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Acid gel formation in (pseudo) alginates with and without G blocks produced by epimerising mannuronan with C5 epimerases

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Abstract

The main scope of this paper is the characterization, in terms of viscoelastic and mechanical properties, of acid gels formed from solutions of mannuronan ALG (0%G/0%GG) and its enzymatically epimerised products. The epimerised products were obtained using recombinantly produced mannuronan C5 epimerases named AlgE1 and AlgE4, which catalyse the conversion of mannuronic residues into guluronic (G) and guluronic–mannuronic (GM) blocks, respectively. The products used in this study resulted from either the action of AlgE1 on mannuronan for 5 and 24 h (named ALG(44%G/32%GG) and ALG (68%G/59%GG), respectively) or AlgE4 on mannuronan (named ALG (47%G/0%GG)). D-gluconic acid-δ-lactone (GDL) was used as H^+-donor to produce acidic gels. ALG (0%G/0%GG) yields strong, stable solid-like structures. As predicted by circular dichroism measurements performed at different pH, gelation of ALG (47%G/0%GG) occurs at lower values of pH (∼1) than those obtainable using GDL. Hydrochloric acid was therefore added to ALG (47%G/0%GG) solutions yielding rapid sol–gel transitions and gels with a remarkable resistance to thermal treatment.

The introduction of guluronic residues along the chain (ALG (44%G/32%GG)) causes a reduction in the storage modulus at the equilibrium with respect to that of ALG (0%G/0%GG) and the occurrence of negligible syneresis at the highest polymer concentrations. The increase in the average length of the G blocks (ALG (68%G/59%GG)) is accompanied by a further increase in the storage modulus without the occurrence of any significant syneresis.

Keywords: Alginates; C-5 epimerase; Acid gels; Rheology; Circular dichroism

1. Introduction

Alginate is a family of polysaccharides made up of (1→4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G). Both the fraction and the sequence of the two residues depend on the source from which the polysaccharide is isolated. This polymer has wide industrial applications due to its viscosifying, gelling, suspending and ion-binding abilities (Ertesvåg & Valla, 1998). The variability in chemical composition and monomer sequence is the key to such wide exploitation.

The mannuronan C5 epimerases extracted from Azobacter vinelandii, are a family of seven enzymes (AlgE1-7) capable of converting M residues into G residues with different patterns of epimerisation (Draget, Skjåk-Bræk, & Smidsrød, 1997). These enzymes can therefore be used to modify alginates in vitro to obtain new alginates with the desired content and distribution of G residues (Ertesvåg, Hoidal, Hals, Rian, Doseth and Valla, 1995).

AlgE1 is a bifunctional C5 epimerase endowed with a two catalytic sites: one introduces single G residues while the other generates G-blocks (Ertesvåg, Høydal, Skjåk-Bræk, & Valla, 1998). Thus, it produces long G-blocks and MG-sequences starting from the mannuronan substrate. The C5 epimerase AlgE4 forms alginates with long strictly alternating sequences (Hartmann, Holm, Johansen, Skjåk-Bræk, & Stokke, 2002).

Mannuronan, an uncommon alginate composed completely of mannuronic residues is produced in large quantities by recombinant *P. fluorescens* strains, and is an excellent substrate to study the effect of increasing the amount of G blocks and MG blocks on the mechanical properties of epimerised mannuronan both in solution and in the gel phase.

In a recent paper, (Dentini, Rinaldi, Risica, Barbetta, & Skjåk-Bræk, 2005) we examined the solution properties of

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mannuronan ALG (0%G/0%GG), mannuronan epimerised by C5 epimerase AlgE1 for 5 and 24 h (ALG (44%G/32%GG) and ALG (68%G/59%GG), respectively) and mannuronan epimerised by C5 epimerase AlgE4 (ALG (47%G/0%GG)). A comparative study on the characteristics of chain flexibility and on the rheological behaviours of both dilute and concentrated solutions of these polymers was carried out. In the present article we focus on the rheological study of both the gelling kinetics and viscoelastic properties of the acid gels obtained from the above epimerised alginates.

2. Experimental

2.1. Materials

High molecular weight mannuronan was isolated from the fermentation of an epimerase-negative AlgG$^-$ strain of P. fluorescens (Ertesvåg & Skjåk-Bræk, 1999). Purification and deacetylation were carried out as described earlier (Gimmestad et al., 2003). Pure mannuronan was epimerized by AlgE1 epimerase for different times (5 and 24 h) and by AlgE4 epimerase as described earlier (Gimmestad, Sletta, Ertesvåg, Ellingsen, Skjåk-Bræk and Valla, 2003).

The fraction of guluronate (G) residues and the mole fraction of GG, MM and GM (MG) diad sequences F_{GG}, F_{MM} and F_{MG} were determined by ^{1}H NMR (Skjåk-Bræk, Grasdalen, & Larsen, 1986) in D_2O at 90 °C. The molecular weights were determined by HPSEC-RI-MALLS using three serially connected columns TSK gel G 6000/5000/4000 PWXL. Detectors were refractive index (RI), UV monitor LKB UV-M II Amersham Pharmacia Biotech and multiple angle light scattering (MALLS) Dawn DSP equipped with a He–Ne laser operating at 632.8 nm, Wyatt Technology Corp. The characterization results are summarized in the Table 1.

