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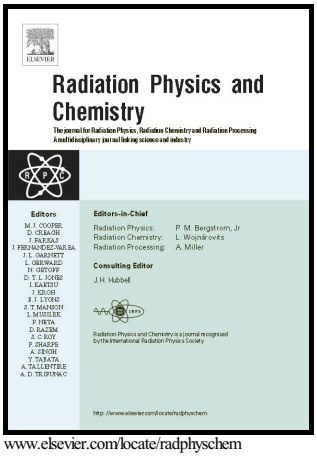

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Rheological analysis of thermo-responsive alginate/PNIPAAm graft copolymers synthesized by gamma radiation

M. M. Soledad Lencina [1*], Chiara Rizzo [2], Christian Demitri [2], Noemí Andreucetti [3] and Alfonso Maffezzoli [2]

[1] Instituto de Física del Sur (IFISUR), Department of Physics, Universidad Nacional del Sur (UNS). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Avenida Alem 1253, (8000) Bahía Blanca, Argentina.

[2] Department of Engineering for Innovation, University of Salento, Via per Monteroni, 73100 Lecce, Italy.

[3] Department of Chemistry, Universidad Nacional del Sur -CONICET, Avenida Alem 1253, (8000) Bahía Blanca, Argentina.

[*] Corresponding author. soledad.lencina@uns.edu.ar

Abstract

Focused on biomedical applications of thermo-responsive polymers, low-doses of gamma radiation from a ^{60}Co source were applied in a simple one-pot method to synthesize graft copolymers of alginate and poly(N-isopropylacrylamide) (PNIPAAm) with different compositions. The molar percentage of grafted NIPAAm (% molar NIPAAm) was determined by thermogravimetric analysis (TGA) and elemental analysis (EA), being the copolymer structure-property relationship studied in terms of thermo-associative and rheological behavior in aqueous solutions.

The addition of more NIPAAm monomer in the initial mixture of reaction, as well as, increasing absorbed dose lead to a greater grafting. However, increasing radiation dose produces copolymers with diminished viscoelastic properties caused by the alginate backbone scission.

From rheological curves, two transition temperatures, Ta and Tgel, were determined as a consequence of the thermo-responsiveness of PNIPAAm side chain. Storage (G') and loss (G'')

modulus curves undergo a slope inversion at Ta temperature, where both moduli begin to increase caused by an associative behavior of PNIPAAm domains. While, Tgel temperature is related to the onset of the gelation process at the G'/G'' crossover.

In order to design samples with liquid-gel transitions close to the human body, able to form gels *in situ* once inoculated, it was possible to tailor both transition temperatures selecting copolymers with an appropriate PNIPAAm content and optimizing the copolymer concentration in the aqueous solution. A good agreement between transition temperatures and viscoelastic properties was achieved for 5 wt% aqueous solutions of copolymers with low NIPAAm content synthesized at the lowest absorbed dose (0.5 kGy).

1. Introduction

Nowadays, gamma radiation is being considered as a green approach to fabricate new materials by means of different chemical reactions in polymers, specially chain crosslinking and graft copolymerization, without chemical reagents, at low temperature, under mild conditions (Flores-Rojas et al., 2018; Flores-Rojas and Bucio, 2016; Yang et al., 2010).

The use of biopolymers has increased in the last decades, as a consequence of the novel biomedical technologies that require biodegradable platform materials. In this sense, an important number of *in situ* forming scaffolds have been reported in the literature, based on injectable aqueous polymer solutions that can be transformed into a gel by the changes in the environmental conditions, such as ionic exchange, electrical stimuli, temperature and pH changes, designed for various biomedical applications, including drug delivery, cell encapsulation, and tissue repair (Nguyen et al., 2015; Yan et al., 2010; Yang et al., 2014).

Among others, thermo-responsive polymer solutions are particularly attractive as specific injectable biomaterials when their spontaneous gelation occurs near physiological temperature of human body (thermo-gelation), without the requirement of any chemical treatment (Kim et al., 2014; Ruel-Gariépy and Leroux, 2004). Injectable gel-forming matrices do not require a surgical procedure for placement and bioactive molecules or cells can be incorporated simply by mixing before injection. After a phase transition, a physically bonded network is formed within the desired tissue, organ, or body cavity, acting as temporary extracellular matrix or scaffold for drug delivery or cell-growth.

Natural biopolymers and semi-synthetic copolymers are exceptionally attractive for thermo-gelling applications due to their chemical flexibility and mechanical and biological properties combined with their availability as renewable resources (Conova et al., 2011; Gupta et al., 2006; Madigan et al., 2009). The strategy usually employed to design thermo-responsive materials is to prepare a copolymer based on a biopolymer backbone, grafted with a synthetic polymer that provides the thermal sensitivity. In this regard, the synthetic PNIPAAm, is frequently selected for its known properties in tissue engineering (R. Liu et al., 2009). The aqueous solutions of PNIPAAm exhibit a lower critical solution temperature (LCST), typically occurring at around 32-34 ºC. Below LCST, PNIPAAm shows a hydrophilic behavior. Above this transition temperature, the polymer becomes hydrophobic, resulting in a notable phase separation with the formation of a reversible physical gel. In this process, a physical aggregation of polymer chains leads to regions with a local order acting as network junction points. The resulting swollen network is termed a "thermo-reversible gel" when regions of local order are thermally reversible (Alemán et al., 2007). The drawback of PNIPAAm homopolymers is that they exhibit poor elastic properties and hold little water at physiological temperature, which is above LCST, due to their hydrophobicity (Schild, 1992).

