound to carboxyl groups, part of it will be bound to polymannuronic residues. Therefore, when the beads are immerse in an aqueous solution containing Na^+ ions, the polymer will undergo an ion-exchange process losing Ca^{2+} ions which are bonded to COO^- groups mainly in the polymannuronate. As a result, the electrostatic repulsion among negatively charged COO^- groups increases which ultimately causes network relaxations enhancing the swelling of the gel. Later on, starts the ion-exchange between Na^+ and Ca^{2+} ions present in the polyguluronate blocks, causing a greater water uptake achieving, in some point, the maximum value of SR. Then, the second step involves the stage where water enters and diffuses into the structure increasing the swelling and a maximum value of SR is achieved. The final step results from a further physically degradation of the structure due to the elimination of Ca^{2+} crosslinking points. At this stage, measurements of SR become difficult as a result of the weakened network and at the end copolymers are again soluble. In some cases, before the degradation of the hydrogels the maximum SR was maintained at least for one hour, due to the competition between swelling and degradation. Also, even when Ca^{2+} ions are replaced by Na^+ the degradation rate of the structure is related to the rate of diffusion of polymer chains out of the bead.

Previous description of the swelling behaviour of all copolymers was based on the ion exchange produced in the beads. However, SR values of hydrogels also depends on other factors such as: the amount of alginate in the copolymer, the temperature of the swelling experiments related to the LCST of PNIPAAm and the number of physical and chemical crosslinks that affect the structure of the formed network.

Chemical composition of obtained copolymers was a result of the initial composition of the reaction mixture and the radiation dose. Swelling of hydrogels, based on copolymers synthesized with 0.5 kGy, in aqueous solutions of NaCl 0.9% (physiological concentration), at two different temperatures are shown in Fig. 3a–b. Plots do not shown the final step associated to the degradation of the gel structure and polymer dissolution since more time would be needed to promote gel destruction by ion exchange.

For 0.5 kGy, increasing the NIPAAm content in the original mixture of reaction from 50% to 80%, grafted copolymers have slightly higher PNIPAAm content, as can be seen in Table 1. However, from swelling curves of Fig. 3, notable differences were observed which can be attributed to different structures. Hydrogel H-COPI-05-80N presented a more compact network, less flexible that leads to lower SR values, which may be the result of a higher amount of entanglements and radiation crosslinks of PNIPAAm side chains. Swelling curves did not present the final step due to network degradation highlighting the stability of the formed structures. Above LCST, the collapsed state of PNIPAAm pendant chains provides the structure with hydrophobicity, reducing the water uptake; thus, swelling ratios are lower at 37 °C than at 24 °C. H-COPI-05-50N presented the maximum SR at 24 °C and 37 °C compared to

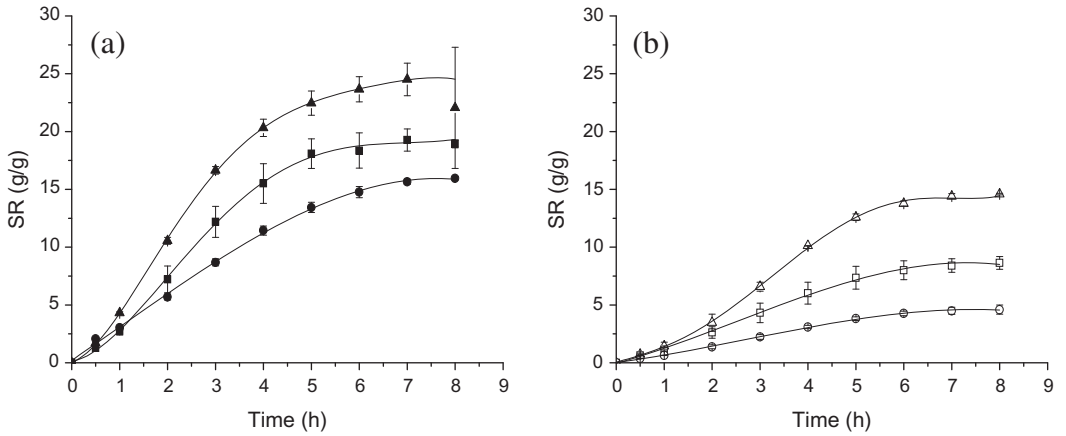

Fig. 3. Swelling ratio as a function of time for hydrogels formed from copolymers synthesized with a 0.5 kGy dose. Swelling was measured in 0.9% NaCl aqueous solution at 24 °C (a) and 37 °C (b). Symbols: (●, ○) H-COPI-05-8 N, (■, □) H-COPI-05-67N, (▲, △) H-COPI-05-50N.

other copolymers at the same temperature. At 37 °C the swelling behaviour is mainly dominated by PNIPAAm content, showing the beads of H-COPI-05-80N a negligible swelling.

