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Post-synthesis modification of hydrogels. Total and partial rupture of crosslinks: Formation of aldehyde groups and re-crosslinking of cleaved hydrogels

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ABSTRACT

Post-synthesis modification of hydrogels is frequently used for chemical functionalization, immobilization of molecules or optimization of mechanical properties. In this work, novel gels were developed using a divinyl compound (+) *N*, *N*′-diallyltartramide (DAT) as a crosslinking agent. DAT possesses a vicinal diol and it was found that can be selectively cleaved by reaction with periodate, obtaining aldehydes as products. The treatment of the gels crosslinked only with DAT leads to complete digestion of the material, obtaining a liquid as a product. The cleavage reaction in gels crosslinked with two agents, one reactive with periodate and another inert, allows modification of the crosslinking degree, maintaining the state of the gel. The change in the mechanical properties of the gels due to periodate reaction was followed by rheological studies. Furthermore, the reaction of periodate with DAT, the aldehyde obtained as a product and its reactivity with adipic acid dihydrazide (ADH) was studied by nuclear magnetic resonance (NMR) spectroscopy.

1. Introduction

Hydrogels are crosslinked polymer networks that have many applications due to their high capability to absorb water and their similarity with biological tissues [1]. These materials have been used for development of biosensors [2], tissue engineering [3], controlled drug release [4], microfluidic devices [5], among others [6]. Hydrogels can be obtained from natural macromolecules, by polymerization of different types of monomers, or by a combination of both. However, in many cases, the synthesized material does not have all the desired properties, thus chemical changes in the synthesis product are required [7]. Post-synthesis modification of hydrogels is often used to introduce new functional groups, to optimize the mechanical properties of the material or to immobilize molecules [8]. An interesting case is the obtention of polymers containing aldehyde groups. This highly reactive functional group is generally afforded by post-synthesis reactions since it is unstable in the usual polymerization conditions [8,10]. Aldehydes in polymers are of great interest because of their easy chemical derivatization and possibility to form reversible bonds such as imines, oximes, acetals and hydrazones [11]. Materials with such reversible linkages are called dynamic polymers [12] and have been extensively studied due to their potential applications [13]. However, even post-modification of synthetic polymers to obtain aldehyde groups is generally tedious, involving reactions with several steps. Frequently, monomers with protected aldehyde groups are used. After polymer synthesis, deprotection reactions need to be performed, sometimes using nonaqueous reagents and strong acids [14–18]. However, a straightforward reaction protocol is followed with polysaccharides. Aldehyde groups are achieved in the polymeric product through the reaction between vicinal diols with periodate in aqueous media, by Malaprade mechanism [19]. This reaction has been commonly used to afford aldehydes in natural polysaccharides such as dextran, chitosan, pectin, hyaluronic acid or alginate [20–24].

An interesting alternative could be the application of this straightforward protocol to synthetic materials, since the hydrogels yielded by polymerization of different monomers generate more versatile materials with predictable properties by controlling the composition and functionality of monomers. Accordingly, glycidol (with an oxirane functional group) has been used to react with

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hydroxyl groups of synthetic polymers producing vicinal diols anchored to the material. Subsequently, these terminal diols react with periodate to produce aldehydes in the polymer chain and formaldehyde as a by-product [9].

Kornysova et al. prepared nonparticulate (continuous or monolithic) beds containing vancomycin as chiral selector for capillary electrochromatography [25]. For this, *N*,*N*′-diallyltartardiamide (DAT) containing diol functionality was used, since can be converted to aldehyde groups via periodate treatment. So, the activation of the matrix to the attachment of vancomycin and the increase of the pore size and porosity of the support were achieved. In other report [26], the preparation of an affinity monolithic capillary column with immobilized α-mannose was performed using 2-hydroxyethyl methacrylate, and both crosslinkers, DAT and piperazine diacrylamide. Later, periodate was used to the oxidation of DAT and, in the following step, the mannose was bound with a spacer to the aldehyde groups of the still partially crosslinked matrix via reductive amination. Thus, an affinity column for the separation, enrichment or binding studies of mannose-specific lectins was achieved. It should be noted that the DAT monomer has also been used in other systems, in which it acts only as a crosslinking agent [27–30].

In this work we present novel strategies for the post-synthesis control of the crosslinking degree of hydrogels and their functionalization. The synthesis of hydrogels using commercial crosslinking agents with vicinal functional groups (diols, 1,2-hydroxy ketones, 1,2-diketones, α-keto acids, α-hydroxy acids, amino acids, 1,2-amino alcohols, 1,2-diamines, and epoxides) allows the development of numerous materials that can be subsequently treated in the presence of periodate to achieve selective cleavage. In particular, gels containing the crosslinking agent DAT (a vicinal diol carrier) and gels with both crosslinkers DAT and *N*, *N*′-methylene bis (acrylamide) (BIS) (nonreactive with periodate) were synthesized. The gels yielded were treated with periodate in aqueous solutions. Changes in mechanical properties of the materials were measured by rheological tests and the reaction products were characterized by NMR. Subsequently, the aldehyde groups afforded reacted with adipic dihydrazide acid (ADH). Therefore, a crosslinked hydrogel by dynamic hydrazone links was obtained.

2. Materials and methods

2.1. Reagents

The following reagents were used as received: acrylamide (AM) (Fluka); *N*, *N*′-methylene bis(acrylamide) (BIS) (Sigma); (+) *N*, *N*′-diallyltartramide (DAT) (Sigma); *N*, *N*, *N*′, *N*′-tetramethylethylenediamine (TEMED) (Sigma); ammonium persulfate (APS) (Anhedra); sodium periodate (Sigma); ethylene glycol (EG) (Anhedra); D_2O (Sigma); ammonium chloride (Cicarelli); glacial acetic acid (Cicarelli); sodium acetate (Anhedra) and adipic acid dihydrazide (ADH) (SIGMA). The solutions were prepared with ultrapure water (18 $M\Omega cm^{-1}$).

2.2. Synthesis

The hydrogels were synthesized by free radical polymerization using the monomers AM, BIS and/or DAT (see Fig. 1). Solutions were prepared dissolving AM in 5 mL of ultrapure water to reach 1.4 M. Then, BIS and/or DAT (crosslinking agents) and APS (initiator) were dissolved in AM solutions using a vial with a rubber cap, in the proportions shown in Table 1. The solutions were cooled on an ice bath and deoxygenated with N_2 for 10 min. To initiate polymerization, an aqueous solution of TEMED (0.5 mL, 0.32 M) was added to the vial and the whole solution was transferred to a 5 mL plastic syringe. The syringe was placed in a thermostatic bath at 37 °C for 18 h. Finally, the hydrogels obtained were cut into discs of 3 mm thick and 12 mm in diameter and washed thoroughly with water. The products were named HG-1-7 as shown in Table 1. All the syntheses were performed in triplicate.