As others biopolymers, alginates can provide biodegradability, biocompatibility and non-toxicity, also showing mucoadhesive properties. It is reported their use for the repair of certain injured areas

of body, such intervertebral disc, scaffolds should possess adhesive properties (Christiani et al., 2016; Wiltsey et al., 2015). Alginate is an anionic linear polysaccharide composed of 1,4´-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G). The main source for alginates is brown algae harvested in the wild, resulting in significant variation of alginate composition depending on the species (Lencina et al., 2013).

Graft copolymerization involving synthetic and natural polymers is an effective method to obtain new materials with hybrid characteristics. Recently, Liu et al. (2017) have synthesized thermo-responsive alginate-*g*-PNIPAAm copolymers with good efficiency in releasing anticancer drug in a sustained manner (Liu et al., 2017). The reported synthesis corresponds to a chemical "grafting on to" method that requires a previous preparation of an amino-terminated PNIPAAm. After that, grafting reaction lead to amide bonds formation between carboxyl group from alginate and the amino group from PNIPAAm-NH_2 (Cheaburu et al., 2013; Kim et al., 2002; Lencina et al., 2014). The major drawback of this type of chemical grafting synthesis is the demanding time and the need of several toxic reagents and solvents that may further require an extensive purification.

In this context, a copolymerization reaction based on gamma irradiation appears as an advantageous environmentally friendly, cleaner and green alternative technique using a simple one-pot method and mild reaction conditions, i.e. employing aqueous solutions at ambient temperature. This "grafting from" technique, involves the growth of PNIPAAm chains directly from the alginate backbone by means of free radicals radioinduced *in situ* along the polysaccharide. Even at low doses of gamma radiation from ^{60}Co, it is possible to obtain thermo-responsive water-soluble copolymers of alginate with different compositions of grafted PNIPAAm (Lencina et al., 2015). Since these copolymers are thermo-thickening at tunable temperature close to physiological conditions, they show promising applications as smart injectable in controlled drug delivery.

The aim of this work is to examine the structure-property relationship of these copolymers in terms of thermo-associative and rheological behavior. Dynamic mechanical analysis is a powerful tool to detect gelation in polymers, related to physical or chemical phenomena, either at low and at high frequency (Gupta et al., 2015; Karakasyan et al., 2008; Lee et al., 2003; Lencina et al., 2014; Lionetto et al., 2005a, 2005b; C. Liu et al., 2009; Martini et al., 2016).

Copolymers composition in terms of % molar NIPAAm was determined by TGA and EA. Thermo-responsive and viscoelastic properties of aqueous solutions of the synthesized copolymers were studied, using a rotational rheometer with Peltier control temperature.

2. Materials and Methods

2.1. Materials

NIPAAm monomer provided by ALDRICH was recrystallized in hot hexane. A low viscosity sodium alginate was provided by Alfa Aesar. The mannuronic/guluronic ratio (M/G) was estimated in 2.2 by ^{1}H-NMR according to the literature (Lencina et al., 2014; Salomonsen et al., 2009).

2.2. Sample preparation

A 5 wt% aqueous solution of NIPAAm was prepared 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; 50/50 and 70/30 (moles of repetitive units). Then, the copolymers were named as 80N, 50N and 30N in function of the initial NIPAAm monomer content in the reaction mixture -nominal NIPAAm composition- respectively.

2.3. Sample irradiation

In order to avoid alginate degradation, oxygen was removed by vacuum from aqueous solutions and they were sealed into glass vials before irradiation. Samples were irradiated with ^{60}Co gamma-rays, at the Atomic Centre of Ezeiza (CNEA, Argentina). The dose rate was selected at 1 kGy/h and doses of 0.5 kGy, 1.5 kGy and 3 kGy were applied. After irradiation, samples were

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

2.4. Copolymer characterization

2.4.1. TGA

TGA assays were carried out by using a TA Instrument Discovery Series equipment under nitrogen atmosphere, with a flow rate of 40 mL/min. The heating rate used was 10 ºC/min, in the range from 30 to 800 ºC. The values of % molar NIPAAm were calculated using first derivative curves.

2.4.2. EA

The CHN (Carbon, Hydrogen, Nitrogen) EA was performed with a Carlo Erba C–H–N–S EA1108 instrument. The C, H, and N content were determined, where N content was used to find % molar NIPAAm in the copolymers.

2.4.3. Rheological measurements

Rheological studies of the synthesized graft copolymers were performed in aqueous solutions. Copolymer concentration is expressed in weight percentage (wt%). Rheological properties of the polymers were studied using a Malvern Kineus Pro+ rheometer equipped with cone/plate geometry (diameter 49.97 mm, angle 0.98º, truncation 95 μm). The experiments were performed in the linear viscoelastic regime, which was established by a preliminary stress sweep test at frequencies between 0.1 and 1 Hz. The rheometer was equipped with a Peltier control system that allows accurate control of temperature (± 0.1 ºC). A solvent trap was used to minimize changes in concentrations due to water evaporation. Temperature ramps were performed at 1 Hz frequency, 1% strain and a heating rate of 1 ºC/min.

3. **Results and discussion**

3.1. Copolymer Characterization

Thermo-sensitive water soluble alginate-*g*-PNIPAAm copolymers were successfully synthesized by low doses and low dose rate gamma radiation from ^{60}Co source. Table 1 shows the copolymers composition in terms of % molar NIPAAm. The results were obtained by two experimental methods: EA and TGA, with an overall good agreement between them.