Also at 37 °C, hydrogels beads remain structurally unaffected during longer periods of time than at low temperature, probably because the exposed hydrophobic methyl groups of PNIPAAm limited the entrance of water and also the Ca^{2+}—Na^{+} ion exchange. This behaviour was similar for all hydrogels studied and it is in good agreement with results reported in the literature for other hydrogels based on NIPAAm copolymers [13,46].

Similar results were obtained for hydrogels prepared with copolymers synthesized with doses of 0.3 and 1 kGy. All hydrogels presented thermo-sensitivity in swelling experiments, with values of SR at 37 °C lower than at room temperature. Fig. 4 shows swelling ratios for COPI-XX-80N hydrogels. Results of swelling measurements at 37 °C reveal that PNIPAAm content is the main factor of water up-take in the formed hydrogels. On the other hand, at 24 °C the swelling behaviour of hydrogels based on radiation grafted copolymers of a natural polysaccharide, is the consequence of several factors such as, polysaccharide content, its possible chain scission, the molar % of NIPAAm grafted to alginate, the degree of permanent radioinduced crosslinking and also the number of entanglements due to the PNIPAAm side chains.

Below LCST of PNIPAAm chains, swelling is a function of the initial concentration of NIPAAm in the mixture of reaction as well as the dose employed. Even for hydrogels obtained from copolymers with approximately the same NIPAAm content, as H-COPI-05-XX (~55% NIPAAm), the swelling behaviour is different. The higher the dose applied in the copolymer synthesis the lower the swelling ratio achieved.

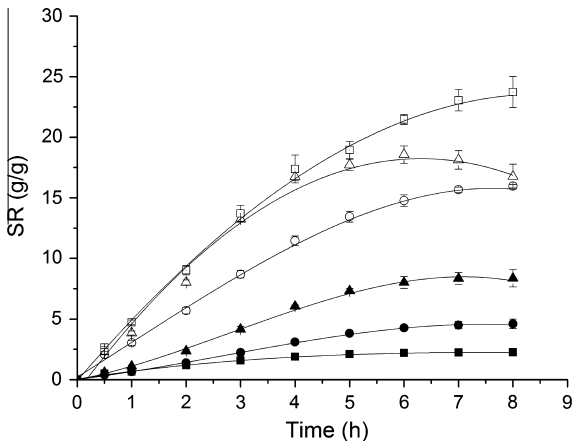

Fig. 4. Swelling ratio as a function of time for hydrogels formed from copolymers synthesized with 80 molar % NIPAAm and different doses radiation. Swelling was measured in 0.9% NaCl aqueous solution at 37 °C (filled symbols) and 24 °C (empty symbols). Symbols: (■, □) H-COPI-1-80N, (●, ○) H-COPI-05-80N, and (▲, △) H-COPI-03-80N.

4. Conclusions

Alginate-*g*-PNIPAAm water soluble copolymers were successfully synthesized using ^{60}Co γ-rays. FTIR, ^{1}H NMR, TGA and elemental analysis confirmed the occurrence of grafting. NIPAAm concentration in the initial reaction mixture and irradiation dose had a direct effect on the grafting percentage; an increasing in NIPAAm concentration in the initial reaction mixture always gives a higher content of grafted PNIPAAm in the copolymers. On the other hand, for a fixed value of NIPAAm in the reaction mixture, the amount of PNIPAAm in the copolymer increases with an increase in dose used.

Hydrogels were obtained by external gelation using calcium ions to crosslink alginate based copolymers. As expected, the thermoresponsive behaviour of hydrogels in swelling experiences at 24 and 37 °C, were a function of the amount of PNIPAAm grafted on to the alginate backbone, changing the polymer structure from extended to collapsed, respectively. Swelling behaviour of hydrogels in normal saline solution was a function of several factors, especially at 24 °C. At this temperature, below the LCST of

PNIPAAm, hydrogels presented a swelling behaviour based on the ion exchange Ca^{2+}—Na^{+}. This process damages the gel structure reducing the number of crosslinking points at the same time that enhance the water uptake. At 37 °C, PNIPAAm moieties in the copolymers are in the collapsed state exhibiting the hydrophobic groups. For this reason, swelling ratios in normal saline solution are lower than those obtained at low temperature and almost negligible in those cases where NIPAAm content in the copolymers was higher than 70 mol%. At this temperature, swelling ratio is a function of both amount of PNIPAAm in the copolymer and dose applied for the synthesis. Higher doses can originate crosslinks among PNIPAAm side chains contributing to more compact and stable networks. However, an extensive study on hydrogels structure will be discussed in a further publication. The synthesis of reversible hydrogels from alginate-*g*-PNIPAAm copolymers obtained by low-doses of gamma radiation offers the possibility of preparing biomaterials with potential applications in biomedicine, as scaffold materials, for drug and growth factor delivery, engineering tissue replacements, among other applications.

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