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ACID-INDUCED GELATION OF SODIUM ALGINATE: EFFECT OF THERMAL ANNEALING AND CONCENTRATION

Agoub A. A.; S. M.Hasan; S. A. Mohamed and S. T. Abousalloum
Department of Food Sciences and Technology, University Omar Almukhtar, Elbeida, Libya.

ABSTRACT

Gelation on slow acidification by hydrolysis of D-glucono-□-lactone (GDL) has been studied for guluronate-rich sodium alginate from *Laminaria hyperborea*. Network formation by alginate–GDL mixtures during holding at 25°C was monitored by low-amplitude oscillatory measurements of storage modulus (G') and loss modulus (G") at 1 rad s^{-1} and 0.5 % strain.

For samples in which the final pH was held constant (at 3.0) and alginate concentration (c) was varied, the slope of log G' versus log c decreased progressively with increasing concentration towards a limiting value of 2 (c^2-dependence), as seen for other gelling biopolymers.

A numerical analysis is reported which demonstrates that the concentration of GDL required to achieve a constant value of final pH increases in direct proportion to the concentration of alginate used.

Thermal annealing (heating to 85°C and cooling back to 25°C) of samples acidified to pH 3.0 caused a large (~10-fold) increase in G' at low concentration (0.5 wt % alginate), attributed to a co-operative disorder–order transition during cooling by chain segments freed by disaggregation during heating. Increase in modulus on cooling became progressively smaller as the concentration of alginate was increased to 1.0 wt %, and at 1.5 wt % was replaced by a sharp decrease, attributed to excessive aggregation (incipient precipitation) of the acidified polymer.

Keywords: Alginate; Alginic acid; GDL; Gelation; Rheology

INTRODUCTION

Alginate is a (1→4)-linked linear co-polymer of two different monosaccharides,β-D-mannuronate and ۩α-L-guluronate, with residues arranged in homopolymeric sequences of both types (polymannuronate and polyguluronate) and heteropolymeric sequences in which the two monosaccharides are present in roughly equal amounts (Moe *et al.,* 1995). It occurs as the main structural polysaccharide in numerous species of marine brown algae (Phaeophyceae) and is expressed as an extracellular polysaccharide by several strains of bacteria, notably *Azotobacter vinelandii* (Larsen and Haug, 1971a; Brivonese and Sutherland, 1989).

In both bacteria (Larsen and Haug, 1971a, 1971b) and marine algae (Madgwick *et al.,* 1973) and, alginate is biosynthesised initially as poly- β -D-mannuronate,with subsequent partial conversion of β -D-mannuronate to α-L-guluronate by enzymes that induce epimerisation at C(5). Seven C(5) epimerases, identified as AlgE1 to AlgE7, have been extracted from *Azotobacter vinelandii*. These have different patterns of epimerisation, and have been used to modify the structure of alginate *in vitro* (Skjåk-Braek et al., 1986; Draget *et al.,* 1997; Hartman *et al.,* 2006). AlgE4 is of particular interest, since it produces heteropolymeric sequences with a regular

alternating structure, in contrast to the irregular distribution found in alginates that occur naturally.

Alginates from marine algae (seaweeds) have extensive industrial applications as thickeners, stabilisers and gelling agents (Sime, 1984). Gelation is normally induced by incorporation of calcium ions. Intermolecular association in calcium alginate gels occurs predominantly by formation of "egg box" junctions (Grant *et al.,*1973; Morris et al.,1978) in which long arrays of site-bound Ca^{2+} ions are sandwiched between poly-α-L-guluronate sequences in the buckled, 2-fold conformation identified by X-ray fibre diffraction (Atkins *et al.,*1973).

Pectin, which has some structural similarities to alginate, being composed predominantly of (1→4)-linked uronic acid residues (α-D-galacturonate), also forms acid-induced gels. Commercial pectins are classified as "high methoxy" or "low methoxy", according to their "degree of esterification" (DE), defined as the percentage of galacturonate residues that are present in the methyl ester form(Rolin, 1993). High methoxy pectins (DE > 50) are normally gelled by replacing a large proportion (typically 60-70 %) of the solvent (water) by sucrose or other co-solutes, and reducing the pH (typically to ~pH 3). Gelation occurs on cooling from high temperature. The resulting gels do not dissociate on heating, which is attributed to hydrophobic association of methyl ester substituents as the temperature is raised(Oakenfull and Scott, 1984; Evageliou *et al.,* 2000).