TGA curves corresponding to pure sodium alginate, PNIPAAm homopolymer and 30N, 50N and 80N copolymers synthesized at 0.5 kGy are shown in Figure 1. All samples have an initial weight loss until 150 ºC, corresponding to the dehydration of the samples. Alginate shows an important mass decrease in the temperature range of 200-300 ºC as a first step of thermal degradation, and also a second weight loss at 550 ºC leaving a residue of about 20 % of the initial mass. In the case of PNIPAAm only one step of mass degradation is observed at 408 ºC of almost all total mass, with a small residue less than 2.5 % of the initial mass. The first derivative of copolymer TGA curves (inset Figure 1) were used to calculate the mass associated with weight loss of alginate and PNIPAAm. Then, % molar NIPAAm values, were calculated from equation 1 (Lencina et al., 2015), where m_{alg} represents the mass loss corresponding to the first peak of the derivative curves plus the mass corresponding to the residue zone; whereas m_2 represents only the mass under the second peak of derivative curves attributable to PNIPAAm.

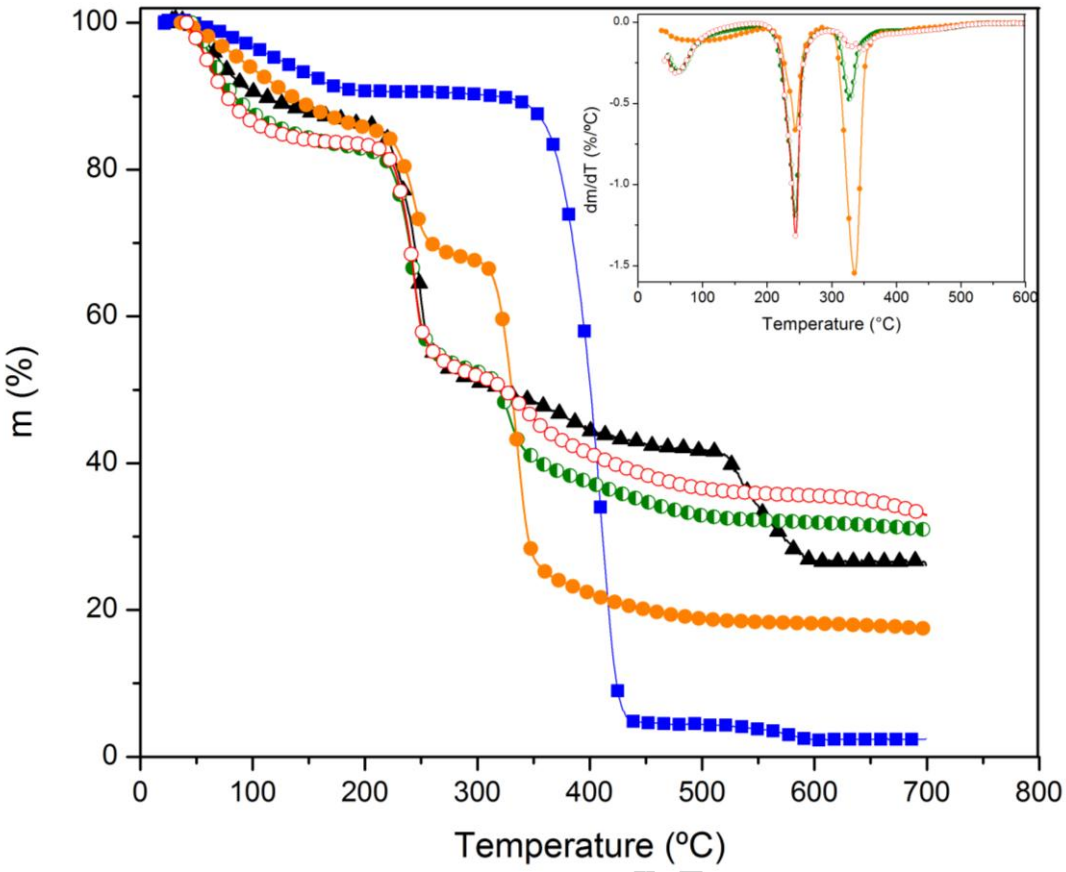

Figure 1. TGA curves of sodium alginate (▲), PNIPAAm (■) and 30N (○), 50N (ϖ) and 80N (●) copolymers synthesized at 0.5 kGy. Inset: only TGA derivative curves of the copolymers are shown.

$$\%\ molar\ NIPAAm = \frac{\frac{m_2}{113.16}}{(\frac{m_{alg}}{198}+\frac{m_2}{113.16})} \times 100 \quad (1)$$

Table 1. Copolymers composition obtained by (a) TGA and (b) EA.

(a)	**% molar NIPAAm by TGA**			**(b)**	**% molar NIPAAm by EA**		
Initial % molar NIPAAm	**0.5 kGy**	**1.5 kGy**	**3 kGy**	**Initial % molar NIPAAm**	**0.5 kGy**	**1.5 kGy**	**3 kGy**
80N	69.1	75.0	79.0	**80N**	66.1	72.3	76.1
50N	28.4	35.1	44.5	**50N**	21.5	30.0	37.3
30N	21.7	27.5	26.0	**30N**	18.2	15.5	18.0

Results in Table 1 confirmed that NIPAAm monomer concentration in the initial reaction mixture and the radiation dose have a direct effect on the grafting percentage. These two variables can be combined to obtain copolymers with adequate composition and properties in view of the desired application.

Copolymers synthesized even at the lowest radiation dose 0.5 kGy presented notable grafting efficiency, where more than 50% of initial NIPAAm was grafted onto alginate. These results show the effectiveness of gamma radiation for alginate/PNIPAAm grafting reactions. Moreover, for the same absorbed dose, samples with the highest initial NIPAAm availability (80N) showed the highest grafting percentage.

Thus, in order to achieve higher grafting percentages, a greater NIPAAm concentration during the synthesis is rather effective than increasing radiation dose. This is related to the monomer availability around the radioinduced active sites. For the lowest initial NIPAAm concentration (30N), a similar % molar NIPAAm is obtained at all doses. In this case, the monomer concentration during the radiation synthesis appears as the limiting condition for grafting reaction.