Table 1
Experimental conditions for the synthesis of hydrogels.

Hydrogel	Monomer	Crosslinker (%)[a]	Initiator (%)[a]
HG-1	AM (1.4 M−5 mL)	BIS (5) - DAT (0)	APS (3.7) - TEMED[b] (1.4)
HG-2	AM (1.4 M−5 mL)	BIS (5) - DAT (1)	APS (3.7) - TEMED (1.4)
HG-3	AM (1.4 M−5 mL)	BIS (5) - DAT (3)	APS (3.7) - TEMED (1.4)
HG-4	AM (1.4 M−5 mL)	BIS (5) - DAT (5)	APS (3.7) - TEMED (1.4)
HG-5	AM (1.4 M−5 mL)	BIS (5) - DAT (7)	APS (3.7) - TEMED (1.4)
HG-6	AM (1.4 M−5 mL)	BIS (5) - DAT (10)	APS (3.7) - TEMED (1.4)
HG-7	AM (1.4 M−5 mL)	BIS (0) - DAT (10)	APS (3.7) - TEMED (1.4)

[a] Molar percentage from AM concentration.
[b] 0.5 mL of 0.32 M solution of TEMED was used.

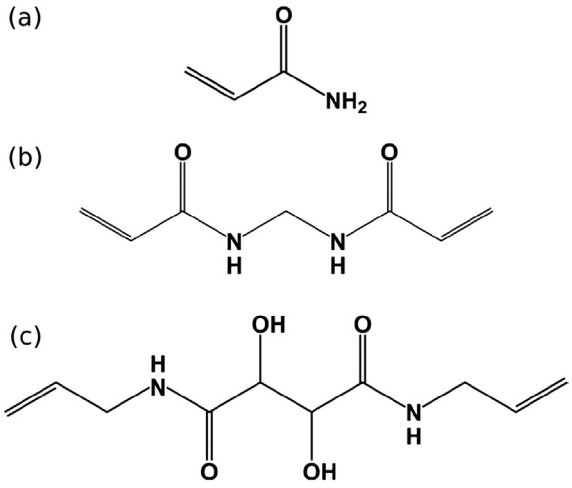

Fig. 1. Monomers used to prepare hydrogels. **(a)** AM, **(b)** BIS and **(c)** DAT.

2.3. Swelling studies

After synthesis, the hydrogel discs swollen at equilibrium in water during 48 h were weighed. Then, the discs were dried in an oven at 37 °C until constant weight (≈80 mg). Equilibrium swelling ratio as percentage (%ESR) was calculated according to equation (1):

$$\%ESR = [me - md\,]/md \times 100 \quad (1)$$

Where m_e is the sample mass at swelling equilibrium and m_d is the dried mass.

The hydrogel was weighed using a Mettler Toledo balance (New classic MF, model MS204S; 0.1 mg precision). All assays were performed in triplicate.

2.4. Rupture of crosslinks of hydrogels with periodate

The moles of periodate (m_{NaIO4}) required for complete rupture of crosslinks produced by DAT in a hydrogel sample were estimated from DAT, BIS, AM and APS molarities used in the synthesis and the mass of each dry hydrogel disc (m_d) according to equation (2):

$$m_{NaIO4} = [(m_{DAT} \times mw_{DAT}) / (m_{DAT} \times mw_{DAT} + m_{BIS} \times mw_{BIS} + m_{AM} \times mw_{AM} + m_{APS} \times mw_{APS})] \times (md / mw_{DAT}) \quad (2)$$

where m_{DAT}, m_{BIS} m_{AM} and m_{APS} are the moles of DAT, BIS, AM and APS used in the synthesis, respectively. Also, mw_{DAT}, mw_{BIS}, mw_{AM} *and* mw_{APS} are the molecular weights of DAT, BIS, AM and APS, respectively.

To estimate the quantity of periodate required to obtain rupture of DAT, it was assumed that all DAT added as reagent was incorporated into the polymer network.

Furthermore, the volume of periodate solution was determined considering the minimal quantity of liquid necessary to swamp the sample of hydrogel disc.

2.4.1. Cleavage of DAT in hydrogels crosslinked with BIS and DAT (HG-2-6)

A weighed hydrogel disc swollen at equilibrium in water was put in a 5 mL glass vial. Then, a solution of $NaIO_4$ ($m_{NaIO4} \times 2$ disolved in 1.25 mL of water) was added. For this reaction, the periodate was always in excess (200%) in relation to amount of DAT used to prepare the hydrogel. The reagents were left in contact during 24 h at 25 °C. Then, the liquid was discarded and the hydrogel treated was thoroughly washed with distilled water. The products obtained were named CHG-2-6. Fig. 2A shows a schematic diagram of hydrogels crosslinked with BIS and DAT before and after reaction with periodate.

2.4.2. Partial cleavage of DAT in hydrogels crosslinked with BIS and DAT (HG-6)

A hydrogel disc swollen at equilibrium in water was treated with $NaIO_4$ during 24 h at 25 °C following the procedure detailed in section 2.4.1. However, the periodate concentration used to obtain a partial cleavage (50%) was lower ($m_{NaIO4}/2$ disolved in 1.25 mL of water). The hydrogel afforded was named pCHG-6.

Fig. 2B shows a schematic diagram of the partial rupture of DAT in hydrogels crosslinked with BIS and DAT.

2.4.2.1. Characterization of pCHG-6 by confocal microscopy. The assayed samples were obtained from synthesis HG-6. A weighed hydrogel disc swollen at equilibrium in water was treated with $NaIO_4$ during 24 h at 25 °C following the procedure detailed in section 2.4.2. Before the measurements, the samples of hydrogels partially modified with periodate were axially cut and freeze-dried. Laser images were taken from partially modified hydrogels in dry state. Differential Interference Contrast (DIC) was used to study unstained hydrogels in hydrated state. All confocal microscopy images were obtained using a confocal microscope OLYMPUS LEXT OLS4000 from LAMARX laboratory, National University of Córdoba.

2.4.3. Complete rupture of crosslinks with periodate (HG-7)

The assayed samples were obtained from synthesis HG-7. A weighed hydrogel disc swollen at equilibrium in water was put in a 5 mL glass vial. Then, a solution of $NaIO_4$ ($m_{NaIO4} \times 1.3$, disolved in 1.25 mL of water) was added. The periodate used for this reaction was in excess (130%) with respect to the amount of DAT estimated for the dried hydrogel disc. The reaction was carried under stirring during 24 h at 25 °C. After reaction, a liquid product (LHG-7) was obtained. Then, 195 μL of ethylene glycol solution (2.48 w/v %) was added to the reaction product and allowed to react during 20 h. Finally, LHG-7 solution was bubbled with N_2 connected to an ammonium chloride trap for 10 min to eliminate the presence of formaldehyde as a cleavage sub-product. Fig. 3 shows a schematic diagram of a complete rupture of the hydrogel crosslinked only with DAT.