2.2. Circular dichroism

The circular dichroism spectra were recorded using a spectropolarimeter Jasco J715-A in the wavelength range 190–300 nm with four repetition scans and a sensitivity of 5 mdeg. All measurements were performed at room temperature using 0.5 cm quartz cells.

The polymer solutions at different pH values at a concentration of 0.2% w/v were prepared from a 1% w/v stock polymer solution and standard HCl and NaOH solutions were used to adjust the final pH value.

2.3. Preparation of acid-induced gels

All experimental polymer solutions were prepared by dilution of stock polymer solutions. Homogeneous acid gels were obtained upon direct addition of solid D-glucono-δ-lactone (GDL, 0.8 M) to the polymer solution. In order to lower the pH of ALG (47%G/0%GG) solutions, a 1.0 M hydrochloric acid solution was used.

2.4. Rheological characterization

Dynamic viscoelastic characterization of the samples was carried out using a concentric cylinder cell of a Bohlin 'CS' Version 4.033 (Couette geometry 'C14', ratio of inner and outer cylinders, $r_1/r_2 = 0.909$).

The gelling kinetics of ALG (0%G/0%GG), ALG (44%G/32%GG) and ALG (68%G/59%GG) solutions in the presence of GDL were studied at $T=25\pm0.1$ °C and $\omega=0.1$ Hz. In the case of ALG (47%G/0%GG) solutions in the presence of HCl, the kinetics were determined at $T=20\pm0.1$ °C and $\omega=0.1$ Hz. G' data were collected at 15 min intervals. A few drops of mineral oil were added on top of the polymer solution in order to minimize solvent evaporation. Dynamic measurements on gels of ALG (0%G/0%GG), ALG (44%G/32%GG) and ALG (68%G/59%GG), the products of AlgE1 epimerase, were performed at 0.2% strain and measurements on gels of ALG (47%G/0%GG) were performed at 1% strain since strain sweeps on a few selected gels demonstrated that these strains produced working deformations well within the linear viscoelastic region. For the dynamic experiments carried out by varying T, the heating rate was set at 0.5 °C/min.

3. Results and discussion

3.1. Study of the effect of pH on the conformation of pseudoalginates by means of circular dichroism (CD) spectra.

Before illustrating the results regarding CD studies on the pseudoalginates it is useful to summarize the main features of the dichroic spectra of alginates (Morris, Rees, Sanderson, & Thom, 1975). In the undissociated form, all D-uronic acids exhibit a positive transition between 200 and 210 nm ($n\rightarrow\pi^*$); the corresponding C5 epimers will therefore show a negative band at about the same wavelength (λ) region. Moreover, the onset of a $\pi\rightarrow\pi^*$ transition is shifted at shorter λ.

Table 1

Chemical composition, sequence parameters and weight average molecular weight of mannuronan (ALG (0%G/0%GG)), mannuronan epimerized by AlgE4 epimerase (ALG (47%G/0%GG)), mannuronan epimerized by AlgE1 epimerase for 5 h (ALG (44%G/32%GG)) and mannuronan epimerized by AlgE1 epimerase for 24 h (ALG(68%G/59%GG)). F_G and F_M are the molecular fractions of guluronic and mannuronic acid respectively. F_{GG}, F_{MM}, F_{MG} ($=F_{GM}$) are the diad frequencies, while $N_{G>1}$ is the average number of guluronate residues in the G blocks

Sample	F_G	F_M	F_{GG}	F_{MM}	F_{MG}	$N_{G>1}$	M_W
ALG (0%G/0%GG)		1	–	1	–	–	496,000
ALG (47%G/0%GG)	0.47	0.53	–	0.06	0.47	–	533,000
ALG (44%G/32%GG)	0.44	0.56	0.32	0.44	0.12	13	444,000
ALG (68%G/59%GG)	0.68	0.32	0.59	0.23	0.09	22	452,000

When the hydroxyl group in C4 is equatorial (as in the case of mannuronic residues) a small band, opposite in sign to the one at $\lambda \sim 205$ nm, can be observed at longer wavelengths. This is because the equatorial hydroxyl induces the local chain segment to assume a particular conformation in which the etheric oxygen lies on the chromofore plane. This leads to a conjugative effect stabilizing the excited state and consequently to a decrease in the energy of transition.

The three kinds of sequences occurring in alginates will therefore exhibit different CD spectra. In particular, poly-L-guluronate yields a totally negative signal; poly-D-mannuronate gives rise to a double-band spectrum (in the range of wavelengths instrumentally accessible) and alternate sequences show an intermediate behaviour. It is worth pointing out that the CD spectrum of alternate sequence is not merely the same as is seen from an equimolar mixture of the two homopolymeric blocks. This is due to the influence of the sequence of residues along the chain on the conformation of the polymer (Morris et al., 1975; Morris, Rees, & Thom, 1980).

The presence of molecules or ions able to induce conformational changes in the polymeric chain under appropriate physical conditions, leads to a more or less pronounced change in the specific ellipticity trace vs wavelength.

It is well known that concentrated solutions of alginates are able to form solid-like structures (gels) in the presence of bivalent ions at suitable values of the ratio *R* (Ca^{++} equivalents/polymer equivalents) (Smidsrød & Draget, 1996). At lower polymer concentrations, the interaction between Ca^{++} and the chain segments gives rise to conformational modifications that can be easily identified by the CD technique.