Low methoxy pectins (DE < 50) are normally gelled by incorporation of calcium ions (as in production of calcium alginate gels). Gilsenan et al., (2000), however, observed gel formation when acidified solutions of low methoxy pectin (DE ≈ 31), with no added Ca^{2+} or co-solute, were cooled from high temperature (85°C). The resulting gels were thermoreversible, although with substantial thermal hysteresis between formation of network structure on cooling and dissociation on heating.

In the present work, the effect of systematic variation of the concentrations of alginate and GDL was studied at constant pH on the formation and rheological properties of acid-induced networks. The inter-relation between alginate concentration, GDL concentration, and the final pH of the networks formed was analyzed. Also explored the possibility of similar thermoreversibility in the acid-induced networks formed by alginate.

MATERIALS AND METHODS

Materials

The alginate used was a typical guluronate-rich preparation from *Laminaria hyyperborea*, purchased from BDH. The sodium alginate content of the sample, as determined by carbon analysis (Butterworth Microanalytical Consultancy Ltd.), was 78 %. GDL was purchased from ADM, Ringaskiddy, Co. Cork, Ireland.

Methods

Solutions were prepared by dispersing the alginate in distilled deionised water and heating to ~85°C, with vigorous overhead stirring throughout. The solutions were then cooled to 25°C, the required amount of GDL was added as solid powder, and the mixtures were homogenised by brief (~3 min) mechanical stirring before being used immediately for the experiments described below.

Oscillatory measurements of storage modulus (G'), loss modulus (G") and complex dynamic viscosity (□*) were made at a fixed strain of 0.5 % using parallel plate geometry (4 cm diameter; 0.5 mm gap) on a CarriMed CSL-100 rheometer. Samples were loaded onto the rheometer at 25°C and coated around their periphery with light silicone oil, to minimise loss of water by evaporation. Development of network structure during holding at 25°C was monitored by measurements of G' and G" at 1 rad s^{-1}. When the moduli had reached constant values, a mechanical spectrum (variation of G', G" and □* with frequency, □ /rad s^{-1}) was recorded (at 25°C). Selected samples were then heated from 25°C to 85°C and cooled from 85°C to 25°C at a fixed rate of 1°C/min, with measurements again being made at 1 rad s^{-1}.

Samples for compression testing were filled (at 25°C) into lubricated cylindrical moulds (diameter 12.6 mm; height 13.5 mm), sealed with lubricated cover slips,and held in a water bath for 18 h at 25°C. The resulting gels were then compressed (at 25°C) on a TA-TX2 texture analyser (from Stable Microsystems), using a cylindrical probe of diameter 50 mm and compression rates of 0.5, 1.0 and 1.5 mm/s. Results for each sample at each compression rate are reported as the average of two replicates. The extent of compression is expressed as "true" (Henky's) strain (□ = ln (H_o / H),where H denotes the current height of the sample and H_o the initial height). Stress (force per unit area) is similarly expressed as "true stress" (□), calculated by assuming constant volume and preservation of cylindrical geometry (i.e. with cross-sectional area increasing inversely with H). Nishinari and Takahashi (2003).

To determine the final pH of the gel networks, the gels were reduced to pastes by manual stirring, and left in contact with the pH electrode (at 25°C) until stable readings were attained (typically after ~15 min) (.

RESULTS AND DISCUSSION

The investigation of acid-induced gelation of sodium alginate by low-amplitude oscillatory measurements was carried out when the final pH of the acid-induced gels was held constant at 3.0, which is just below the pH range of the steepreduction in tan □ shown in Fig. 1, and the concentration of alginate was varied.The alginate concentrations studied were 0.5, 0.6, 0.8, 1.0, 1.5, 2.0 and 3.0 wt %.The concentrations of GDL required to achieve a final pH of 3.0 are listed in Table 1 and increase linearly with increasing concentration of alginate.