2.4.4. Swelling ratio of hydrogels (HG-1-7) during the reaction with periodate

The sweeling ratio of hydrogels obtained from the synthesis HG-1-7 was followed during its reaction with a sodium periodate solution. Each hydrogel was initially swollen at the equilibrium in water. Then, it was submerged into 12 mL of a 0,1 M $NaIO_4$ solution and its swelling change was followed gravimetrically over time. In order to carry the measurements, the hydrogel was momentanously removed from the periodate solution, dried with drying paper and weighed to be submerged again into the periodate solution.

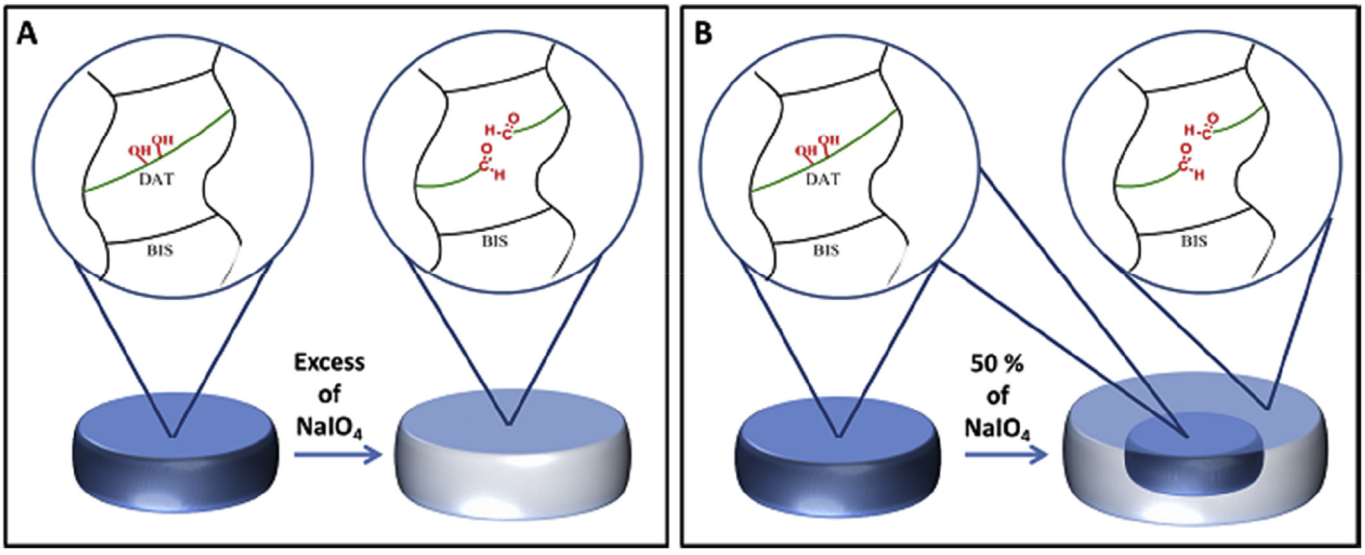

Fig. 2. A). Schematic diagram of hydrogels crosslinked with BIS and DAT before and after reaction with periodate. **B)** Schematic diagram of the partial rupture of DAT in a hydrogel crosslinked with BIS and DAT.

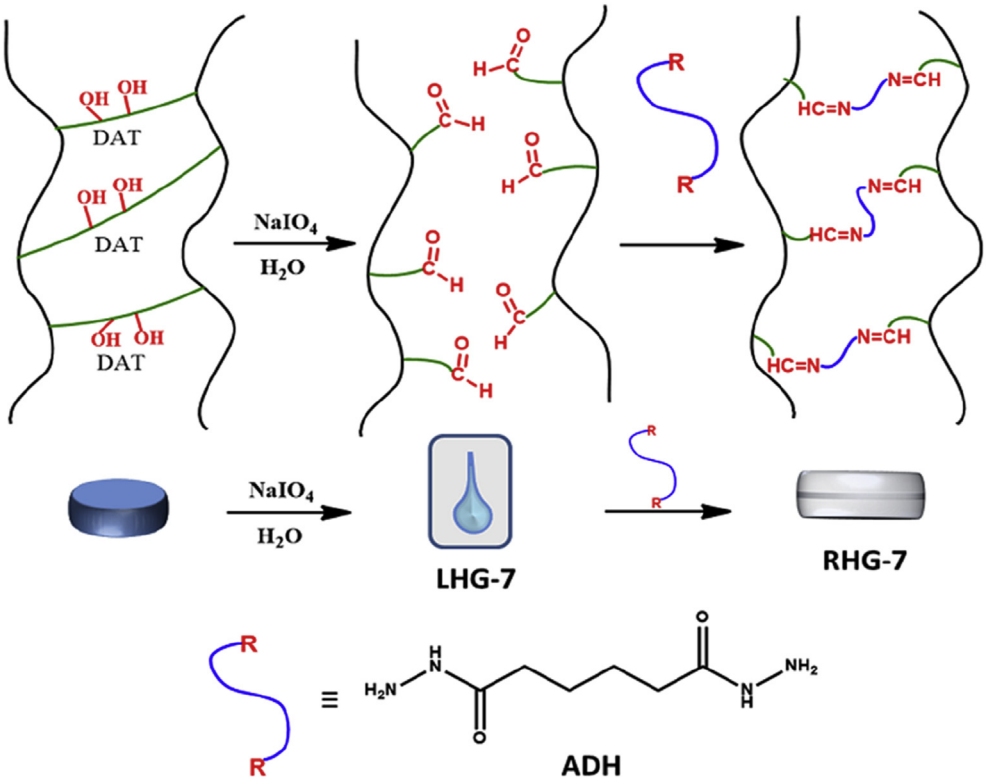

Fig. 3. Schematic diagram of a complete rupture of hydrogel HG-7 to obtain a liquid product (LHG-7) following of re-crosslinking of LHG-7 solution with ADH to obtain the hydrogel RHG-7.

2.5. Re-crosslinking of cleaved hydrogels

Solutions of LHG-7 were used to obtain re-crosslinked hydrogels. For this purpose, the reaction between LHG-7 and adipic acid dihydrazide was studied. To each assay, 150 μL of LHG-7 was mixed with 50 μL of ADH. The ADH solutions were prepared by dilution of 0.55 M stock solution. The moles of ADH (m_{ADH}) were calculated according to equation (3):

$$m_{ADH} = [(m_{DAT} \times mw_{DAT})/(m_{DAT} \times mw_{DAT} + m_{AM} \times mw_{AM} + m_{APS} \times mw_{APS})] \times [md/mw_{DAT}] \quad (3)$$

The new hydrogel was named RHG-7. Fig. 3 shows a schematic diagram of formation of RHG-7 from liquid solution (LHG-7) after reaction with ADH.