The CD spectra of uronic acids are also influenced by the degree of neutralization of the carboxylic groups (i.e. by the solution pH). The ionisation introduces a new plane of symmetry in the chromofore which gives rise to π and π^* orbitals delocalisation (Morris et al., 1980). The CD spectra of ALG (0%G/0%GG) recorded at different values of pH are reported in Fig. 1(a). An isodichroic point can be noted at about $\lambda = 242$ nm. This feature is indicative of an equilibrium existing between dissociated and undissociated states of the carboxylic groups.

Thus, the CD spectrum is sensitive to the ratio [–COO$^-$]/[–COOH] with no evident spectral perturbations attributable to conformational variations. At $pH < 3$ the formation of a transparent gel leads to a sharp variation in the trace of specific ellipticity vs wavelength. Probably, the polymer chains undergo a process of association which results in the formation of a thick network of inter and intramolecular hydrogen bonds spanning over the whole solution's volume.

This modified spatial arrangement of chain molecules is the basis for the remarkable variation in the observed ellipticity. Analogous optical behaviour can be observed for ALG (47%G/0%GG) in Fig. 1(b). In this case the isodichroic point occurs at $\lambda \sim 200$ nm. The formation of a gel at $pH \sim 1.3$ corresponds to a noticeable decrease in the band's intensity. As far as ALG (44%G/32%GG) and ALG (68%G/59%GG) (Fig. 1(c) and (d)) are concerned, they tend to form macroscopic gel structures at $pH < 3$ and 4, respectively.

In comparison with polymer chains rich in homomannuronic sequences, pseudoalginates with a higher content in G residues arranged in a blockwise fashion undergo gel formation at higher pH. MG blocks are rather soluble at

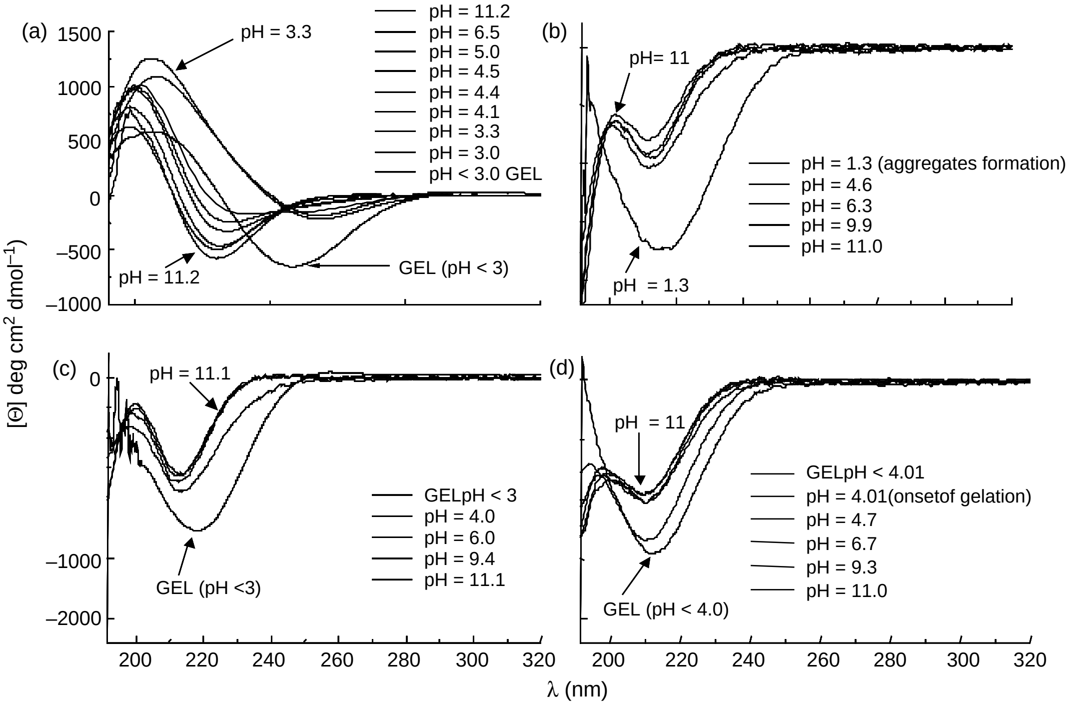

Fig. 1. pH dependence of the CD spectrum of (a) ALG (0%G/0%GG), (b) ALG (47%G/0%GG), c) ALG (44%G/32%GG) and (d) ALG (68%G/59%GG). The polymer concentration is 0.2% w/v.

relatively acidic conditions and need lower values of pH to produce macroscopic supramolecular structures. This result is in agreement with previous studies (Smidsrød & Draget, 1996).

3.2. Dynamic rheology of pseudoalginic acid gels: ALG (0%G/0%GG), ALG (44%G/32%GG) and ALG (68%G/59%GG)

A gel is a soft, solid-like material that consists of two or more components, one of which is a liquid. Gelation occurs when the polymeric chains effectively interact with each other (either physically or chemically) yielding a cross-linked network spanning the whole volume.

Alginate solutions may form gels by lowering the pH value below the pK_a of the uronic acid residues (Morris, Rees, & Thom, 1978). The pK_a values of β-D mannuronic and α-L-guluronic residues are 3.38 and 3.65, respectively (Morris et al., 1975). Gel formation results from the establishment of intra and intermolecular hydrogen bonds (Haug & Karsen, 1963).