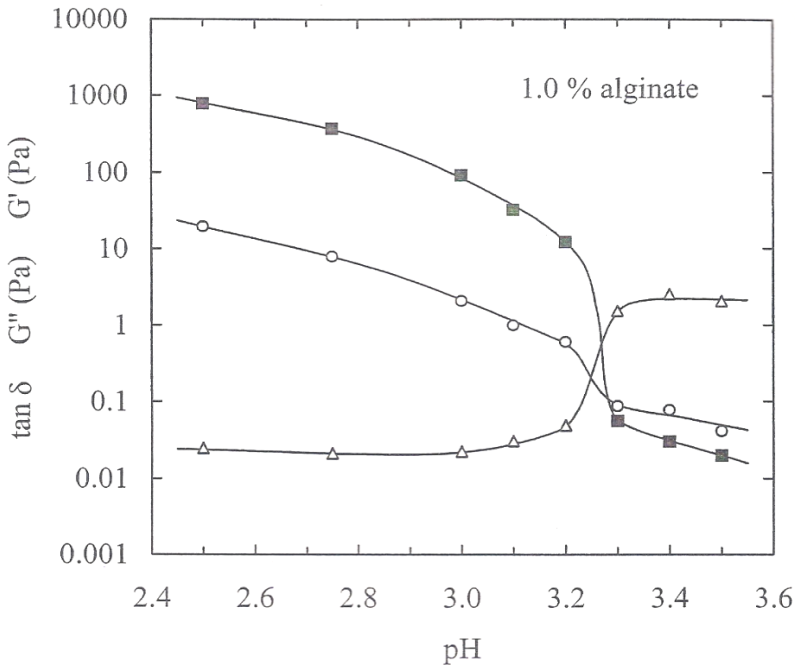

Fig. 1. pH-dependence of G' (■), G" (O) and tan □ (Δ), measured at 1 rad s^{-1} and 0.5 % strain, for 1.0 wt % alginate acidified with GDL at 25°C.

As shown in Fig. 2, gel formation, characterised by a sharp increase in moduli, with G' rising above G", occurred during holding at 25°C for even the lowest concentration of alginate studied (0.5 wt %). The changes in G' during reduction in pH at 25°C observed for higher concentrations of alginate (0.6 to 3.0wt %)are shown in Fig. 3a, and the corresponding changes in G" are shown in Fig. 3b.In all cases, there is a sharp increase in both moduli, indicative of network formation, which moves to progressively shorter times as the alginate concentration is raised.

Table1.Relationship between alginate concentration, GDL concentration and pH

[Alginate] (wt %)	**0.5**	**0.6**	**0.8**	**1.0**	**1.5**	**2.0**	**3.0**
pH	**[GDL] wt%**	**[GDL] wt %**	**[GDL] wt %**	**[GDL] wt %**	**[GDL] wt %**	**[GDL] wt %**	**[GDL] wt %**
2.50	4.24	5.09	6.79	8.48	12.7	17.0	25.4
2.75	2.32	2.78	3.71	4.64	6.95	9.27	13.9
3.00	1.25	1.50	2.00	2.50	3.75	5.00	7.50
3.10	0.97	1.17	1.56	1.94	2.92	3.89	5.83
3.20	0.75	0.91	1.21	1.51	2.26	3.02	4.53
3.30	0.58	0.70	0.94	1.17	1.75	2.34	3.51
3.40	0.45	0.54	0.72	0.91	1.36	1.81	2.72
3.50	0.35	0.42	0.56	0.70	1.05	1.40	2.10

GDL D-glucono-□-lactone wt% percentage of weight

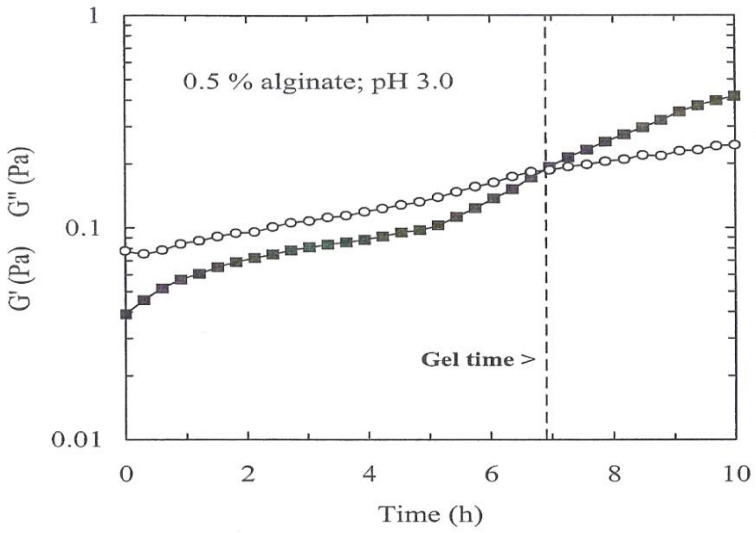

Fig. 2. Variation of G' (■) and G" (O), measured at 1 rad s^{-1} and 0.5 % strain during holding at 25°C, for 0.5 wt % alginate acidified with GDL to a final pH of 3.0.