It is known that, besides grafting, gamma radiation could promote others radioinduced reactions in aqueous solution, such as unattached PNIPAAm homopolymer, scission of alginate backbone and crosslinking of pendant PNIPAAm side chains (Lencina et al., 2015). Then, increasing radiation dose, and when almost all NIPAAm monomer was incorporated, competitive reactions could be predominant, as it will be discussed in section 3.2.1.

3.2. Rheological analysis

3.2.1. Viscoelastic properties

The study of the viscoelastic behavior of alginate-*g*-PNIPAAm copolymers in aqueous solution was focused on the evolution of the G’ and G’’ moduli when increasing temperature.

The viscoelastic properties of 30N, 50N and 80N copolymers synthesized with different radiation doses are shown in Figure 2. a) 0.5 kGy, b) 1.5 kGy and c) 3 kGy. At each radiation dose, G' and G'' moduli of 30N copolymers are slightly lower than those of 50N copolymers, whereas the moduli of 80N becomes far greater than the others. Setting a fixed radiation dose, a certain number of radioinduced sites are produced where NIPAAm monomers can be grafted onto alginate backbone. By increasing monomer concentration in the original mixture, more monomers are available for being incorporated at the growing PNIPAAm side chains. Thus, the increment of both moduli is a result of the longer length of side chains, producing more physical entanglements.

Apart from grafting, secondary reactions as crosslinking and chain scissions, must be taken into account. In this sense, comparing G' and G'' curves in Figure 2, from one plot to the other (different doses) for each copolymer composition, both moduli diminish increasing the radiation doses. This effect is observed from 0.5 kGy to 1.5 kGy, and even more at 3 kGy, being attributable to the scission of alginate backbone. As an exception, the sample of 80N at 3 kGy showed the highest moduli where crosslinks of PNIPAAm side chains were produced, offsetting the alginate scission effect.

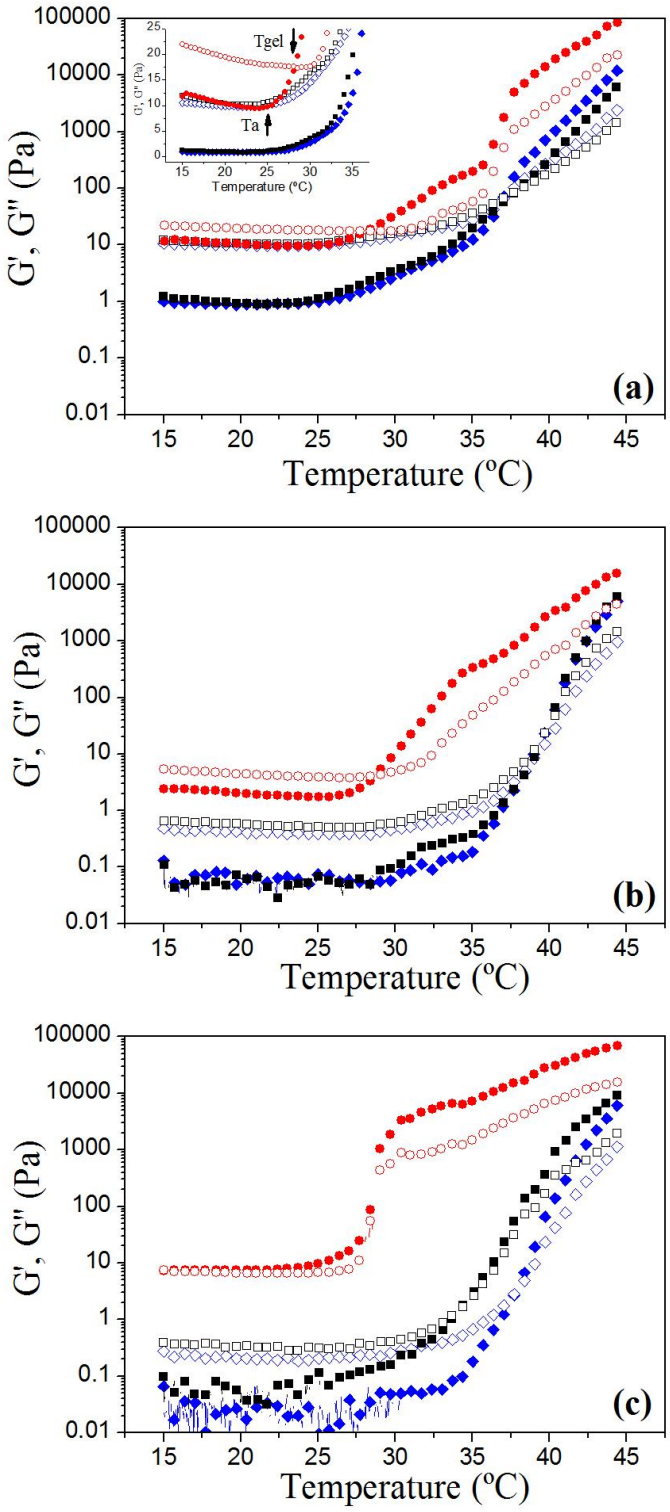

Figure 2. Viscoelastic properties of 5 wt% copolymer aqueous solutions increasing NIPAAm content at different doses: (a) 0.5 kGy, (b) 1.5 kGy and (c) 3 kGy. References: (♦,◊) G' and G'' 30% NIPAAm; (■,□) G' and G'' 50% NIPAAm and (●,○) G' and G'' 80% NIPAAm.