2.6. Rheological studies

2.6.1. Cleavage of DAT in hydrogels crosslinked with BIS and DAT

The mechanical characterization of the hydrogels during reaction with sodium periodate was carried by rotational rheology. The elastic modulus (G′) of HG-1-6 during their reaction with $NaIO_4$, was recorded over time during 2700s. For this experiment, the hydrogels swollen until equilibrium in water were cut on 8 mm diameter and 2.3 mm thickness. Each sample was centrally placed between two 8 mm diameter parallel plates of the rheometer Anton Paar Physica MCR−301. To determine the linear viscoelastic region (LVR) of the hydrogels, an oscillatory stress sweep was performed at a frequency of 1 Hz and 20 °C.

Subsequently, an oscillatory test at constant frequency (ω = 1 Hz) and amplitude (γ = 1%) was carried out over time. During this test, each hydrogel was placed between the plates and submerged into water during 2 min to begin the measurement, after that, the excess of water was removed. Then, a $NaIO_4$ (0.2 M) solution was added over minute 3.6, swamping the hydrogel until the end of the measurement. A scheme of the measurement process can be found in the supporting information as Fig. S1. All tests were performed in triplicate at a constant temperature of 20.0 ± 0.1 °C.

2.6.2. Re-crosslinking of cleaved hydrogels

To perform this study, 150 μL of a LHG-7 sample were placed between the plates (gap = 0.3 mm) of a circular geometry of 25 mm of diameter. The elastic modulus (G′) at 20 °C was recorded over time at constant frequency (1 Hz) and strain (1%) during 10 min. Subsequently 50 μL of a ADH solution (calculated according to equation (3)) were added and the measurement was followed during 180 min.

2.7. ^1H NMR spectroscopy

The measurements were taken using the nuclear magnetic resonance spectrometer NMR Bruker Advance 400 MHz D_2O was used as the solvent in all cases.

2.7.1. Characterization study of DAT cleavage

57 mg of DAT (2.5×10^{-4} mol) was dissolved in 1 mL of ultrapure water. Then, 106.9 mg of $NaIO_4$ (5×10^{-4} mol) was added. The reaction proceeded for an hour at 20 °C. The solution obtained was afterward freeze-dried. The dry product was redissolved in D_2O to perform NMR measurements.

2.7.2. Characterization study of HG-7 cleavage

A disc of HG-7 was immersed into solution of $NaIO_4$ ($m_{NaIO4} \times 3$ disolved in 1.5 mL of D_2O). Periodate was estimated as a three-time excess with respect to the moles of diol groups in the mass of hydrogel treated. The reaction proceeded during 12 h at 20 °C. Then, sodium acetate and acetic acid were added to achieve an equimolar buffer (0.1 M) at pH = 4.7.

Afterward, ^1H NMR measurements were performed with incorporation of 0; 30; 70 and 120% of ADH ($m_{ADH} \times 0$; 0.3; 0.7 and 1.2). ADH was directly dissolved into the measurement tube with the reaction mixture and then stirred. Each measurement was done 5 min following ADH addition.

3. Results and discussion

3.1. Synthesis and characterization of hydrogels (HG-1-7)

In all cases, hydrogels based on AM were obtained (Table 1). DAT and BIS were used as crosslinking agents, between 0–10% and 0–5% molar (with respect to AM moles), respectively. Fig. 4 shows the products yielded. The hydrogels afforded with BIS and DAT simultaneously (HG-1-6) were whitish, while that obtained only with DAT as a crosslinker (HG-7), was translucent.

The swelling properties of the hydrogels obtained were studied. Fig. 5 shows the equilibrium swelling ratio (%ESR) of HG-1-6 versus %DAT used for the synthesis. In all cases, the hydrogels showed high capacity of water absorption (>700%). Furthermore, the hydrogels prepared with larger concentrations of DAT showed lower %ESR. Therefore, the swelling response could be related to growing incorporation of DAT in the networks from synthesis 1 to 6.

The effect of DAT percentage on the swelling equilibrium of the hydrogel (%ESR) can be estimated using a non linear fitting formula (equation (4)):

$$\%ESR \cong \%ESR_0 \Big/ (1 + \%DAT)^k \quad (4)$$

Where $\%ESR_0 = (9.8 \pm 0.2) \times 10^2$ is the swelling equilibrium of HG-1, in the absense of DAT (crosslinked only with BIS 5%) and $k = 0.10 \pm 0.01$ is a constant. The trendline of %ESR data is shown in Fig. 5 with a dashed line. It can be estimated that the subsequent increases of %DAT (greather than 10%) would not produce a significant decrease in %ESR. This result is consistent with the relation between ESR and crosslinker concentration observed previously [31,32].

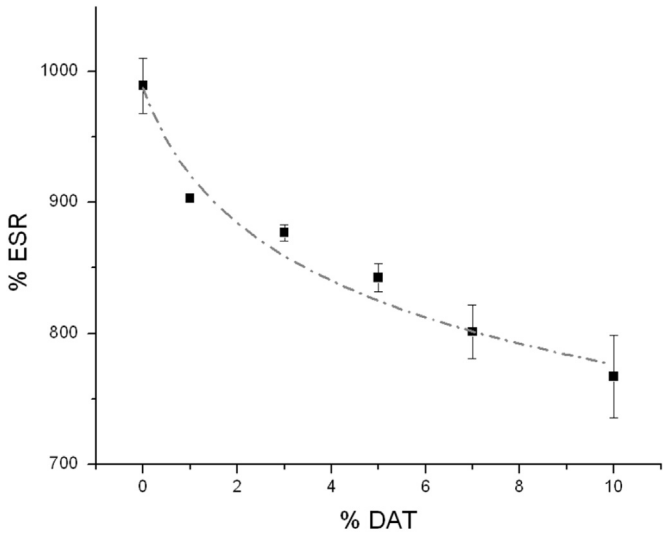

Fig. 5. %ESR vs. %DAT added to hydrogel synthesis.