Gel time was estimated as the time at which the curves for G' and G" (Figs. 2 and 3) crossed one another. There is a smooth reduction in gel time (Fig. 4) with increasing concentration of alginate. This can be attributed, at least partially, to the proportion of chain segments that must form intermolecular junctions to give a continuous network decreasing progressively as the polymer concentration is increased. Another factor, however, may be the accompanying increase in the concentration of GDL required to give a final pH of 3.0, leading to faster initial reduction in pH.

Figs. 5 and 6 show mechanical spectra (pH 3.0; 25°C; 0.5 % strain) recorded for illustrative concentrations of alginate. At the lowest concentration studied (0.5 wt %; Fig. 5a), G' shows only slight variation with frequency, as observed for typical gel networks (Ross-Murphy, 1984), and at low frequencies G" also shows little frequency-dependence, and is substantially lower than G', which is again characteristic of gel-like response. At higher frequencies, however, G" rises steeply above G', which, as mentioned previously, is indicative of a substantial sol fraction of chain segments that do not form part of the continuous network. As the concentration of alginate is increased to 0.6 wt % (Fig. 5b) and then to 0.8 wt % (Fig. 6a) the increase in G" at high frequency becomes progressively less evident, and by 1.5 wt % (Fig. 6b) the spectra have the form typical of a strong gel, with G' > G" and little frequency-dependence of either modulus.

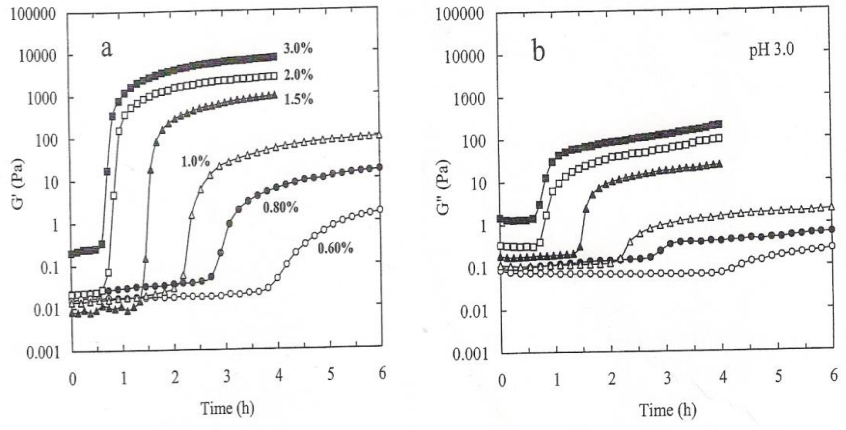

Fig. 3. Variation of (a) G' and (b) G", measured at 1 rad s^{-1} and 0.5 % strain during holding at 25°C, for samples acidified with GDL to a final pH of 3.0 at alginate concentrations (wt %) of 0.6 (O), 0.8 (●), 1.0 (Δ), 1.5 (▲), 2.0 (□) and 3.0 (■).

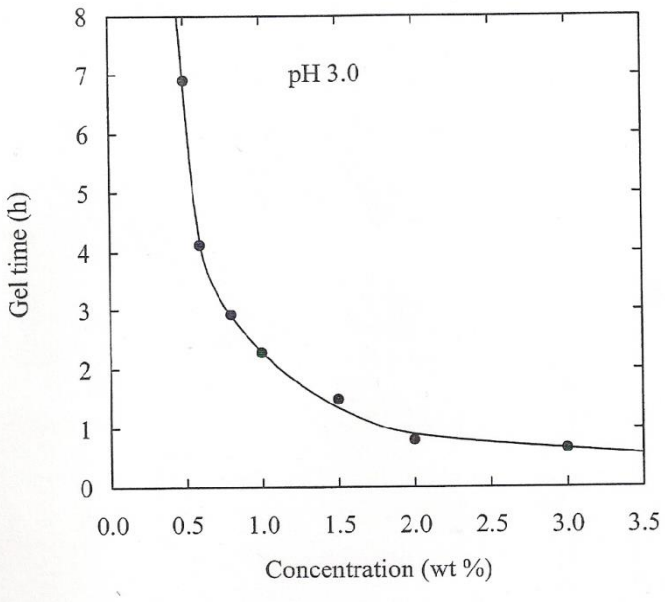

Fig. 4. Variation of gel time with alginate concentration for samples acidified with GDL at 25°C to a final pH of 3.0.