Moreover, scission effect is particularly observable in Figure 3, where rheology curves of samples with the lowest initial monomer concentration (30N) are shown. Regarding the similar composition among these samples (Table 1), G' and G'' moduli diminish with the absorbed dose. In this case, the limited availability of monomer concentration produces that, once all NIPAAm monomer was grafted, the scission of alginate backbone becomes more probable, causing lower values of storage and loss moduli. Alginate, as others irradiated polysaccharides, is broken by gamma radiation at glycosidic bonds diminishing their molecular weight and mechanical properties (Lee et al., 2003).

Hence, the adjustment of initial NIPAAm monomer concentration and radiation dose will determine the composition and chemical structure of the synthesized copolymers. According to these characteristics, viscoelastic and thermo-responsive properties of the copolymers in aqueous solution will depend on the selected copolymer but also on their wt% concentration, as it will be discussed in section 3.2.2.

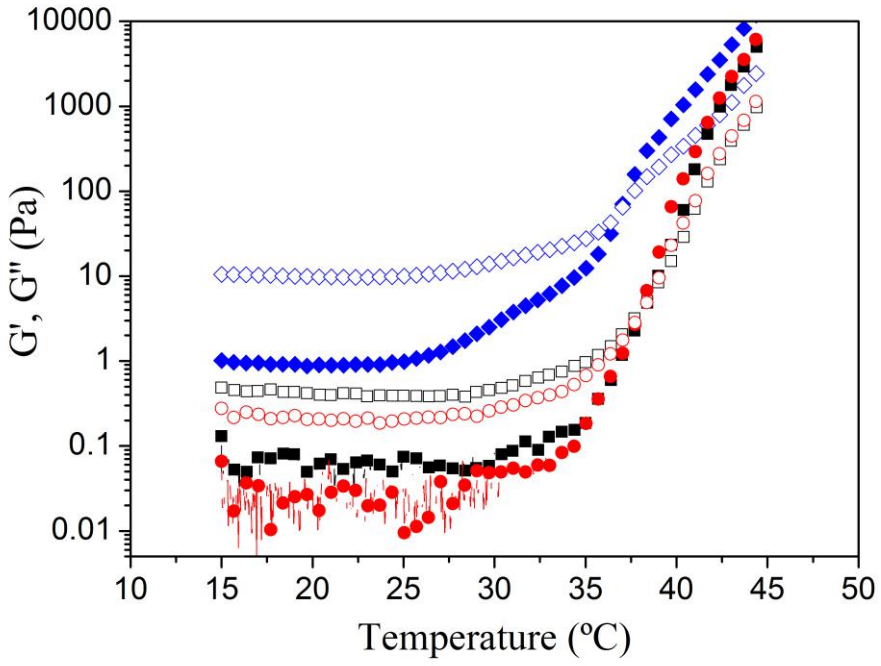

Figure 3. Viscoelastic properties of 5 wt% copolymer aqueous solutions with ~ 30 % molar NIPAAm synthesized at different radiation doses. References: (♦,◊) G' and G'' 0.5 kGy ; (■,□) G' and G'' 1.5 kGy and (●,○) G' and G'' 3 kGy.

3.2.2. Thermo-responsive properties

Thermo-responsive properties of copolymers were studied from G' and G'' rheology curves. In Figure 4, two temperature transitions are distinguished strongly related to thermo-responsive properties of copolymers. The first transition, named Ta, ideally divides the thermogram in two temperature ranges: below Ta, viscoelastic properties exhibit an initial behavior corresponding to materials that are governed by the Arrhenius law, where elastic and loss moduli decrease with temperature (Figure 2.a) inset, where y-axis is displayed in linear scale). Then, above Ta the slope of the modulus becomes positive; at this temperature, the grafted PNIPAAm side chains start to exhibit their hydrophobicity as a consequence of the conformational coil-globule transition associated with the LCST. Then, G' increases more sharply than G'' until reaching the G'/G'' crossover point, which is the second critical temperature, Tgel. From this temperature, G' starts to prevail over G'', related to the sol-gel transition (Gupta et al., 2015; Lencina et al., 2014; R. Liu et al., 2009; Stabenfeldt et al., 2006).

In view of the potential application of these materials as injectable gel-forming matrices, gelation must be close to the physiological temperature, around 37 ºC, allowing the injection of the solubilized thermo-sensitive copolymers. Thus, it is important to establish adequate Ta and Tgel transition temperatures to fulfill this requirement. In this sense, Ta and Tgel values of 5 wt% aqueous copolymer solutions are shown in Figure 4, as a function of % molar NIPAAm and radiation dose.

At each absorbed dose, Ta does not vary significantly increasing the PNIPAAm content in the copolymer while Tgel values showed an abrupt decrease only for those samples with the major grafting percentage. As an exception, the 80N copolymer at 3 kGy (Figure 4.c) presented the lowest Ta value, because the temperature at which the material undergoes a hydrophilic-hydrophobic transition is lowered due to the longer PNIPAAm side chains (Lencina et al., 2014). Even more, for this sample no Tgel is reported since at room temperature is already a gel (G' > G''), making evident the crosslinking reactions among PNIPAAm side chains.