3.2. Rupture of the crosslinkings of hydrogels with periodate (HG-2-6)

3.2.1. Cleavage of dat in hydrogels crosslinked with BIS and DAT

Hydrogels simultaneously crosslinked with DAT and BIS, were studied after addition of periodate ion. DAT units inside the polymeric network were expected to react with periodate since this crosslinker has a vicinal diol in its structure. Rheological studies were conducted to determine the elastic modulus of gels during their reaction with periodate (Fig. 6a). Before reaction (at time = 0), the elastic modulus was proportional to the amount of DAT used in the synthesis. The G′ initial were 9.6 and 4.3 kPa for HG-6 and HG-1, respectively. This is in agreement with results of %ESR studies. However, after addition of a periodate solution (250 s), the elastic modulus decreased drastically when the hydrogels were prepared with DAT. In less than half an hour, the elastic modulus of the different gel samples was stabilized at a value of G′ similar to gels obtained from HG-1 (prepared only with BIS 5%). The time required for 95% decrease of the elastic modulus ($t_{95\%}$) was in all cases less than 21 min. Fig. 6b shows a comparison between the elastic modulus of hydrogels (HG-1-6) as a function of the concentration of DAT before and after reaction with periodate. Regardless of the initial concentration of DAT, the final values of hydrogel elasticity

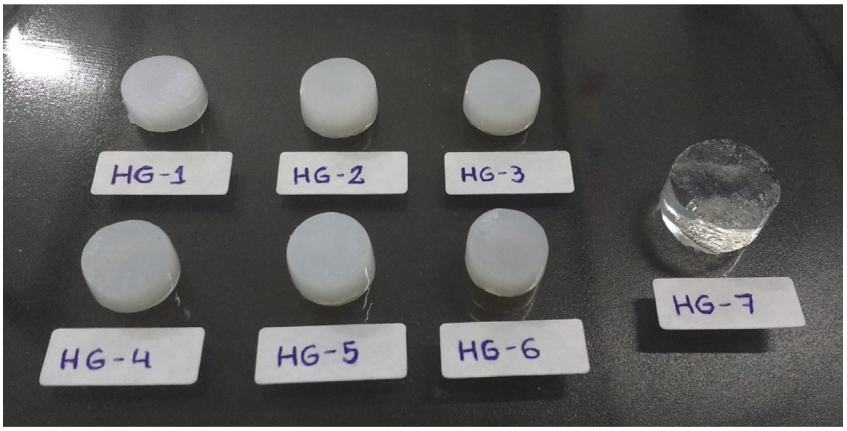

Fig. 4. Photographs of HG-1-7 hydrogels.

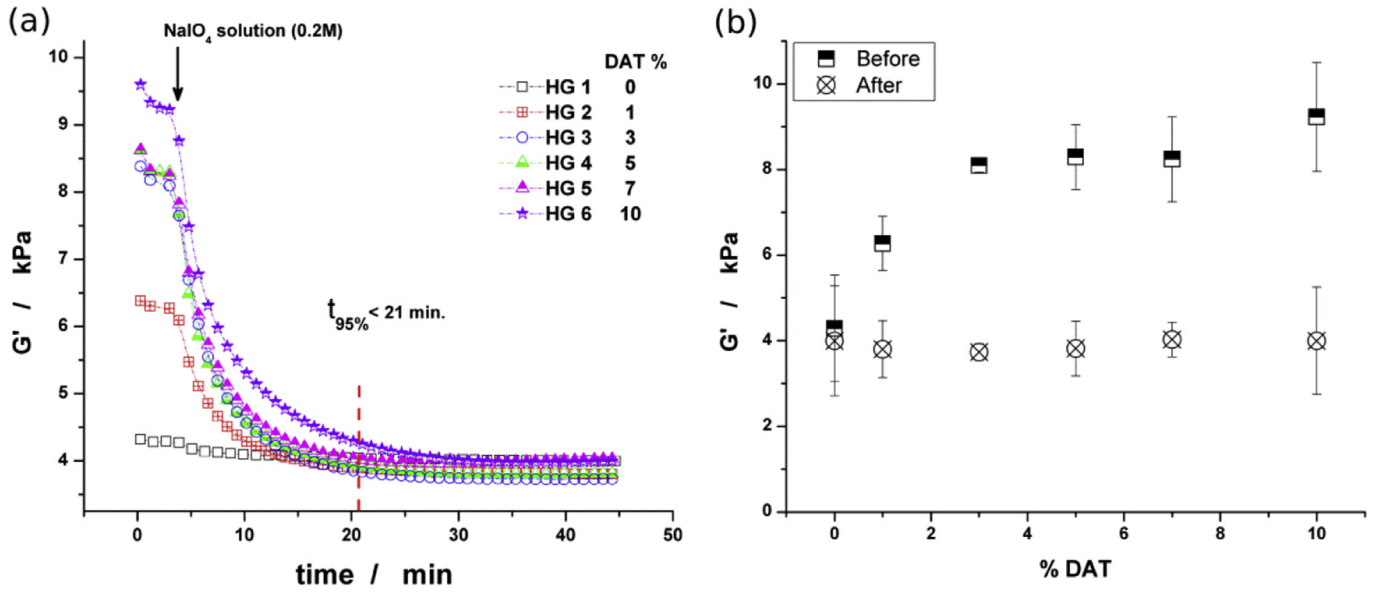

Fig. 6. **(a)** G′ vs. time (s) for HG-1-6 in the presence of periodate. The arrow shows the time of addition of periodate. The dotted line indicates the time in which the elasticity has been reduced 95%. **(b)** G′ vs. %DAT for hydrogels before and after contact with periodate.

decrease to the 2.5—5.5 kPa interval, equivalent to the hydrogel that was not crosslinked with DAT (HG-1).

The kinetics of the cleavage reaction was also studied by measuring the rate of swelling at different times (SR_t) with respect to the equilibriun swelling ratio (ESR) in water of HG-1-6. For clarity, in this assay ESR vas named SR_i (initial swelling rate). Fig. 7 shows the change in the ratio (SR_t/SR_i) as a function of time. It is observed that the hydrogels sintesized with DAT present an increase of the swelling index between 10 and 40%. Instead, HG-1 (which does not have DAT) does not show appreciable changes. Such volume increase is approximately proportional to the amount of DAT used to prepare each hydrogel. In addition, as observed by rheology, the cleavage produces a decrease in the elasticity of the network, which would allow more solvation of the polymer, producing an increase of the hydrogel swelling degree.

These results suggest that crosslinks due to DAT units in the network have been cleaved with periodate without changing the crosslinks of BIS units. Moreover, it was observed that the effect of periodate ion on DAT containing hydrogels was not simultaneous across all the network. By contrast, it was found that the interior of the gels was slightly more opaque in the first minutes of the cleavage reaction. Hence, studies in which DAT crosslinks were partially cleaved by periodate were carried out.