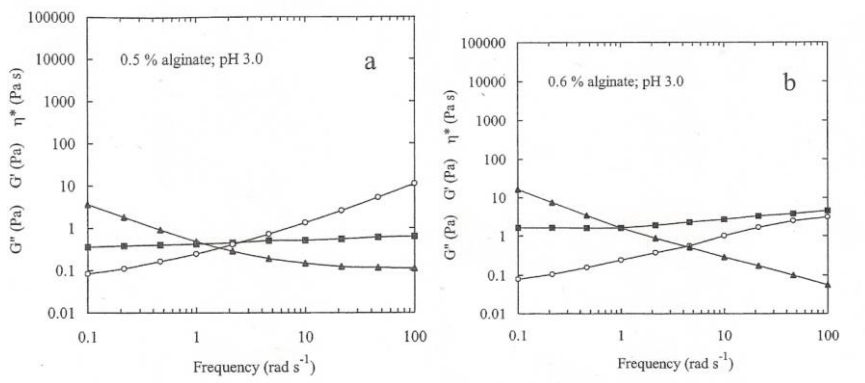

Fig. 5. Mechanical spectra (25°C; 0.5 % strain) showing the frequency-dependence of G' (■), G" (O) and □* (▲) for samples acidified with GDL to a final pH of 3.0 at alginate concentrations of (a) 0.5 wt % and (b) 0.6 wt %.

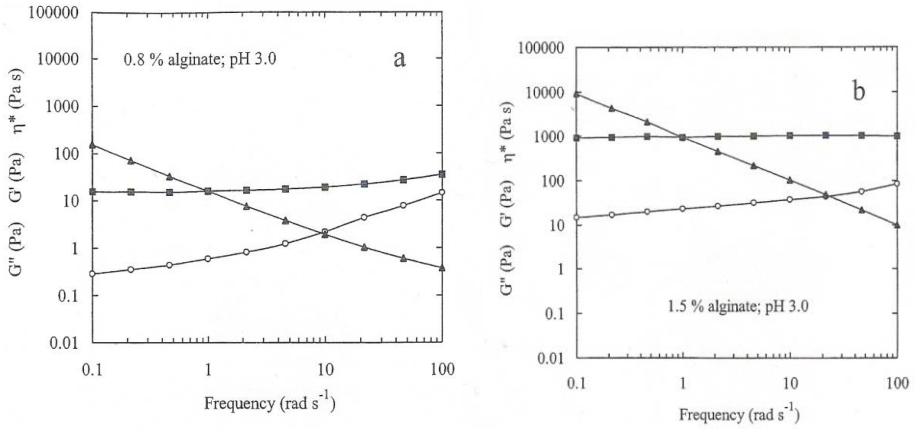

Fig. 6.Mechanical spectra (25°C; 0.5 % strain) showing the frequency-dependence of G' (■), G" (O) and □* (▲) for samples acidified with GDL to a final pH of 3.0 at alginate concentrations of (a) 0.8 wt % and (b) 1.5 wt %.

Fig. 7 shows the values of G' and G" obtained (at 1 rad s^{-1} and 0.5 % strain) on completion of reduction in pH to 3.0, plotted double-logarithmically against alginate concentration (c). As would be anticipated from Fig. 5a, there is little separation between G' and G" at the lowest concentration studied (0.5 wt %). As the concentration is raised, however, G' becomes progressively higher than G", and the slope of log G' versus log c at high concentration (Fig. 7) approaches the limiting value of 2 (c^2-dependence) characteristic of most physically-crosslinked biopolymer gels (Clark and Ross-Murphy, 1985).

