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Oxidation of sodium alginate and characterization of the oxidized derivatives

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Abstract

Oxidation reactions on –OH groups at C-2 and C-3 positions of the uronic units of sodium alginate were performed with sodium periodate, and the influence of degree of oxidation on physical properties of the oxidized derivatives was analyzed. The aim of this work was to find new active functional groups on alginates, which is a polymer of interest as support in drug-controlled delivery systems. The molar mass decreases rapidly until an oxidation of 10 mol% and then remains nearly constant. Polymers with a degree of oxidation higher than 10 mol% were no more able to form gels with calcium ions. Both elastic modulus (G') and swelling degree (SD) of alginate gels decrease as the degree of oxidation increases. This behavior was attributed to the decreasing in the cooperative interactions between calcium ions and carboxylate groups due to a decrease in both the molar mass and the number of unreacted G units.

Keywords: Alginates; Hydrogels; Biopolymers; Sodium periodate; Oxidation

1. Introduction

Polymer delivery systems have been used for several applications in food, therapeutic, cosmetic and agriculture (Bouhadir, Alsberg, & Mooney, 2001). Such delivery systems have many advantages over conventional ones especially for medical applications. When a drug is injected or absorbed, its level in plasma increases reaching a maximum and then rapidly decreases. Above and below some critical level, the drug can be either toxic or inefficient. Polymer drug carriers allow a controlled release of a bioactive substance over a long period of time in the plasma. Such system is thus adapted for sustained release of bioactive molecules. (Ain, Sharma, Khuller, & Garg, 2003; Kikuchi & Okano, 2003; Singh & O'Hagan, 1998; Xing, Dawei, Liping, & Ronqing, 2003). Besides, the polymers used for delivery systems are usually hydrogels, which can offer an interesting property as bioerodible materials. Biopolymers and several synthetic degradable polymers have been used for the preparation of such materials (Ain et al., 2003). Among these compounds, naturally occurring polysaccharides are very attractive. Many polysaccharides like alginates may form physical gels under specific conditions, and their functional groups (–OH and $–COO^-$) also allow different chemical and physical modifications.

Alginate, a water-soluble linear polymer obtained from brown algae, is composed of (1–4)-β-D-mannuronic acid (M) and (1–4)-α-L-guluronic acid (G) units in the form of homopolymeric (MM- or GG-blocks) and heteropolymeric sequences (MG- or GM-blocks). Bearing in mind their gelling ability, stabilizing properties and high viscosity in aqueous solutions, alginates and their derivatives are widely used in the food, cosmetics and pharmaceutical industries (Drury & Mooney, 2003; Ci et al., 1999; Kong, Smith, & Mooney, 2003). In addition, oxidized alginates present more reactive groups and a faster degradation when these ones are used in supports for drug controlled delivery (Boontheekul, Kong, & Mooney, 2005; Kong,

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Kaigler, & Mooney, 2004). Taking into account previous considerations, our objective was to improve the reactive properties of native alginates by studying the effect of chemical modifications on the polymeric chain and its relation with the ability to form hydrogels in the presence of calcium ions.

In the present work, oxidation reactions with sodium periodate on –OH groups at C-2 and C-3 positions of the uronic units were investigated and the influence of degree of chain oxidation on physical properties was analyzed.

2. Experimental

2.1. Reagents

The following chemicals were purchased and used: sodium alginate (NaAlg) (Fluka, Switzerland, No. 71238); sodium periodate (Anedra, Argentine, p.a.); ethylene glycol (Eclaire, Argentine, p.a.); monobasic sodium phosphate (Anedra, Argentine, p.a.); iodine (Merck, Germany, p.a.); potassium iodide (Merck, Germany, p.a.); sodium chloride (Merck, Germany, p.a.); soluble starch (Anedra, Argentine); sodium borohydride ($NaBH_4$) (Fluka, Switzerland, p.a.), sodium cyanoborohydride ($NaBH_3CN$), (Fluka, Switzerland, 95%); acetic acid (CH_3COOH), (Merck, Germany, p.a.); sodium chloride, (Cicarelli, Argentine, p.a.).

2.2. Oxidation reaction on sodium alginate

Alginate oxidation reactions were carried out in aqueous solution at room temperature during 24 h. In a dark bottle, NaAlg (10.00 g) was solubilized with distilled water (600 mL) and then an aqueous solution of sodium periodate (100 mL) was added under stirring, reaching a final volume of 1 L with distilled water. Different ratios of sodium periodate to number of repetitive units (5, 10, 19, 25, 38, 50 or 75 mol%) were used. After 24 h, the reaction was quenched by the addition of ethylene glycol (3.50 mL) under stirring for 0.5 h. The oxidized alginate was purified by precipitation with the addition of NaCl (3.00 g) and ethanol (1 L). The polymer was again dissolved in water (500 mL) and re-precipitated by the addition of ethanol (500 mL) in the presence of NaCl (1.00 g). The process was repeated using NaCl (0.50 g) and the polymer was precipitated with acetone (1000 mL) under its sodium salt form. Finally, the precipitate was washed in ethanol (500 mL) under stirring during 15 min, isolated and dried at room temperature under vacuum.