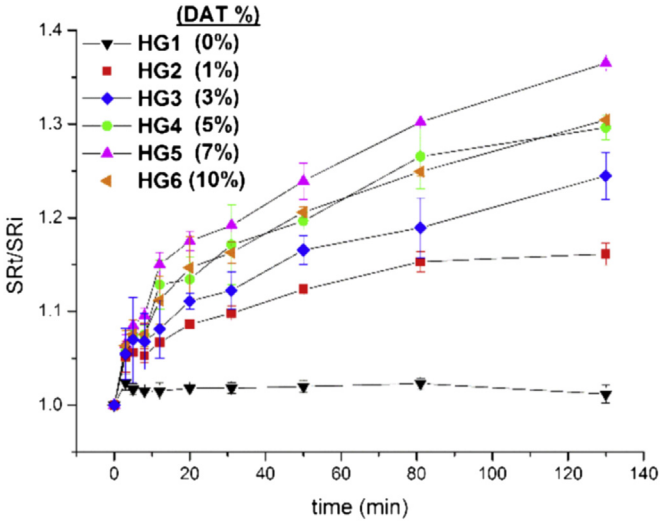

Fig. 7. (SR_t/SR_i) vs. time (min) for hydrogels (HG-1-7) during the reaction with periodate.

3.2.2. *Partial cleavage of DAT in hydrogels crosslinked with BIS and DAT*

Fig. 2B shows a schematic diagram of HG-6, after reaction with periodate as a limiting reagent. Hydrogels were cleaved with periodate ion using it as a limiting reagent. In this case, the periodate ion was consumed before cleaving all the vicinal diols present in the network of the hydrogels containing DAT. The reaction was evidenced by the color change of the external zone from whitish to transparent, leaving a whitish central zone in the material. Fig. 8a shows a photograph of a hydrogel disc (HG-6) after reaction with periodate as a limiting reagent. The dotted circle shows an internal zone more opaque presumably with absence of cleavages. Later, an axial cut of the modified hydrogel was performed for the microscopic study. A cross-section of a dehydrated hydrogel disc after partial cleavage of DAT was observed by confocal microscopy (Fig. 8b). The dotted line circle encloses the central part of the hydrogel, showing an internal zone morphologically different to the external area. In order to observe clearly the difference between the external and the internal zones, an assay with 2,4-dinitrophenylhidrazine (wich reacts selectively with aldehydes an ketones, giving coloured products) was performed and it can be found in the S.I. as Fig. S8. Furthermore, Figs. 8c and d show a confocal image of external (c) and internal (d) zones of the hydrated hydrogel obtained using a DIC accessory. The external area shows higher roughness than the internal zone, probably caused by the presence of many pores. Other images of confocal microscopy can be observed in the Supporting Information (S.I. Figs. S6-7). The presence of higher roughness in the outer zone of the material could be related with the decrease of elasticity (crosslinks), observed by rheology.

The reaction of rupture could be produced while the diffusion

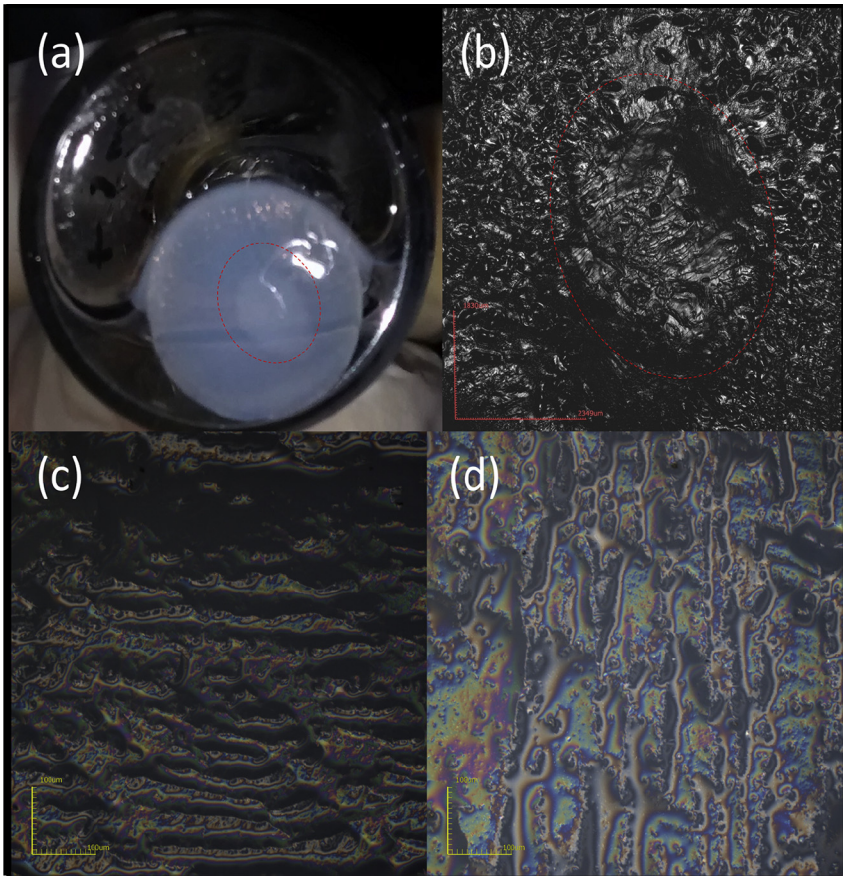

Fig. 8. **(a)** Photograph of a hydrogel disc (HG-6) after reaction with periodate as a limiting reagent. The dotted line circle shows an internal zone more opaque presumably with absence of cleavages. **(b)** A cross section of a freeze-dried hydrogel disc after partial cleavage of DAT observed by confocal microscopy. The dotted circle encloses an internal zone morphological different to external area. Confocal image (with a DIC accessory) of external (c) and internal (d) zones of this hydrated hydrogel.

front of periodate was moving throughout the gel, as observed in other studies of modification of hydrogels with periodate [33,34]. This controlled modification could be used as a post-synthesis method to control the crosslinking degree or porosity of a gel, also with spatial control of the required properties.

3.3. Total rupture of hydrogels crosslinked using periodate (HG-7)

The tridimensional network of HG-7 obtained with AM and DAT is assumed to be supported exclusively by the presence of crosslinks originated by DAT units incorporated into the hydrogel structure. The reaction of periodate with HG-7 would lead to cleavage of all crosslinks. Fig. 9 shows the images of HG-7 before (a) and after (b) reaction with periodate. The product obtained from the reaction was a translucent viscous liquid (LHG-7). As observed previously, the hydrogels containing different amounts of DAT and BIS did not produce liquid products. Accordingly, the results of this study confirm that the crosslinks produced by DAT are those holding the structure and are the targets of cleavage by periodate.