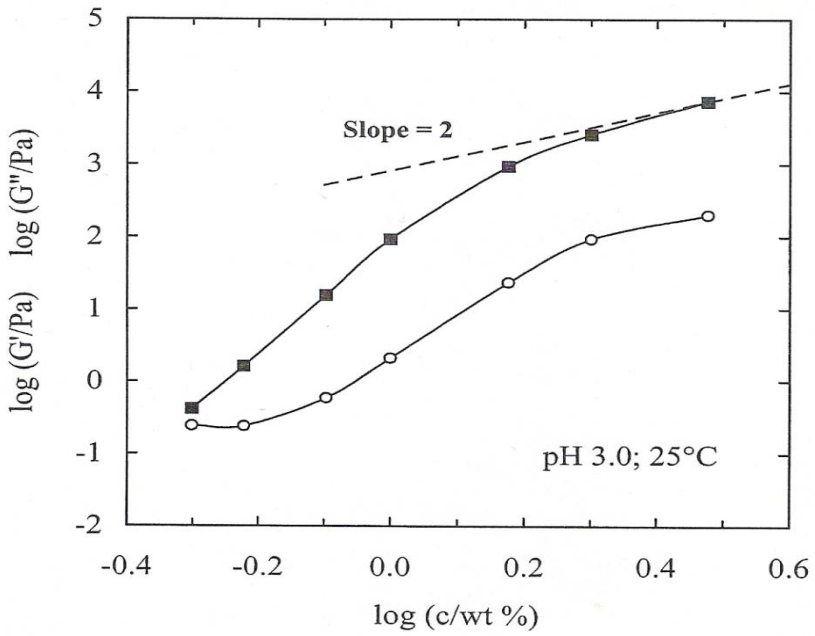

Fig. 7. Variation of G' (■) and G" (O), measured at 1 rad s^{-1} and 0.5 % strain, with alginate concentration (c) for samples acidified with GDL at 25°C to a final pH of 3.0.

Effect of heating and cooling

The investigation of acid-induced gelation of sodium alginate by low-amplitude oscillatory measurements was confined to samples acidified to a final pH of 3.0, at alginate concentrations of 0.5, 0.6, 0.8, 1.0 and 1.5 wt %. Gelation of these samples during holding at 25°C in the presence of the required concentration of GDL (Table 1), and the mechanical spectra obtained when G' and G" had reached constant values.Immediately after the mechanical spectra had been recorded, the gels were heated from 25°C to 85°C at 1°C/min, and then immediately cooled back to 25°C, again at 1°C/min. The changes in G' (1 rad s^{-1}; 0.5 % strain) observed during heating and cooling are shown in Figs. 8 (a and b) and 9 (a and b).

For the sample prepared at 0.6 wt % alginate (Fig. 8a), there was little variation in G' during heating from 25°C to 85°C, but on cooling there was a pronounced increase as the temperature dropped below ~75°C, and the final modulus on completion of cooling to 25°C was about an order of magnitude higher than the initial value at the same temperature. Closely similar traces (although with lower absolute values of G') were obtained at the lowest concentration of alginate studied (0.5 wt %). On increase in concentration to 0.8 wt % (Fig. 8b), there was a slight, progressive decrease in G' during heating, and the increase on cooling below ~75°C was smaller. Both effects were more evident (Fig. 9a) on further increase in

alginate concentration to 1.0 wt % (greater decrease in G' during heating; smaller increase on cooling). At 1.5 wt % alginate (Fig. 9b) the trend to greater reduction in G' during heating continued; on cooling, however, the increase in G' as the temperature dropped below ~75°C was followed by a sharp decrease below ~50°C. These temperature-induced changes in moduli are summarised in Fig. 3, which shows the values of G' (1 rad s^{-1}; 0.5 % strain) recorded at 25°C before heating, on reaching 85°C, and after cooling again to 25°C plotted against alginate concentration (c).