In other experiments, the kinetics of oxidation reaction was studied employing a molar ratio 1/3 of monomeric unit to sodium periodate to demonstrate the degradation of the polymeric backbone. The experiments were performed at room temperature, where 75 mL of alginate aqueous solution (4 g/L) and 15 mL of sodium periodate solution (0.3 M) were left under stirring, into a dark flask, taking samples of 5.0 mL every 30 min. After that, three drops of ethylene–glycol were added to quench the reaction and the sample was stirred for 30 min at room temperature. Aldehyde groups of oxidized alginates were reduced by the addition of $NaBH_4$ (excess) leaving to react 1 h more. Finally, the reaction was neutralized with CH_3COOH (0.3 N) and analyzed by SEC.

2.3. Determination of oxidation degree by UV–Vis absorption spectroscopy

The indicator solution was prepared by mixing equal volumes of 20% (w/v) KI and 1% (w/v) soluble starch solutions, using buffer phosphate pH 7 as solvent.

Before adding the quencher into oxidation reaction, 1.00 mL of solution was diluted to 250 mL with distilled water. Then, 3.00 mL of this diluted solution was mixed with 1.50 mL of indicator solution and the volume was completed to 5.00 mL with distilled water. The absorbance of triiodine–starch complex was rapidly measured with a spectrophotometer Shimadzu UV-160A at 486 nm. The periodate concentration in the sample was obtained using the molar absorption coefficient previously calculated from absorbance of the complex versus IO_4^- concentration (0.8–2.10^{-5} M). The difference between initial and final amount of IO_4^- corresponds to glycol moieties transformed into aldehyde groups.

2.4. Modification and determination of aldehyde groups on alginates

Aldehyde groups on the polymeric chain were reacted with *n*-propylamine in the presence of $NaBH_3CN$ by reductive-amination reaction. Alkyl side chains generated on backbone were used in the determination of free aldehyde groups by ^1H NMR spectrometry. A sample of oxidized alginate (0.2 g) was solubilized with water (67 mL) at room temperature under stirring. Depending on the polymer degree of oxidation (5, 10, 19, 25, 38 or 50%), 1-propanamine (14.8, 29.6, 56.2, 73.9, 112.3 or 147.8 mg) was slowly dropped into polymer solution with high stirring, and the pH was adjusted to a value of 6 by the addition of HCl 0.1 N. Next, $NaBH_3CN$ (15.7, 31.4, 59.7, 78.5, 119.3 or 157 mg), previously solubilized in 3 mL of water, was slowly added to the reaction mixture and stirred at 25 °C for 24 h. Then, the reaction mixture was adjusted to pH 7.3 by the addition of 0.1 N NaOH solution and NaCl was added up to a 1 N salt solution in order to screen the electrostatic interactions between chains and to exchange counter ions other than sodium. The polymer was precipitated by using ethanol (slow addition under high stirring) and washed with pure ethanol. Finally, the polymer was dried until constant mass at room temperature.

2.5. Characterization by SEC

Purified alginates isolated under the sodium salt form were characterized by size exclusion chromatography (SEC) using a Waters Alliance GPCV2000 (USA) equipped

with three detectors on line: a differential refractometer, a viscometric detector and a multiangle laser light scattering (MALLS) detector from Wyatt (USA). The concentration injected was in the range of 0.5–5 g/L (depending on the range of sample molar mass), with an injection volume of 108 μL using two columns in series (Shodex OH-pack 805 and 806). The eluent used was 0.1 M $NaNO_3$ (containing 0.1 g/L NaN_3 as bactericide) at 30 °C as elution temperature and a flow rate of 0.5 mL/min. Weight-average molar mass (M_w), polydispersity index ($I = M_w/M_n$) and molar mass distribution were obtained as characteristic of the polymers. From the SEC data, the intrinsic viscosity of solutions at a given molar mass (M) is determined as a test of the polymer stiffness. For native and purified-native alginates, concentrations of 0.5 and 1 g/L were used, whereas for oxidized alginates (5, 10, 19, 25, 38 and 50% of oxidation) the concentration was 5 g/L. The polymers were left overnight in SEC solvent at room temperature and total dissolution was reached. Samples were filtrated on a 0.2 μm pore membrane (Sartorius A.G. cellulose acetate filter) before injection.

2.6. Rheology

The rheological behavior of gels was studied using an "AR 1000 rheometer from TA Instruments (USA)" at 25 °C. For gel characterization, 2 cm diameter parallel plate geometry was used within the viscoelastic region in dynamic experiments (strain approximately 0.02%). The elastic (G') and loss (G'') moduli were determined as a function of the frequency (ω, expressed in Hz). Temperature dependence was measured at a frequency of 1 Hz and at a given temperature rate (1 or 5 °C/min) using the Peltier plate. A silicone oil film protected the sample in order to avoid water evaporation.

2.7. Elemental analysis

Sodium and calcium contents of the different samples were determined at the CNRS central microanalysis Service at Solaize (France). Initial commercial sample had 1200 ppm of residual calcium content, while all the modified samples have calcium content lower than 600 ppm. Low calcium content is necessary to avoid aggregation of alginate molecules in order to obtain the true molar mass and solution viscosity.