The studies confirm that the reaction between periodate and the DAT units present in the network of the hydrogel occurs. However, it was necessary to perform additional studies to determine the chemical composition of the cleaved product. For this purpose, both the rupture of crosslinks of HG-7 and the formation of new functional groups in the polymer were characterized by ^{1}H NMR studies (Fig. 10a and b). Also, the monomer DAT was studied in the absence and presence of periodate by ^{1}H NMR spectroscopy as shown in Fig. 10c and d, respectively. In the absence of periodate, DAT shows a

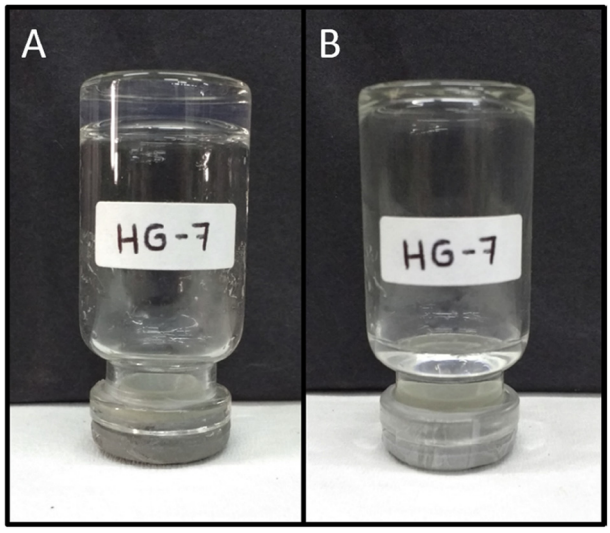

Fig. 9. Image of HG-7 before **(a)** and after **(b)** reaction with periodate. The product obtained from the reaction was a translucent viscous liquid (LHG-7).

particular signal at 4.6 ppm which can be assigned to the carbinolic H of the vicinal diol (indicated as H_a in the structure proposed, Fig. 10c). As expected, this signal disappeared when DAT was treated with an excess of periodate (Fig. 10d), due to C-C bond cleavage of the vicinal diol. Furthermore, a new signal at 5.35 ppm

appears which can be assigned to the aldehyde hydrate (H_b, Fig. 10d) indicating the generation of two identical aldehyde molecules as products of DAT cleavage. Indeed, in the literature, aliphatic aldehyde molecules of similar characteristics are commonly found in their hydrated form [28].

Subsequently, a characterization by ^{1}H NMR of HG-7 before and after its reaction with periodate was performed (Fig. 10a and b, respectively). Fig. 10a shows the spectrum of HG-7 which has broad signals. Such response can be associated with low molecular mobility in the hydrogel caused by the crosslinks of polymer chains. Despite this, the signal at 4.6 ppm (H_a) can be clearly identified. Moreover, Fig. 10b shows the product resulting from treating HG-7 with excess of periodate. The product was liquid, and consequently, the signals observed by ^{1}H NMR become narrower. Furthermore, we can see the disappearance of the peak at 4.6 ppm and the appearance of the signal at 5.35 ppm, attributed to the presence of hydrated aldehyde in the product. The ^{1}H-NMR spectra can be found in the S. I. as Figs. S2-5.

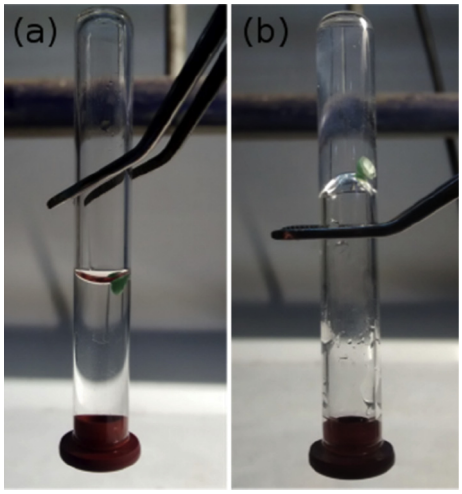

Fig. 11. Re-crosslinking of cleaved HG-7. **(a)** Cleaved product of HG-7 (LHG-7) with periodate; **(b)** Regeneration of hydrogel (RHG-7) using ADH.

3.4. Re-crosslinking of cleaved hydrogels

Gels crosslinked only with DAT (HG-7) were treated with periodate. The rupture of crosslinks generates a cleaved product (LHG-7), which is a viscous liquid having aldehyde functional groups, as observed by ^{1}H NMR (Fig. 10d). The reactivity of these aldehyde groups was studied using ADH. In Fig. 11a, an inverted tube containing LHG-7 in liquid state is shown. However, the addition of a bifunctional hydrazide, ADH, causes the formation of new covalent crosslinking points between the polymer chains in less than 5 min, leading to a translucent RHG-7 gel (Fig. 11b).

The re-crosslinking kinetics of LHG-7 with ADH to obtain RHG-7 was studied by rheology as is shown in Fig. 12. It is observed that the elastic modulus of LHG-7 is approximately 0 Pa, because the polymer is initially in the liquid state. Subsequently, after the addition of ADH (t = 10 min) the elastic modulus increases exceeding 10^2 Pa in less than 5 min. Subsequently, the rate of increase of G′ is reduced and consecutive increases of $\approx 10^2$ Pa require 15; 30 and 120 min of reaction, respectively.

Subsequently a ^{1}H NMR characterization of the chemical reaction between LHG-7 and ADH was performed and shown in Fig. 13. In this study, different amounts of ADH were added to a solution of LHG-7 in D_2O. LHG-7 in the absence of ADH shows the