In the case of ALG (0%G/0%GG), ALG (44%G/32%GG) and ALG (68%G/59%GG), the products of AlgE1 epimerase, D-glucono-δ-lactone (GDL) is used as H^+ donor. GDL slowly hydrolyses in solution and brings about a gradual decrease in pH, thus leading to the formation of a homogeneous gel phase. It was found that a 0.8 M GDL solution was sufficient to obtain maximum acid gel strength at an alginate concentration of about 1% w/v (King, 1983).

Fig. 2 shows the gelling kinetics of solutions of ALG (0%G/0%GG) (a), ALG (44%G/32%GG) (b) and ALG (68%G/59%GG) (c) in the presence of GDL 0.8 M. In the case of ALG (0%G/0%GG), the dependence of the storage modulus G′ on time is monotonic with an apparent equilibrium state attained within about 250 min after introduction of GDL at all the tested concentrations (Fig. 2(a)). The point at which the mechanical properties of the system change from those of a concentrated solution to those typical of a solid-like system (i.e. $G'=G''$) occurs within 45 min since, GDL introduction. In particular, at the lowest values of polymer concentration (0.1 and 0.2% w/v) the gel point occurs within 30 min. It is probable that longer times are required for gelation to occur at higher polymer concentrations because of the reduced diffusive ability of GDL, added in solid form, through the polymer solution (Atkins, Nieduszynski, Mackie, Parker, & Smolko, 1973; Haug, 1964).

ALG (44%G/32%GG) solutions undergo sol→gel transition (Fig. 2(b)) within 30 min at all the tested concentrations. The time necessary for the phase transition decreases with increasing polymer concentration to ~15 min at a polymer concentration of 0.8% w/v.

This trend is opposite to the one observed for ALG (0%G/0%GG). It is likely that, in the case of ALG (44%G/32%GG), the slow hydrolysis of GDL at the highest polymer concentrations is compensated for by a quicker rearrangement of the chain-segments in response to variations in pH. In particular, if compared with M and G blocks, the alternate ‘elastic’ sequence is characterized by the shortest relaxation times (Draget, Strand, Hartmann, Vala, Smidsrød & Skjåk–Bræk, 2000). The large set of accessible angles around the MG glycosidic linkage may allow stiffer chain segments (M and G blocks) to be directed toward preferential orientations amenable to mutual physical interactions.

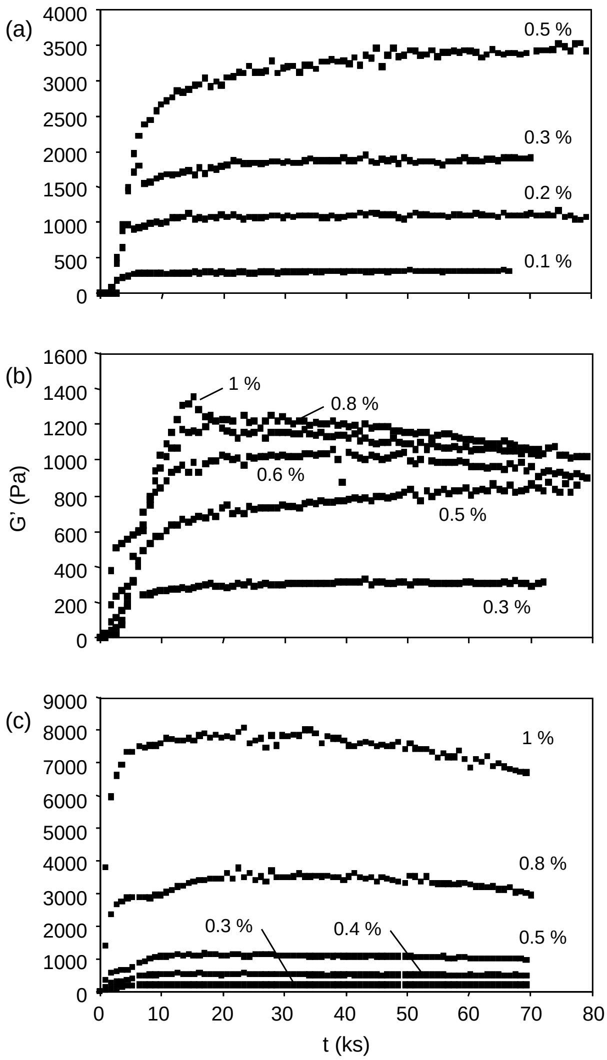

Fig. 2. Time dependence of storage modulus (G′) for solutions of (a) ALG (0%G/0%GG), (b) ALG (44%G/32%GG) and (c) ALG (68%G/59%GG) at different concentrations (%, w/v) and in presence of 0.8 M D-glucono-δ-lactone (GDL) at $T=25$ °C, $\omega=0.1$ Hz and γ (target strain) $=0.2\%$.

A relevant feature characterising the trace of the storage modulus vs time of ALG (44%G/32%GG) is the presence of a flex-zone at about 2 h after the introduction of GDL. As the pK_a of α-L-guluronic acid is higher than the pK_a of β-D-mannuronic acid, we may conclude that the segments capable of interaction are, at least at the shortest times, the ones characterized by a greater presence of G residues.