2.8. NMR analysis

Alginate composition was determined by ^{1}H NMR spectrometry, using polymer samples dissolved in D_2O at a concentration of 6 mg/mL. ^{1}H NMR experiments were performed using a "Bruker AC 300 spectrometer" at 80 °C, with 24 and 50,000 scans number for both proton and carbon, respectively. The delay adopted was 20 s. Chemical shifts are given relative to an external reference of tetramethylsilane signal (TMS = 0 ppm). Deuterium oxide was obtained from SDS (Vitry, France).

^{1}H NMR spectroscopy is suitable for characterizing both the composition and the distribution sequence of the two-uronate residues in alginate samples. We have assigned the signals at 5, 4.6 and 4.37 ppm like that corresponding to H_1-G (peak 1), H_1-M + H_5-$\underline{G}$M (peak 2) and H_5-$\underline{G}$G (peak 3), respectively. Taking into account that proton area of H_1-G and H_5-G are equals, it is possible to calculate H_5-$\underline{G}$M from the difference between peaks 1 and 3. Then, H_1-M area can be obtained from the difference between peak 2 and H_5-$\underline{G}$M area. From those values, the M/G ratio is calculated as H_1-M/H_1-G, whereas the fraction GG is obtained as H_5-$\underline{G}$G/H_1-G.

2.9. Gelation ability of alginates

Polymer gelation in the presence of calcium ions was obtained by dialysis through a porous cellulosic membrane "Spectra/Por" (exclusion limit $M = 12$–14,000). Alginate solutions at concentration from 2 to 20 g/L under sodium form were introduced in the dialysis bag against 1 M $CaCl_2$ during 48 h to assure equilibrium. Slides of gel (3 mm thick and a diameter near to 20 mm) were cut and placed between the plates of the rheometer. The same technique was used with oxidized-alginate solution prepared at 20 and 60 g/L and dialyzed against $CaCl_2$.

2.10. Swelling and re-swelling of alginate gels

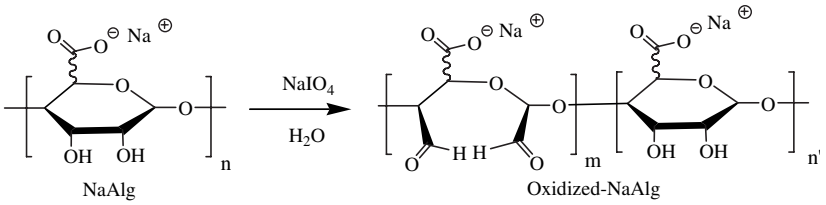
Swelling degree (SD) was calculated as water mass per gram of dried polysaccharide under its sodium form. The amount of water was determined by the difference of weight between the swollen gel as it was formed and the dried weight of polymer under sodium form on the basis of the amount of solution introduced in the bag.

The formed gel in presence of $CaCl_2$ was dried and then swollen again in water. Before determining the swelling degree value, the gel was equilibrated in water and it was exchanged 5 times in order to eliminate the excess of $CaCl_2$ included in the gel. The swollen gel was weighted and then dried at 70 °C until constant mass. In this case, the value of swelling degree was also expressed as water mass per gram of dried polymer under calcium form.

3. Results and discussion

3.1. Influence of periodate concentration on oxidation reaction

Sodium alginates were oxidized at room temperature during 24 h, in aqueous solution, using sodium periodate in the dark. The ratio of sodium periodate to number of repetitive units of alginate used in each reaction was varied to reach different degrees of oxidation. Hydroxyl groups on carbons 2 and 3 of the repetitive unit were oxidized by sodium periodate (Scheme 1) which leads, by rupture of carbon–carbon bond, to the formation of two aldehyde groups in each oxidized monomeric unit. Therefore, larger



Scheme 1. Oxidation of sodium alginate. Formation of aldehyde groups on main chain.

rotational freedom and new reactive groups along the backbone are obtained. As can be seen in Table 1, sodium periodate was quantitatively consumed in all conditions except for a 75 mol% of sodium periodate. In this case, only 90% of sodium periodate was consumed. This behavior can be related to hemiacetal formation for high concentration of aldehyde groups in oxidized uronates. These groups can react with alcohol groups in the vicinity as suggested in the literature (Frollini, Reed, Milas, & Rinaudo, 1995; Painter & Larsen, 1970; Smidsrød & Painter, 1973). The formation of hemiacetals protects some –OH groups from further oxidation.

The introduction of aldehyde groups on the polymer gives new reactive groups for chemical modification especially by reductive-amination as suggested previously (Kang, Jeon, Lee, & Yang, 2002).

3.2. Characterization of oxidized alginates

Different techniques were used to determine the aldehyde content in the modified polysaccharides. Taking into account assigned signals described previously (Heyraud et al., 1996). ^{1}H NMR was performed to characterize the native alginate, where a M/G ratio equal to 0.47 and a fraction of 0.63 for G groups in the GG blocks were found. The M/G ratio was also confirmed by ^{13}C NMR, on a partially depolymerized sample, as shown in Fig. 1(A).

Oxidized samples show changes in the ^{1}H NMR spectrum with modification of the H-1 and H-5 signals for M and G units. In addition, the appearance of two new signals at 5.15 and 5.4 ppm, which would correspond to a hemiacetalic proton formed from aldehyde and neighbors hydroxyl groups, also confirm the proposed modification.