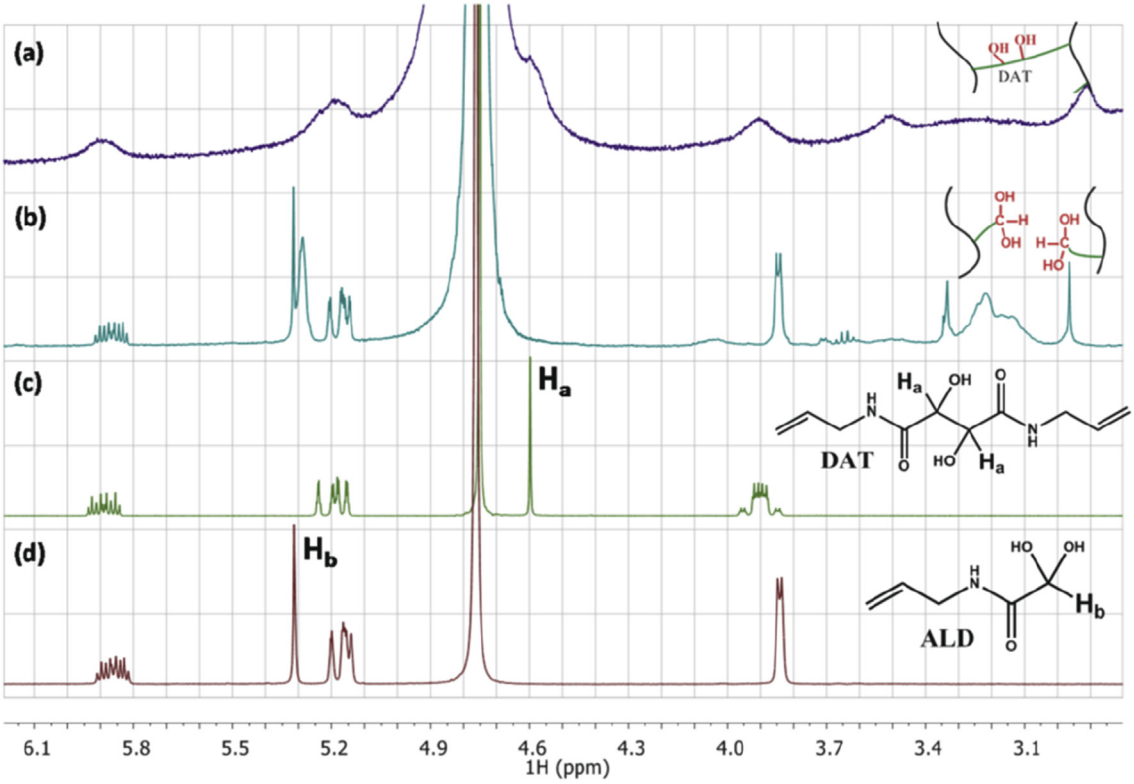

Fig. 10. ^{1}H NMR of DAT and HG7 before and after reaction with periodate. **(a)** HG-7; **(b)** HG-7 + $NaIO_4$ ($NaIO_4$ in excess); **(c)** DAT; and **(d)** DAT + $NaIO_4$ ($NaIO_4$ in excess). On the right: molecular structure of DAT and proposal structure of product ALD. H_a and H_b are relevant protons to analyze the reaction changes.

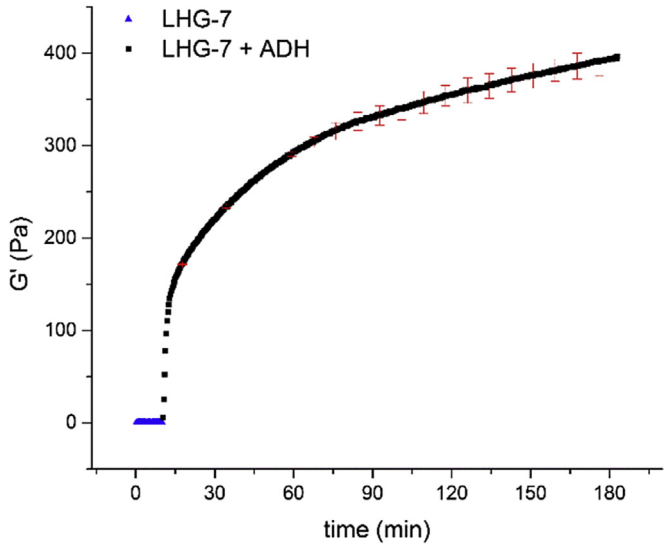

Fig. 12. G′ vs. time for LHG-7 before (blue) and after (black) the addition of ADH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

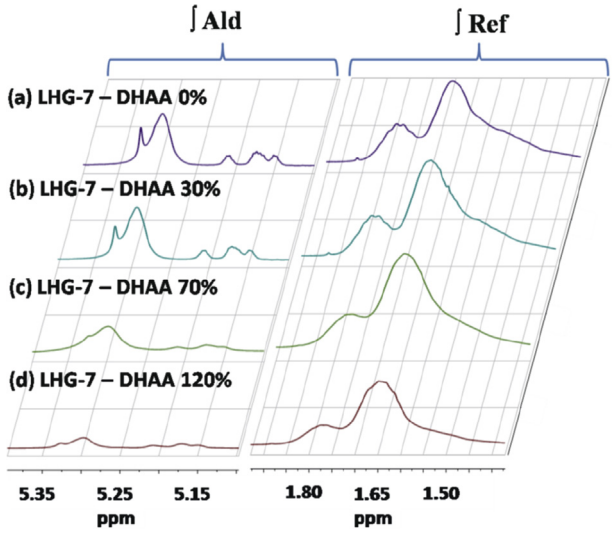

Fig. 13. Reaction between LHG-7 and ADH studied by ^{1}H NMR. Response of different additions of ADH (0; 30; 70 and 120%) to a solution of LHG-7 in D_2O.

characteristic signals attributed to hydrated aldehyde at 5.35 ppm. Furthermore, the gradual addition of ADH produces a broadening of the NMR signals, which indicates that the material passes from a liquid state to a gel state, because the polymer chains have less freedom of movement. In addition, we can observe the relative decrease of the aldehyde signals with respect to those belonging to AM (1.4 a 1.9 ppm, used as reference signal).

This was confirmed by the measurement of the integral area of the signals. Table 2 shows the integral area of aldehydic protons (∫Ald, 5.25–5.4 ppm) and the ratio between ∫Ald and reference protons (∫Ref, 1.4–1.9 ppm) for LHG-7/ADH 0; 30; 70 and 120%. The results indicated that the protons belonging to aldehyde decreased 60% in relation to their initial area, as a result of reaction with ADH.

4. Conclusion

In this paper, novel gels using crosslinking agents such as DAT and BIS were synthesized. Studies performed on gels crosslinked with increasing concentrations of DAT (0–10%) and 5% BIS showed that swelling equilibrium volume in water decreased, and the elastic modulus increased. Moreover, DAT has a vicinal diol and was found that it can be selectively cleaved by reaction with periodate. After this reaction, the elastic modulus of the different gel samples was stabilized at a value of G′ similar to that of the gels obtained only with BIS 5%. This finding indicated that the DAT incorporated into the gel was cleaved while the gel state was maintained only by BIS. However, it was observed that the effect of periodate ion on hydrogels containing DAT was not simultaneous across the entire network. By contrast, the interior of the gels remained less porous during cleavage reaction, when periodate was used as a limiting reagent. This finding can be used for the development of materials with controlled porosity or elasticity. Furthermore, gels crosslinked only with DAT produced a viscous liquid after reaction with periodate. By NMR studies, it was found that DAT can be selectively cleaved by periodate when it reacts as a monomer or incorporated into the polymeric network, obtaining hydrated aldehyde groups as products. Furthermore, the reactivity of the aldehyde groups was verified by reaction with ADH, producing new hydrogels. These products were also verified by NMR studies. The results indicated that the incorporation of DAT in the gels and then a cleavage with periodate would be a useful tool to yield polymers having aldehyde groups. This method could be useful to attach a variety of molecules to a matrix or to functionalize diverse synthetic polymers.

Table 2
Integral area of protons calculated from ^{1}H NMR of Fig. 13.

ADH (%)	∫Ald	∫Ref/∫Ald
0	0.047	21.28
30	0.043	23.26
70	0.038	26.32
120	0.019	52.63

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymer.2017.03.068.

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