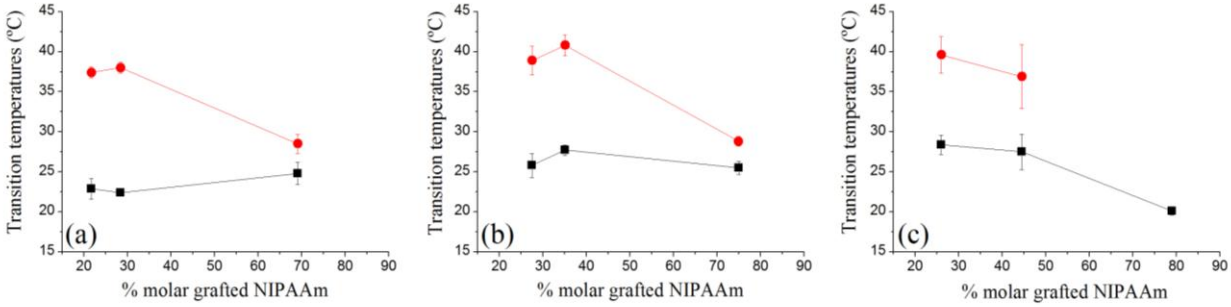

Figure 4. Ta and Tgel of 5 wt% copolymer aqueous solutions increasing grafted NIPAAm at different doses: (a) 0.5 kGy, (b) 1.5 kGy and (c) 3 kGy. References: (■) Ta and (●) Tgel

In general, the associative onset point Ta and the cross-over point Tgel are shifted towards lower temperatures by increasing the copolymer concentration in the solutions. In this sense, the higher amount of copolymer molecules are capable of forming entanglements starting a physical gel immediately above Ta; this is consistent with the increase of the moduli (Lencina et al., 2014). Results for aqueous solutions with 1.5, 3 and 5 wt% concentration of 30N, 50N and 80N copolymers (0.5 kGy) are shown in Figure 5.a), 5.b) and 5.c), respectively.

In this context, varying the solution concentration it is possible to tailor the Tgel transition close to body temperature. Gelation does not occur in the range of temperature evaluated for 1.5 wt% solutions of 30N and 50N copolymers, Figures 5.a) and 5.b), where copolymer concentration is not enough. On the other hand in Figure 5.c) the copolymer with the highest PNIPAAm content shows Tgel values below 37 ºC at all concentrations. Then, the most adequate sample for injectable purpose seem to be 5 wt% aqueous solution of 30N (Ta = 24.1 ± 0.7 Tgel= 36.6 ± 0.7) and 50N copolymers (Ta = 23.7 ± 0.3; Tgel = 37.6 ± 0.4) if the concentration is slightly increased to diminish Tgel.

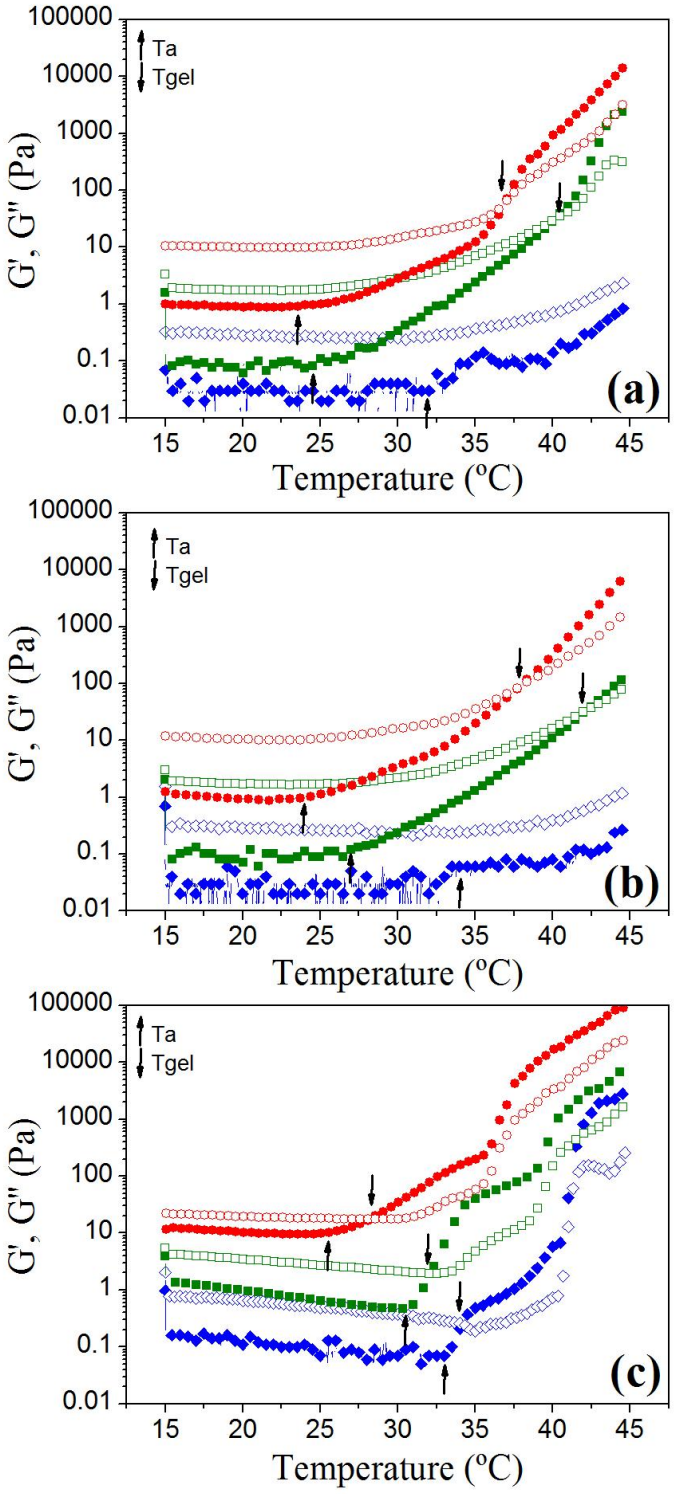

Figure 5. Viscoelastic properties increasing copolymer concentration in the aqueous solutions for (a) 30N, (b) 50N and (c) 80N copolymers synthesized at 0.5 kGy. References: (♦,◊) G' and G'' 1.5 wt% ; (■,□) G' and G'' 3 wt% and (●,○) G' and G'' 5 wt%.