An interesting conclusion from the comparison of A and B ^{13}C NMR spectra (Fig. 1) is obtained. For a degree of oxidation of 19 mol%, the signal corresponding to G-1 becomes smaller than M-1, which indicates that G units react preferentially. However, no quantitative determination of the degree of oxidation was possible to reach by this technique. The spectrum is progressively modified when the degree of oxidation increases with appearance of new signals in the region of 90–95 ppm, which should be attributed to hemiacetalic carbons corresponding to aldehyde groups generated.

Table 1
Oxidation reaction of Na-alginate with sodium periodate at room temperature

Oxidized alginates (%)	$NaIO_4$ (mol%)		Oxidation yield (mol%)
	Added	Consumed	
5	5.0	4.9	98
10	10.0	9.7	97
19	19.0	18.7	98
25	25.0	24.8	99
38	38.0	37.6	99
50	50.0	49.5	99
75	75.0	67.5	90

This behavior would indicate that not all the aldehyde groups remain free. Consequently, only a fraction of those groups are available for reaction with propylamine choose as a reference for a reductive amination reaction on the available aldehyde groups. The alkyl side chains attached to oxidized alginates were determined by ^{1}H NMR spectrometry (Fig. 2), where the signal for $-CH_3$ and $-CH_2-$ appear at 1.5 and 0.8 ppm, respectively. Considering that G units react first, the integral for $-CH_3$ of the propyl chain was compared to that of the H-1 corresponding to G units located at 4.9 ppm (labeled G-1 on the spectrum). A relatively important fraction of the oxidized units seems to be unreacted in agreement with some previous data, as can be seen in the values given in Table 2 (Painter & Larsen, 1970; Smidsrød & Painter, 1973).

On the other hand, the weight-average molar mass (M_w) and the molar mass distribution were obtained by SEC measurements, after control of the residual calcium in the sample. As shown in Fig. 3, M_w of oxidized samples decreases very rapidly as the degree of oxidation increases. Also, the molar mass distribution becomes similar when larger degrees of oxidation (25 and 38 mol%) are compared (Fig. 4). All the results obtained were summarized in Table 2, where a large decrease in M_w as the degree of oxidation increases can be observed. This behavior is attributed to scission of the main chain as oxidation occurs, which indicate that degradation depends directly on the concentration of sodium periodate in the solution. A free radical-independent degradation has been attributed to the cleavage of the main chain of alginate.

3.3. Kinetics of oxidation reaction

The kinetics of oxidation was followed using an excess of sodium periodate. The samples were isolated from reaction media at different times and quenched by addition of ethylene glycol. The aldehyde groups of oxidized alginates were reduced with excess of $NaBH_4$ and then neutralized and analyzed by SEC. The evolution of the number and weight average molar mass is given in Table 3. Both M_w

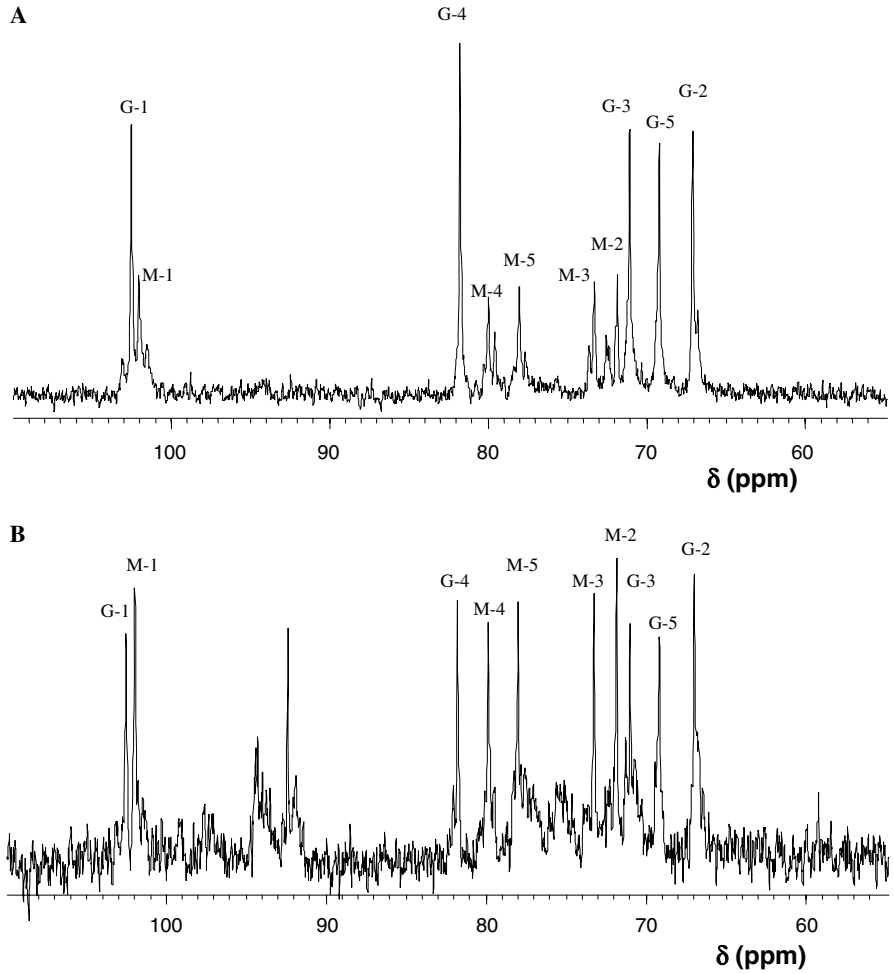


Fig. 1. ^{13}C NMR spectrum of native alginate (A) and 19 mol% oxidation (B) with identification of the main signals. G-i and M-i are the different carbons of the guluronic and mannuronic units, respectively.