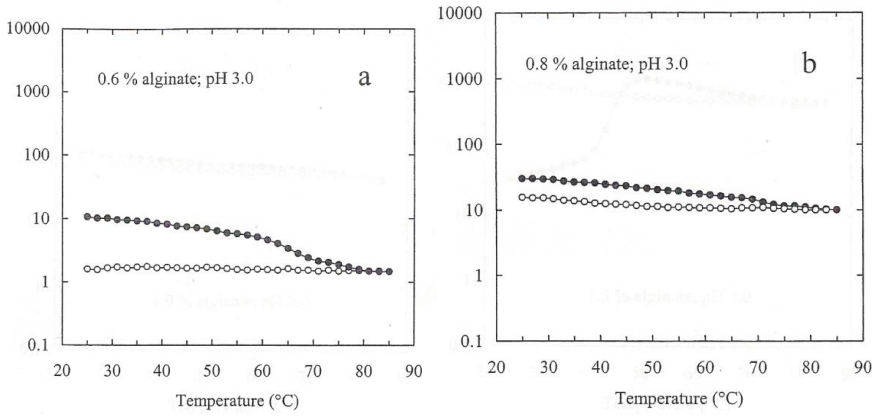

Fig. 8. Values of G' (1 rad s^{-1}; 0.5 % strain) recorded during heating (O) and cooling (●) at 1°C/min for samples acidified with GDL to a final pH of 3.0 at alginate concentrations of (a) 0.6 wt % and (b) 0.8 wt %.

A possible trivial explanation of the sharp decrease in G' shown in Fig. 9b is that it was caused by slippage, perhaps arising from slight syneresis, rather than by genuine weakening of the sample. This possibility was explored by compression testing, where no slippage artefacts can occur. Gels of the same composition (1.5 wt % alginate; final pH 3.0) were prepared (at 25°C) in cylindrical moulds and subjected to a time–temperature regime matched as closely as possible to that used in the oscillatory studies. Fig. 4 shows the compression curve (mean of 2 closely-similar replicates) recorded after completion of cooling to 25°C, in comparison with the corresponding curve for samples withdrawn from the moulds before heating. Although the difference between the two curves is smaller than the drop in G' shown in Fig. 9b. it is evident that heating and cooling, at this comparatively high concentration of alginate, causes a substantial decrease in both yield stress and yield strain.

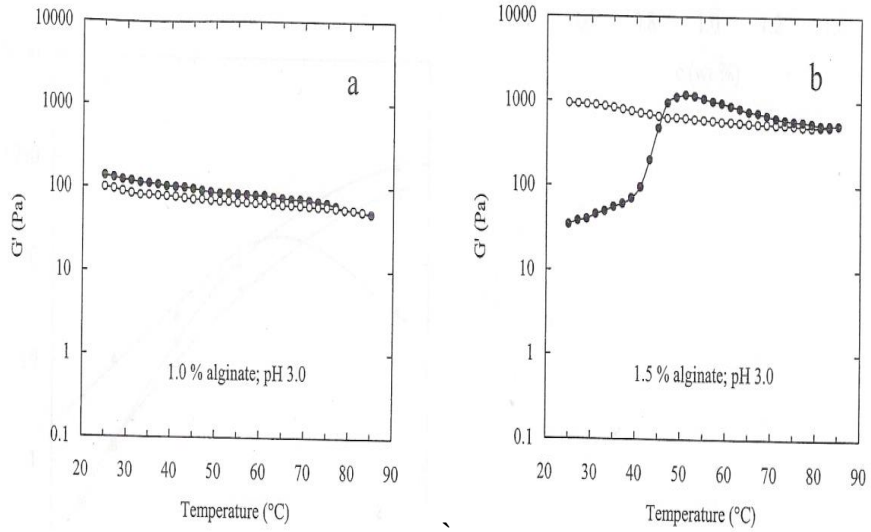

Fig. 9. Values of G' (1 rad s^{-1}; 0.5 % strain) recorded during heating (O) and cooling (●) at 1°C/min for samples acidified with GDL to a final pH of 3.0 at alginate concentrations of (a) 1.0 wt % and (b) 1.5 wt %.

We offer the following, speculative, interpretation of the results presented in Figs. 7 - 11. Heating causes progressive dissociation of the random aggregates proposed by Draget et al., (2006), with consequent progressive reduction in G'.The extent of initial aggregation increases with increasing concentration of polymer, and the effect of disaggregation therefore also increases (Fig. 10) as the alginate concentration is raised. On cooling, chain sequences liberated by disaggregation during heating undergo a co-operative disorder–order transition analogous to that observed (Gilsenan et al., 2000) for acidified solutions of low methoxy pectin, giving rise to the sharp increase in G' shown in Fig. 8a. The increase in modulus from formation of these new, better ordered, junctions, however, occurs in competition with a subsequent decrease due to excessive aggregation (incipient precipitation) of the acidified alginate. The extent of aggregation during cooling increases with increasing concentration of polymer. At alginate concentrations in the range 0.5 - 1.0 wt % it causes a progressive reduction in the overall magnitude of the increase in G' during cooling (Fig. 10); at 1.5 wt % it can be seen (Fig. 11) as a sharp reduction in modulus following the initial increase.