4. Conclusions

Gamma radiation is a simple and eco-friendly technique to synthesize graft copolymers with adequate properties to be used as injectable aqueous polymer solutions. Whether the grafting is the main radioinduced outcome other phenomena may take place, such us backbone scission and crosslink of grafted side chains, which determine the rheological behavior of these copolymers aqueous solutions.

In order to design samples with liquid-gel transitions close to the human body, able to form gels *in situ* once inoculated, it was possible to tailor both, Ta and Tgel, transition temperatures selecting those copolymers with appropriate PNIPAAm content and optimizing the copolymer concentration in the aqueous solutions. In view of this application, 5 wt% aqueous solutions of copolymers synthesized with low NIPAAm content (30N and 50N) at the lowest radiation dose 0.5 kGy, seems to fulfill the requirement aforementioned. Furthermore, applying the lowest radiation dose the alginate scission is minimized resulting in better viscoelastic properties.

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References

Alemán, J. V., Chadwick, A. V., He, J., Hess, M., Horie, K., Jones, R.G., Kratochvíl, P., Meisel, I., Mita, I., Moad, G., Penczek, S., Stepto, R.F.T., 2007. Definitions of terms relating to the structure and processing of sols, gels, networks, and inorganic-organic hybrid materials (IUPAC Recommendations 2007). Pure Appl. Chem. 79, 1801–1829. https://doi.org/10.1351/pac200779101801

Cheaburu, C.N., Ciocoiu, O.-N., Staikos, G., Vasile, C., 2013. Thermoresponsive sodium alginate- g -poly(N -isopropylacrylamide) copolymers III. Solution properties. J. Appl. Polym. Sci. 127, 3340–3348. https://doi.org/10.1002/app.37789

Christiani, T.R., Toomer, K., Sheehan, J., Nitzl, A., Branda, A., England, E., Graney, P., Iftode, C., Vernengo, A.J., 2016. Synthesis of Thermogelling Poly(N-isopropylacrylamide)-graft-chondroitin Sulfate Composites with Alginate Microparticles for Tissue Engineering. J. Vis.

Exp. 1–11. https://doi.org/10.3791/53704

Conova, L., Vernengo, J., Jin, Y., Himes, B.T., Neuhuber, B., Fischer, I., Lowman, A., 2011. A pilot study of poly(N-isopropylacrylamide)-g-polyethylene glycol and poly(N-isopropylacrylamide)-g-methylcellulose branched copolymers as injectable scaffolds for local delivery of neurotrophins and cellular transplants into the injured spinal cord. J. Neurosurg. Spine 15, 594–604. https://doi.org/10.3171/2011.7.SPINE11194

Flores-Rojas, G.G., Bucio, E., 2016. Radiation-grafting of ethylene glycol dimethacrylate (EGDMA) and glycidyl methacrylate (GMA) onto silicone rubber. Radiat. Phys. Chem. 127, 21–26. https://doi.org/10.1016/j.radphyschem.2016.05.015

Flores-Rojas, G.G., López-Saucedo, F., Quezada-Miriel, M., Bucio, E., 2018. Grafting of glycerol methacrylate onto silicone rubber using γ-rays: Derivatization to 2-oxoethyl methacrylate and immobilization of lysozyme. MRS Commun. 8, 199–206. https://doi.org/10.1557/mrc.2018.16

Gupta, D., Tator, C.H., Shoichet, M.S., 2006. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. Biomaterials 27, 2370–2379. https://doi.org/10.1016/j.biomaterials.2005.11.015

Gupta, N.R., Arun Torris, A.T., Wadgaonkar, P.P., Rajamohanan, P.R., Ducouret, G., Hourdet, D., Creton, C., Badiger, M. V., 2015. Synthesis and characterization of PEPO grafted carboxymethyl guar and carboxymethyl tamarind as new thermo-associating polymers. Carbohydr. Polym. 117, 331–338. https://doi.org/10.1016/j.carbpol.2014.09.073

Karakasyan, C., Lack, S., Brunel, F., Maingault, P., Hourdet, D., 2008. Synthesis and rheological properties of responsive thickeners based on polysaccharide architectures. Biomacromolecules 9, 2419–29. https://doi.org/10.1021/bm800393s

Kim, D.Y., Kwon, D.Y., Kwon, J.S., Kim, J.H., Min, B.H., Kim, M.S., 2014. Stimuli-responsive injectable in situ-forming hydrogels for regenerative medicines. Polym. Rev. 0, 1–46. https://doi.org/10.1080/15583724.2014.983244

Kim, J.H., Lee, S.B., Kim, S.J., Lee, Y.M., 2002. Rapid Temperature / pH Response of Porous Alginate-g-poly (N- isopropylacrylamide) Hydrogels. Polymer (Guildf). 43, 7549–7558.

Lee, D.W., Choi, W.S., Byun, M.W., Park, H.J., Yu, Y.-M., Lee, C.M., 2003. Effect of gamma-irradiation on degradation of alginate. J. Agric. Food Chem. 51, 4819–23. https://doi.org/10.1021/jf021053y

Lencina, M.M.S., Ciolino, A.E., Andreucetti, N.A., Villar, M.A., 2015. Thermoresponsive hydrogels based on alginate-g-poly(N-isopropylacrylamide) copolymers obtained by low doses of gamma radiation. Eur. Polym. J. 68, 641–649. https://doi.org/10.1016/j.eurpolymj.2015.03.071

Lencina, M.M.S., Gómez, C.G., Andreucetti, N.A., Villar, M.A., 2013. Recent Studies on Alginates Based Blends, Composites, and Nanocomposites, in: Sabu, T., Visakh, P.M., Aji, P.M. (Eds.), Advances in Natural Polymers. Springer Berlin Heidelberg, pp. 193–254.