and M_n decrease very rapidly until 30 min (50%), going to an apparent limit after 300 min.

A linear dependence of $1/M_n$ as a function of time is obtained (Fig. 5) in agreement with a statistic degradation of the backbone following the relationship:

$$1/M_n - 1/M_{n,0} = Kt, \quad (1)$$

where M_n is the number-average molar mass at a given time (t), $M_{n,0}$ is the initial number-average molar mass of the polymer and K is the rate constant under the experimental conditions. M_n drops to a value of approximately 20,000 g/mol after 5 h of reaction.

3.4. Stiffness of modified alginate chains

Different authors previously described this aspect for the oxidation of relatively low molar mass polyguluronate (Painter & Larsen, 1970; Smidsrød & Painter, 1973; Lee, Bouhadir, & Mooney, 2002). It was demonstrated that stiffness parameters B become higher when the degree of oxidation increases in agreement with enhance in the flexibility (Painter & Larsen, 1970; Smidsrød & Painter, 1973). Nevertheless, the persistence length of chains determined by light scattering with so low molar mass (from $M_w = 7100$ g/mol for the initial sample to 5900 g/mol for 85.6% of oxidation) is questionable (Lee et al., 2002).

Due to the decrease of M_w with the degree of oxidation, we considered that radius of gyration values obtained from light scattering were not precise enough to conclude on its basis about the evolution of chain stiffness with the oxidation degree. Then, from a more sensitive method such as viscometric detection, intrinsic viscosity values $[\eta]$ (mL/g) for a given molar mass were taken for each sample. The evolution of $[\eta]$ as a function of the degree of oxidation is given in Fig. 6 for $M = 100{,}000$ g/mol.

At a given molar mass if the conformation of the polymer and the thermodynamic parameters are the same, the intrinsic viscosity must be the same. We had discussed this behavior for other semi rigid chains and alginates in previous papers (Rinaudo, 1992; Rinaudo, 1993a, 1993b; Rinaudo, Roure, & Milas, 1999). The local stiffness of polysaccharides is an original property and can be also predicted from molecular modeling (Mazeau, Perez, & Rinaudo, 2000; Mazeau & Rinaudo, 2004; Petkowicz et al.,