In this respect, a distinction has to be made between concentrated and dilute solutions. The former (1% w/v) showed the presence of a transitory plateau (the expanded view is shown in Fig. 3), which might be due to the progressive neutralization of guluronic carboxylic groups.

As the polymer concentration decreases the extension of this plateau decreases and at the lowest concentration (0.3% w/v)

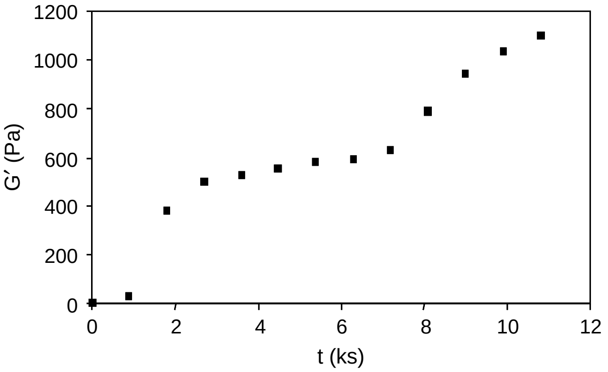

Fig. 3. Initial development of G′ after addition of GDL to a 1% w/v ALG (44%G/32%GG) solution.

the transitory plateau is absent. This behaviour may be attributed to the higher diffusion rates of GDL leading to a faster decrease in pH and, consequently, to a lower differentiation between the effects caused by the neutralization of M and G residues on the mechanical properties of the growing network.

As it can be observed (Fig. 2(b)), the difference in G′ at times longer than ∼40 ks for 1, 0.8, 0.6 and 0.5% w/v solutions becomes increasingly smaller. Furthermore, the gelling kinetics of the solution at the highest polymer concentration (1% w/v) shows a peculiar dependence of G′ on time. After a rapid increase in G′ during the initial 15 ks, the storage modulus reaches a point of maximum after which the trend inverts: a rapid decrease in G′ occurs during the following 5 ks. From $t=20$ ks onwards, the decrease in G′ is more gradual (Fig. 2(b)).

The change in viscoelastic properties during the initial 15 ks can be attributed to interactions among homo-polymeric blocks, leading to a preliminary framework. The following event might be the aggregation of alternate MG sequences causing local distortions of the pre-formed macromolecular architecture and a consequent decrease in the storage modulus. The occurrence of a slight degree of syneresis for the gel formed by the 1% w/v solution accompanied by a sharp drop in G′ shown in Fig. 2(b), strengthen this hypothesis.

Although less marked, the decrease in G′ was also observed for the 0.8 and 0.6% w/v solutions. On the contrary, when the polymeric concentration is 0.5% w/v the effect of the interaction among MG sequences appears to strengthen the gel structure. The similar extrapolated values of the storage modulus at long times after GDL introduction at the higher concentrations might be explained by a common polymer chain packing arrangement in the gels.

From the comparison of the storage modulus of the gels obtained from ALG (0%G/0%GG) and ALG (44%G/32%GG),

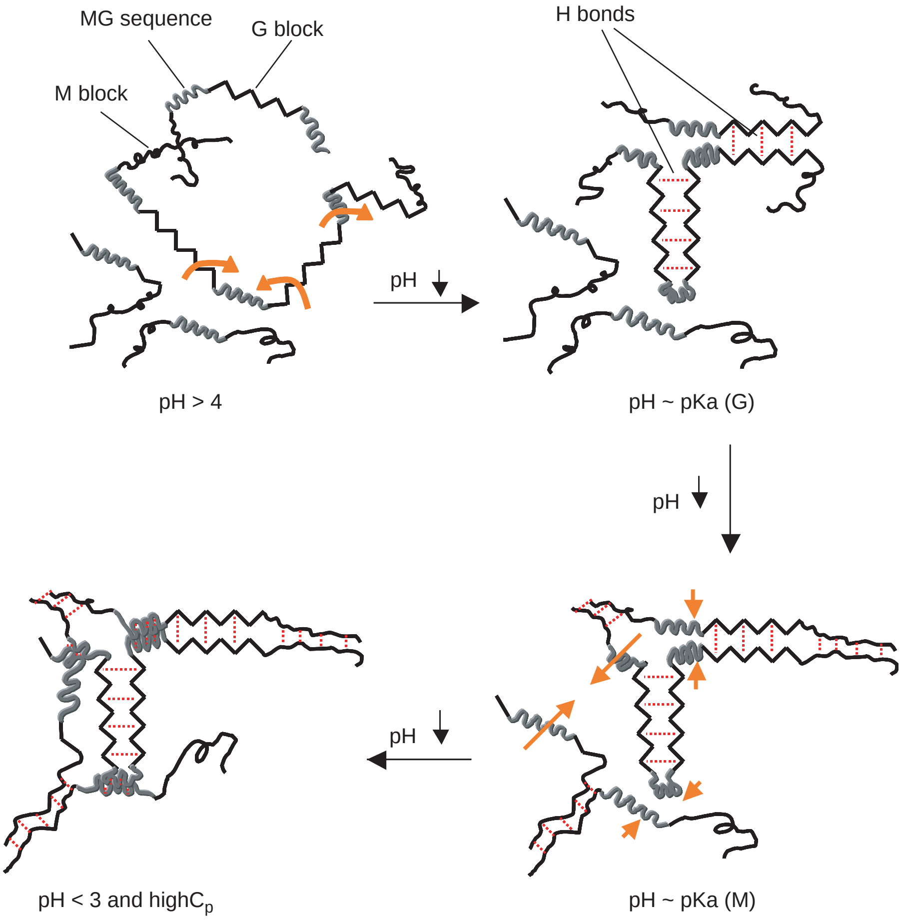

Fig. 4. Role of the M and G and MG sequences in the formation of the network in the case of ALG (44%G/32%GG) and ALG (68%G/59%GG).