Lencina, M.M.S., Iatridi, Z., Villar, M.A., Tsitsilianis, C., 2014. Thermoresponsive hydrogels from alginate-based graft copolymers. Eur. Polym. J. 61, 33–44. https://doi.org/10.1016/j.eurpolymj.2014.09.011

Lionetto, F., Montagna, F., Maffezzoli, A., 2005a. Ultrasonic Dynamic Mechanical Analysis of Polymers Abstract : Appl. Rheol. 15, 326–335. https://doi.org/10.3933/ApplRheol-15-326

Lionetto, F., Sannino, A., Maffezzoli, A., 2005b. Ultrasonic monitoring of the network formation in superabsorbent cellulose based hydrogels. Polymer (Guildf). 46, 1796–1803. https://doi.org/10.1016/j.polymer.2005.01.008

Liu, C., Guo, X., Cui, H., Yuan, R., 2009. Journal of Molecular Catalysis B : Enzymatic An amperometric biosensor fabricated from electro-co-deposition of sodium alginate and horseradish peroxidase 60, 151–156. https://doi.org/10.1016/j.molcatb.2009.04.015

Liu, M., Song, X., Wen, Y., Zhu, J.-L., Li, J., 2017. Injectable Thermoresponsive Hydrogel Formed by Alginate- *g* -Poly(*N* -isopropylacrylamide) That Releases Doxorubicin-Encapsulated Micelles as a Smart Drug Delivery System. ACS Appl. Mater. Interfaces acsami.7b12849. https://doi.org/10.1021/acsami.7b12849

Liu, R., Fraylich, M., Saunders, B.R., 2009. Thermoresponsive copolymers: From fundamental studies to applications. Colloid Polym. Sci. 287, 627–643. https://doi.org/10.1007/s00396-009-2028-x

Madigan, N.N., McMahon, S., O'Brien, T., Yaszemski, M.J., Windebank, A.J., 2009. Current tissue engineering and novel therapeutic approaches to axonal regeneration following spinal cord injury using polymer scaffolds. Respir. Physiol. Neurobiol. 169, 183–199. https://doi.org/10.1016/j.resp.2009.08.015

Martini, B., Dimida, S., De Benedetto, E., Madaghiele, M., Demitri, C., 2016. Study on the degradation of chitosan slurries. Results Phys. 6, 728–729. https://doi.org/10.1016/j.rinp.2016.10.012

Nguyen, Q.V., Huynh, D.P., Park, J.H., Lee, D.S., 2015. Injectable polymeric hydrogels for the delivery of therapeutic agents: A review. Eur. Polym. J. 72, 602–619. https://doi.org/10.1016/j.eurpolymj.2015.03.016

Ruel-Gariépy, E., Leroux, J.C., 2004. In situ-forming hydrogels - Review of temperature-sensitive systems. Eur. J. Pharm. Biopharm. 58, 409–426. https://doi.org/10.1016/j.ejpb.2004.03.019

Salomonsen, T., Jensen, H.M., Larsen, F.H., Steuernagel, S., Engelsen, S.B., 2009. Alginate Monomer Composition Studied by Solution- and Solid-State NMR – A Comparative Chemometric Study. Food Hydrocoll. 23, 1579–1586. https://doi.org/10.1016/j.foodhyd.2008.11.009

Schild, H.G., 1992. Poly (N-isopropylacrylamide): Experiment, Theory and Application. Prog. Polym. Sci. 17, 163–249.

Stabenfeldt, S.E., García, A.J., LaPlaca, M.C., 2006. Thermoreversible laminin-functionalized hydrogel for neural tissue engineering. J. Biomed. Mater. Res. Part A 718–725. https://doi.org/10.1002/jbm.a

Wiltsey, C., Christiani, T., Williams, J., Scaramazza, J., Van Sciver, C. V., Toomer, K., Sheehan, J., Branda, A., Nitzl, A., England, E., Kadlowec, J., Iftode, C., Vernengo, J., 2015. Thermogelling bioadhesive scaffolds for intervertebral disk tissue engineering: Preliminary in vitro comparison of aldehyde-based versus alginate microparticle-mediated adhesion. Acta Biomater. 16, 71–80. https://doi.org/10.1016/j.actbio.2015.01.025

Yan, J., Yang, L., Wang, G., Xiao, Y., Zhang, B., Qi, N., 2010. Biocompatibility Evaluation of Chitosan-based Injectable Hydrogels for the Culturing Mice Mesenchymal Stem Cells In Vitro.

J. Biomater. Appl. 24, 625–637. https://doi.org/10.1177/0885328208100536

Yang, C., Xu, L., Zhou, Y., Zhang, X., Huang, X., Wang, M., Han, Y., Zhai, M., Wei, S., Li, J., 2010. A green fabrication approach of gelatin/CM-chitosan hybrid hydrogel for wound healing. Carbohydr. Polym. 82, 1297–1305. https://doi.org/10.1016/j.carbpol.2010.07.013

Yang, J.A., Yeom, J., Hwang, B.W., Hoffman, A.S., Hahn, S.K., 2014. In situ-forming injectable hydrogels for regenerative medicine. Prog. Polym. Sci. 39, 1973–1986. https://doi.org/10.1016/j.progpolymsci.2014.07.006

Highlights

- Low doses of γ radiation allow to synthesize alginate-g-PNIPAAm copolymers
- Structure-properties relationship was well defined by rheology in aqueous solution
- Copolymers sol-gel transitions could be tailored close to body temperature
- We obtained injectable solutions to form gels *in situ* once inoculated