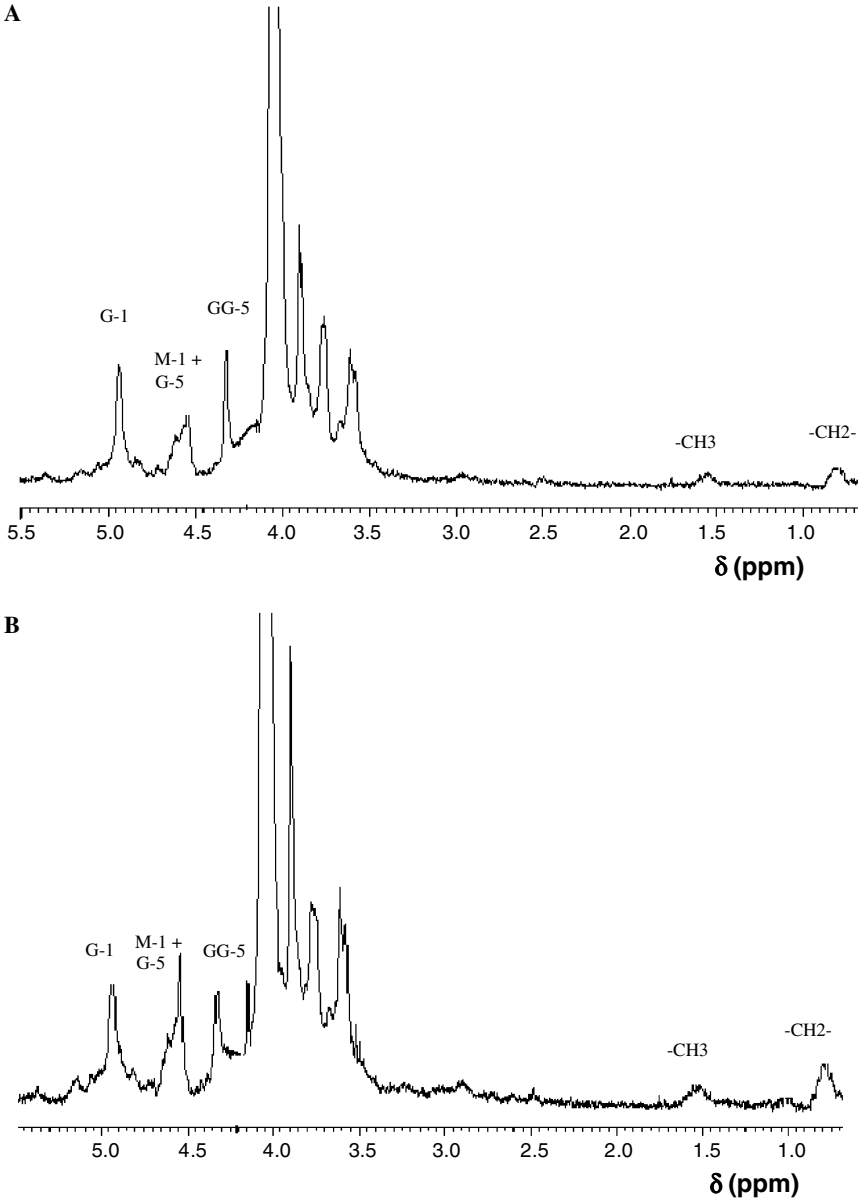


Fig. 2. ^{1}H NMR spectrum for propylated oxidized alginate in D_2O at 80 °C with identification of the main signals. G-i and M-i are the protons in i position in the guluronic and mannuronic units, respectively. GG-5 represents the proton in 5-position in GG block. (A) and (B) correspond to 19 and 25 mol% oxidation, respectively.

Table 2
Characterization of modified alginates by oxidation with $NaIO_4$

Oxidation degree (%)	M_w (g/mol)[a]	M_n (g/mol)[a]	M_w/M_n	Ca^{2+} content ppm)	Free aldehyde groups (%)[b]
0	231,500	107,700	2.15	1200	–
5	60,400	26,600	2.27	⩽500	~0
10	35,800	18,900	1.89	⩽500	2.1
19	31,200	17,000	1.84	⩽500	6.5
25	30,200	16,500	1.83	520	18.2
38	31,200	16,400	1.90	1500	nd
50	34,800	18,500	1.88	600	nd

[a] By SEC.
[b] By ^{1}H NMR (nd, not determined due to the deep modification of the spectrum).

1999; Rinaudo, 1993a, 1993b). In the same thermodynamic conditions, the intrinsic viscosity depends directly on persistence length as it was described in the Yamakawa–Fujii theory (Yamakawa & Fujii, 1974).

From our results (Fig. 6), we can conclude that stiffness as well as persistence length of the oxidized samples decreases with an increase in the degree of oxidation. This is related to the transformation of anhydroglycosidic units to a more flexible linear chain when C_2–C_3 linkages are broken. These results are in agreement with previous data (Painter & Larsen, 1970; Smidsrød & Painter, 1973), but we are not able to calculate seriously the persistence length value.

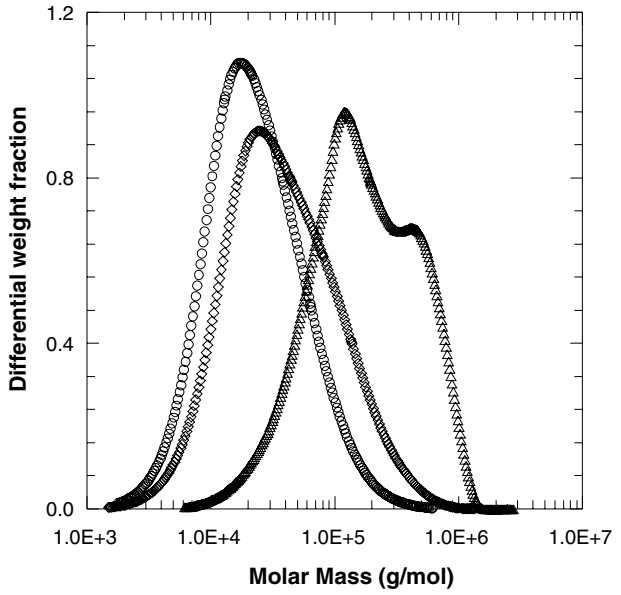


Fig. 3. Molar mass distribution for the (△) native alginate, (◇) 5 and (○) 10 mol% of oxidation.

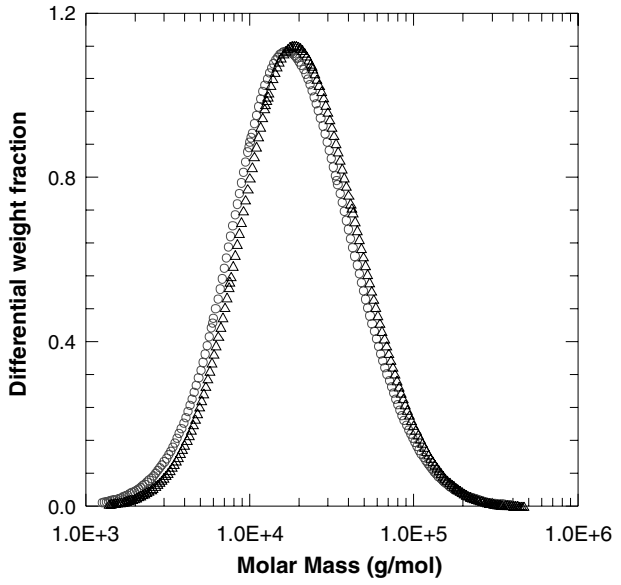


Fig. 4. Molar mass distribution for (○) 25 and (△) 38 mol% oxidation.