it is evident that weaker structures are obtained in the latter case. This may be attributed to a less ordered primary structure (see Table 1), which may impair to some extent the intermolecular interaction among G and M bocks. Another explanation relies on the higher solubility of MG sequences in acidic environment at $pH > 2$ (Hartmann, Dentini, Draget & Skjåk-Bræk, in press). For this reason the higher the content of Mg blocks the lower the crosslinking density.

In Fig. 2(c) the dependence of G' on time for ALG (68%G/59%GG) is shown. As for ALG (44%G/32%GG), the time from the onset of the sol → gel transition, decreases with increasing polymeric concentration. It may be envisaged that the presence of more extended G blocks (Table 1) permits the formation of more regular structures, while the shorter extension of alternate sequences perturbs the homo-polymer network made up of hydrogen bonds to a lesser extent.

This brings two main consequences:

1. At the same polymer concentration the storage modulus of ALG (68%G/59%GG) is higher than that of ALG (44%G/32%GG) (effect of the increased structural regularity);
2. Absence of the maximum zone in ALG (68%G/59%GG) (as a result of the shorter extension of MG-blocks).

Furthermore, it is worth noting, in the case of ALG (68%G/59%GG), the remarkable extension of the first 'pseudo' stationary state. The slow decrease in pH leads to a differential neutralization of M and G residues, and the large fraction of guluronic residues which have a higher pK_a leads to an extended pseudo stationary state with respect to gel mechanical properties.

The slight decrease in G' observed at 1 and 0.8% w/v concentrations may be due to the establishment of additional interaction between the residual MG sequences. In Fig. 4 a schematic representation of the establishment of crosslinking junctions mediated by hydrogen bonding formation among GG, MM and MG blocks as the pH is lowered is depicted.

Strain-sweep experiments were performed on the gels made from the samples considered in this section, in order to ensure a linear stress–strain response during the dynamic rheological measurements. In Fig. 5 is reported, as an example, the strain sweep relative to the acid gel obtained from a solution of MANNA in the presence of GDL. The target strains chosen for the oscillatory tests (0.2 or 1%) were well within the linear response range.

Mechanical spectra of gels obtained from solutions of ALG (0%G/0%GG) (Fig. 6(a)), ALG (44%G/32%GG) (Fig. 6(b)) and ALG (68%G/59%GG) (Fig. 6(c)) were recorded at different polymer concentrations. No frequency dependence of G' or G'' (loss modulus) was observed.

Moreover, the storage modulus was more than an order of magnitude higher than the loss modulus at all the tested concentrations, therefore these structures can be categorised as 'strong gels'.

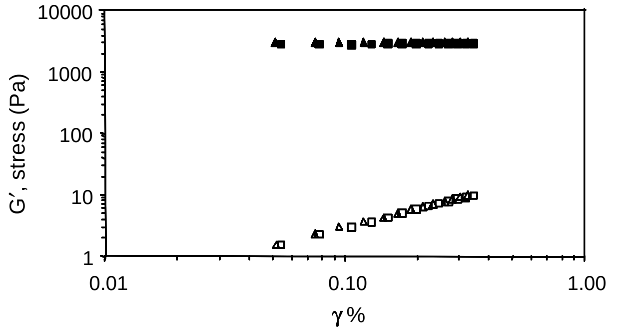

Fig. 5. Strain sweep on the acid gel obtained from a solution of MANNA (0.5% w/v) in the presence of 0.8 M GDL. Key: (▲) and (■), storage modulus recorded at $\omega = 0.1$ and 0.01 Hz, respectively. (△) and (□), stress recorded at $\omega = 0.1$ and 0.01 Hz, respectively.

The rheological behaviour of these gels was characterised with respect to thermal treatment (Fig. 7). Their mechanical properties are stable in a relatively wide range of temperatures (25–45 °C). Above 45 ° C, the gels undergo shrinking