Table 3
Evolution of number and weight average molar mass as a function of the reaction time at room temperature ($C_p = 15$ g/L and molar ratio (1:3) uronate/sodium periodate)

T (min)	M_w (g/mol)	M_n (g/mol)
0	231,500	107,700
30	163,500	84,500
90	126,300	61,500
120	78,900	34,900
150	86,500	46,000
180	73,500	38,400
240	58,000	31,100
270	36,700	20,200
300	49,500	24,500
330	45,400	24,800
390	43,600	23,600
1470	20,700	12,800

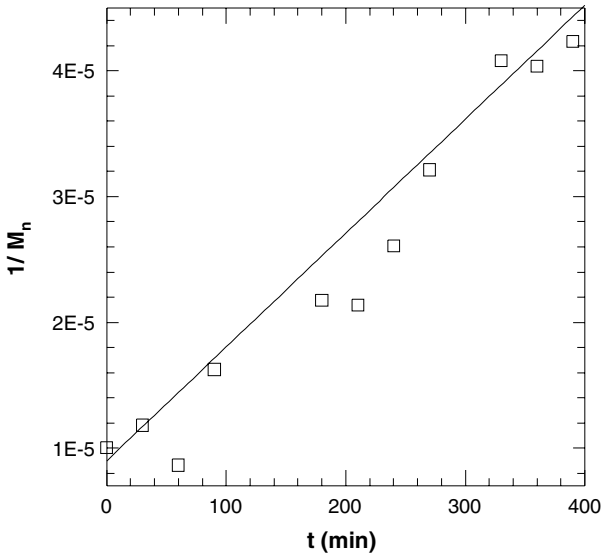


Fig. 5. Inverse of the number-average molar mass as a function of reaction time.

3.5. Ability for gelation

3.5.1. Native alginate

Gels of alginate were prepared by dialysis against 1 M calcium chloride aqueous solution having a polymer concentration from 2 to 20 g/L. The diffusion of calcium counter ions was left for 2 days at room temperature and then the mechanical behavior was tested in dynamic experiments with parallel plate geometry. The dependence of the elastic modulus (G') as a function of polymer concentration (C_p) is shown in Fig. 7. In a log–log plot the slope is nearly equal to 2 (Section 2.2), as we obtained previously on different polysaccharide physical gels (Rinaudo, 1993a, 1993b). The degree of swelling related to the crosslinking density of the gels was determined and it is shown in Fig. 8. It decreases very rapidly when the polymer concentration increases up to 5 g/L and then a little change is obtained for higher concentrations.

3.5.2. Oxidized alginates

Gels obtained with 20 g/L aqueous solution of native alginate show relatively good mechanical properties (high G' and low SD). For this reason, the solutions of oxidized alginates were prepared at 20 g/L and equilibrated with calcium chloride. In these conditions, gels were formed only for alginates with 5 and 10 mol% oxidation. The mechanical properties and swelling degree of these gels were compared with those obtained for the native alginate (Fig. 9). A decreasing in both G' and SD was found when the degree of oxidation increases. This behavior can be related to the decrease of both the molar mass and the number of GG

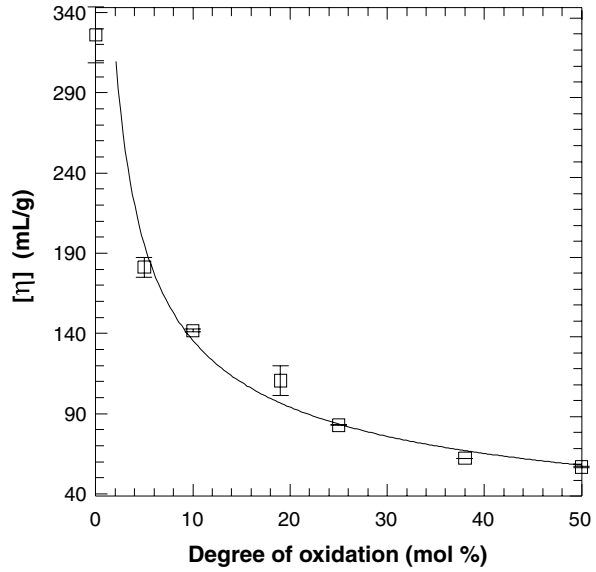


Fig. 6. Intrinsic viscosity as a function of the degree of oxidation for a constant molar mass ($M = 100{,}000$ g/mol) in two separated series of SEC experiments.