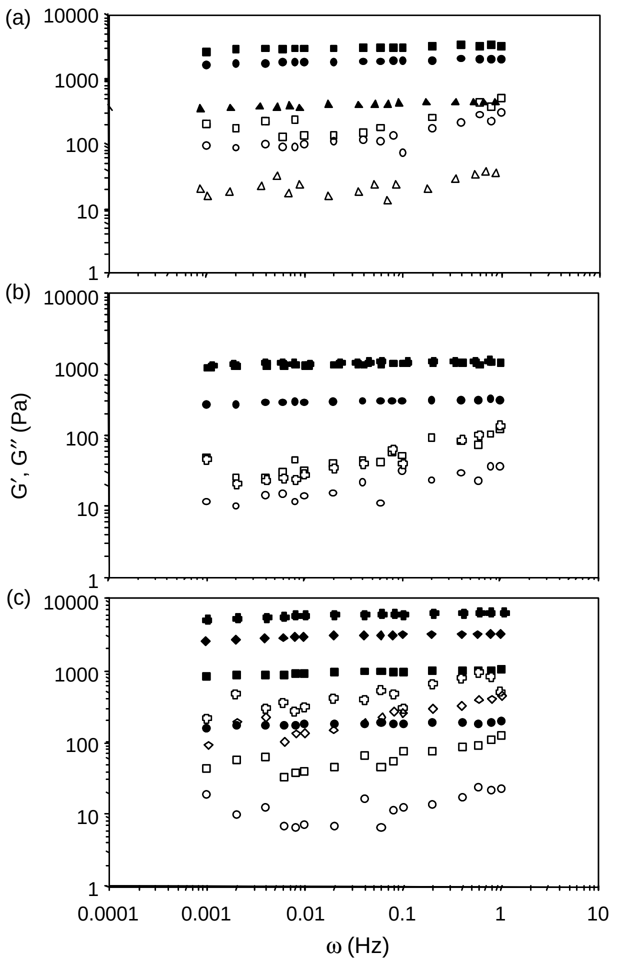

Fig. 6. Mechanical spectra of gels obtained from solutions at different polymeric concentrations (C_p) of (a) ALG (0%G/0%GG), (b) ALG (44%G/32%GG) and (c) ALG (68%G/59%GG) in the presence of 0.8 M GDL. Filled symbols G', empty symbols G''. C_p (✚) 1%, (◆) 0.8%, (■) 0.5%, (●) 0.3% and (▲) 0.1% (w/v). $T = 25$ °C; $\gamma = 0.2\%$.

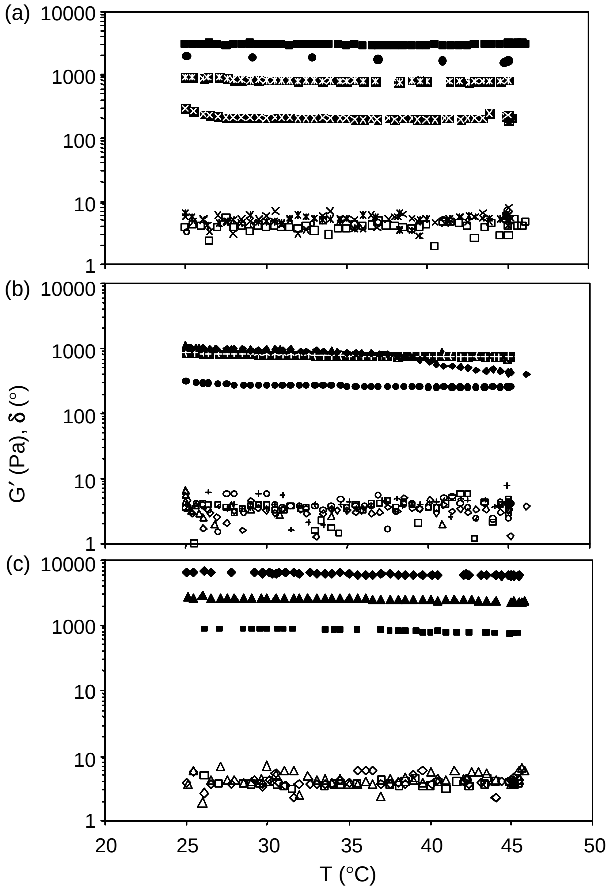

Fig. 7. Elastic modulus G′ (full symbols) and phase angle δ (empty symbols) as a function of temperature of (a) ALG (0%G/0%GG), (b) ALG (44%G/32%GG) and (c) ALG (68%G/59%GG in the presence of 0.8 M GDL. C_p: (◆) 1%, (▲) 0.8%, (▣) 0.6% (■) 0.5%, (●) 0.3%, (☒) 0.2%, (☒) 0.1% (w/v), $\gamma=0.2\%$.

phenomenon which endangers the mechanical properties and stability of the matrix.

3.3. Dynamic rheology of pseudoalginic acid gels: ALG (47%G/0%GG)

When the same conditions of polymer and GDL concentrations established for the pseudoalginates studied in Section 3.2 were applied to ALG(47%G / 0%GG), no sol–gel transition occurred within 24 h of the introduction of GDL. As expected from CD spectra (Section 3.1), in the case of ALG (47%G/0%GG) pH values around 1 are required to induce the formation of macroscopic structures while the minimum value of pH accessible with GDL is 2–2.5 (Draget, Skjåk–Bræk & Smidsrød, 1994).

In order to reach a pH value of about 1, hydrochloric acid was used as an acidogen. In Fig. 8, the kinetics of gelation of ALG (47%G/0%GG) solutions at pH = 1 are reported.

The initial increase in storage modulus G′ is relevant: the transition from solution to gel phase occurs within 10 min from the introduction of HCl. The 'stationary' state is reached within 40 ks for all tested concentrations and the storage modulus equilibrium value is considerably lower than those obtained for the previous samples at the same concentrations.