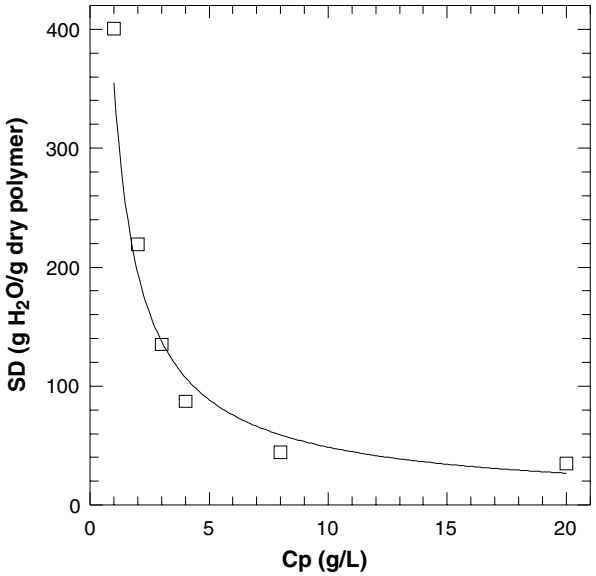


Fig. 8. Swelling degree (SD) of native-alginate gels as a function of polymer concentration (C_p) in the initial solution.

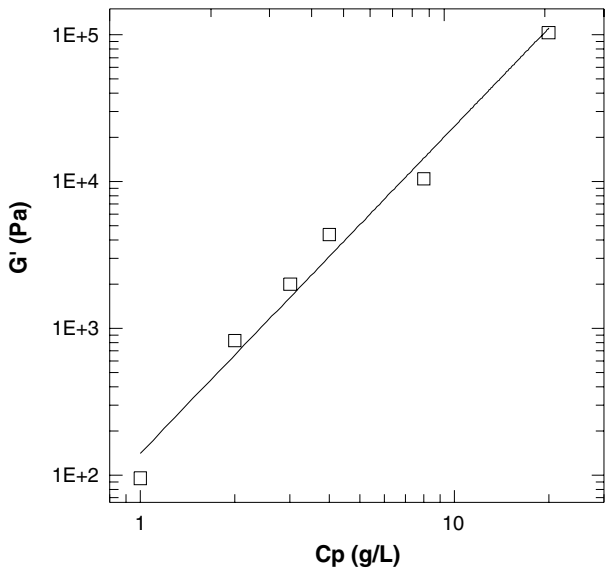


Fig. 7. Elastic modulus (G') as a function of polymer concentration (C_p) for Ca-alginate formed gels.

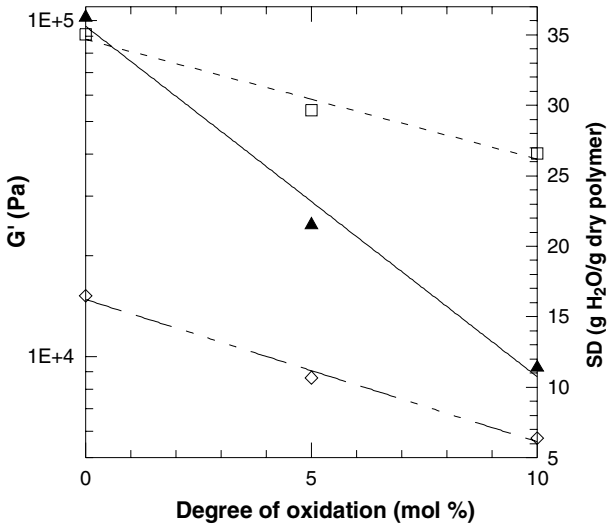


Fig. 9. Elastic modulus (G', ▲), and both swelling (□) and re-swelling degree (◇) as a function of % oxidation. Gels prepared with a $C_p = 20$ g/L of oxidized alginate against 1M $CaCl_2$.

blocks, which give as result a reduction of the cooperative ability for calcium interaction (Martinsen, Skjaak-Braek, & Smidsrød, 1989). This result is important because the derivatives will have a limited application as hydrogel of calcium or the use of covalent crosslinkers will be needed (Bouhadir et al., 2001). To confirm the role of the oxidation and decrease of the carboxylic group density on the ability for gelation, a higher polymer concentration ($C_p = 60$ g/L) was used for the higher degrees of oxidation such as to preserve the condition of being over the overlap concentration. Even, in these conditions, no gels were formed over 10 mol% oxidation.

On the other hand, the low molar mass obtained for oxidized alginates is interesting for biomedical applications since it was found that, for $M_w < 80{,}000$ g/mol, alginates can be cleared from the human body (Al-Shamkhani & Duncan, 1995).

4. Conclusions

The chemical modification of alginates by sodium periodate and the influence of oxidation degree on the characteristics of modified polymers were studied. The weight-average molar mass decreases rapidly with the degree of oxidation even for low degree of oxidation such

as 5 mol%. M_w decreases with an increase on both concentration of sodium periodate and reaction time. Oxidation of alginate chain produces a decreasing in the stiffness of the polymer by breaking C_2–C_3 bond with a chain scission as a simultaneous reaction. Using a triple detection in SEC, we clearly demonstrated that the stiffness of the molecule decreases as calculated from viscosity measurements. No calculation of the persistence length was proposed, since the theoretical treatment cannot strictly be applied to low molar mass polymers. The ability for gelation was tested and it was observed that over 10 mol% oxidation no more gels are formed in excess of calcium even over their respective overlap concentration. Two effects can be proposed for this behavior: a relatively low molar mass of the resulting polymer due to oxidation and decrease of the stiffness reducing the cooperative interaction between carboxylate groups and calcium ions especially if the guluronic units are first oxidized.

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