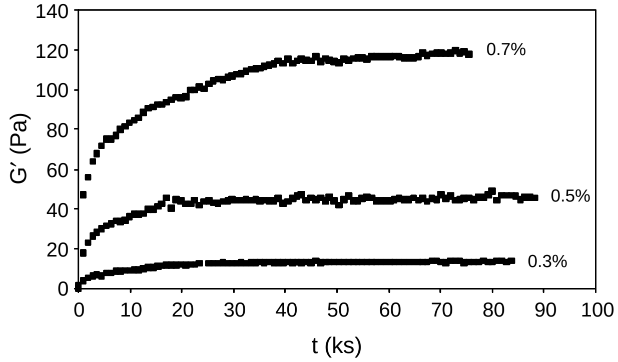

Fig. 8. Time dependence of the storage modulus for solutions of ALG (47%G/0%GG) at different concentrations (%, w/v) and pH = 1. $T=20$ °C; $\omega=0.1$ Hz and $\gamma=1\%$.

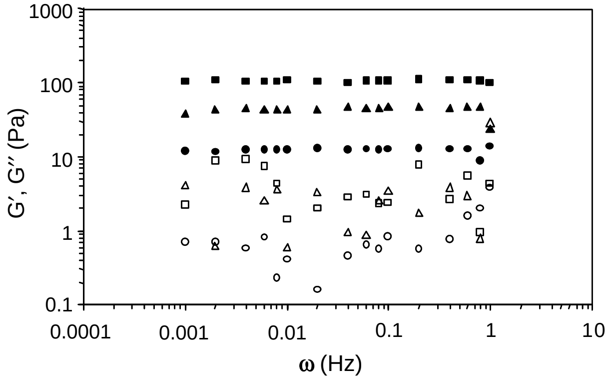

Fig. 9. Mechanical spectra of gels obtained from solutions at different C_p of ALG (47%G/0%GG) at pH = 1. Full symbols: G′. Empty symbols: G″. Concentrations (% w/v): ■/□, 0.7; ▲/△ 0.5 and ●/○, 0.3. $\gamma=1\%$; $T=20$ °C.

Some slight syneresis was observed 24 h after gel formation. Once again, this can be interpreted as the result of re-arrangement of the framework of polymeric segments constituting the gel matrix.

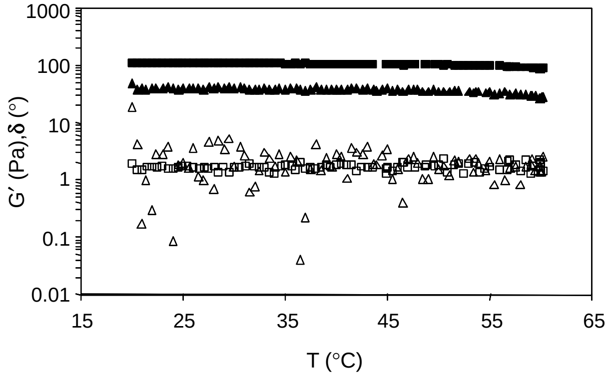

Fig. 10. Elastic modulus G′ (full symbols) and phase angle δ (empty symbols) as a function of temperature of gels obtained from solutions at different polymer concentration of ALG (47%G, 0%GG) at pH = 1. C_p (% wv): ■/□, 0.7; ▲/△, 0.5. $\omega=0.1$ Hz, $\gamma=1\%$.

Fig. 9 shows the mechanical spectra of the gels formed starting from ALG (47%G/0%GG) solutions. The lack of any dependence of the storage modulus on the applied frequency and the marked differences between G′ and G″ values are typical of true gels, that is a networks crosslinked by permanent junctions.

Fig. 10 illustrates the response of the mechanical properties of ALG (47%G/0%GG) gels to thermal treatment. The increase in temperature up to 60 °C does not modify the viscoelastic properties of these structures whereas the gels obtained from ALG (0%G/0%GG) and its epimerised products by AlgE1 ALG (44%G/32%GG) and ALG (68%G/59%GG) undergo a phenomena of water-release from their core for temperatures above 45 °C.

4. Conclusions

Circular dichroism spectra performed on solutions of mannuronan and mannuronan epimerised with AlgE1 epimerase for 5 and 24 h both at different values of pH have shown conformational changes at the polymer level.

At higher concentrations, mannuronan and its epimerised products by AlgE1epimerase actually produce strong gel structures after direct addition of D-glucono-δ-lactone. The influence of both polymer concentration and residues sequence along the chain has been studied.

ALG (0%G/0%GG) can be reputed as a high quality gelling material (in the presence of H^+-donor agents), because of the stability and strength of the resultant gels. Solutions of ALG (44%G/32%GG) in presence of GDL yields acid gels with a somewhat extended degree of syneresis at polymer concentrations higher than 0.8% w/v. This kind of gel, by virtue of its incremented compactness may be exploited in the formulation of immunoprotective capsules for drug delivery and cell-therapy.

At the tested concentrations, ALG (68%G/59%GG) forms strong acid gels with no syneresis and properties similar to those of natural alginates with high contents of G residues.

As predicted by circular dichroism spectra recorded at several pH, mannuronan epimerised by AlgE4 epimerase needs lower values of pH than those obtainable by using GDL as acidogen to form gels. Therefore, ALG (47%G/0%GG) acid gels have been prepared by using HCl as a proton donor. The subsequent mechanical characterization has demonstrated viscoelastic characteristics typical of strong gels. The resistance to thermal treatment is higher than that observed for the other acid gels.

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