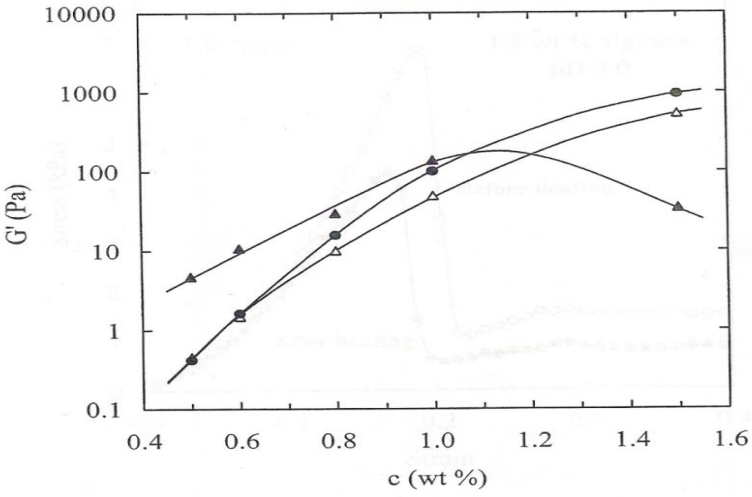

Fig.10. Variation of G' (1 rad s^{-1}; 0.5 % strain) with alginate concentration (c) for samples acidified with GDL to a final pH of 3.0, after reaching constant moduli at 25°C (●), on reaching 85°C after heating at 1°C/min (Δ), and after cooling backto 25°C at 1°C/min (▲).

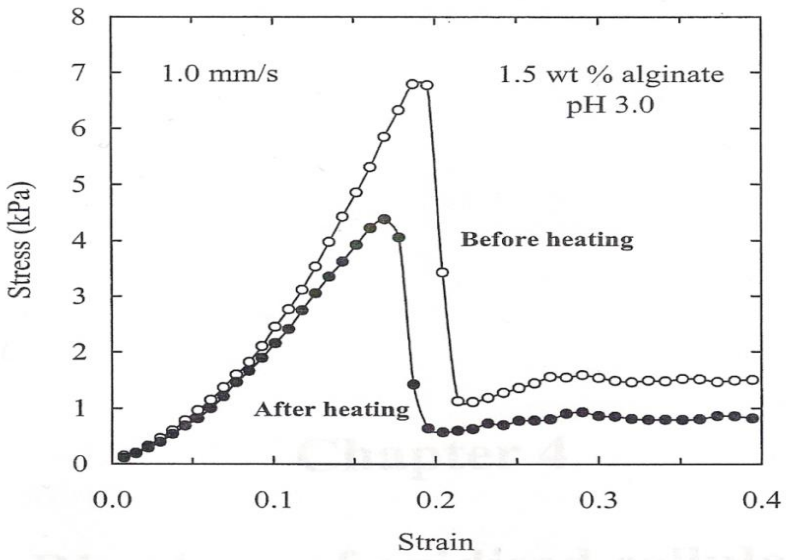

Fig. 11. Compression curves (1.0 mm/s) for 1.5 wt % alginate acidified with GDL to a final pH of 3.0, recorded at 25°C before (O) and after (●) heating to 85°C.

Conclusions

The concentration-dependence of G' for samples acidified to the same final pH (3.0)
has the form characteristic of physically-crosslinked biopolymer networks, approaching a c^2-dependence at high concentrations.
`The concentration of GDL required to achieve a constant value of final pH increases in direct proportion to the concentration of alginate used.

Heating causes partial disaggregation, allowing new associations to form by a co-operative disorder–order transition on cooling. For samples acidified to a final pH of 3.0, thermal annealing increases gel strength at alginate concentrations up to ~1.0 wt % , but causes a reduction at higher concentration (1.5 wt %), which can be attributed to excessive aggregation (incipient precipitation) during cooling.

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تكوين جل من الجينات الصوديوم بالتحميض : تأثيرالحرارة (التسخين والتبريد) والتركيز

عقوب عبدالله عقوب , صلاح محمد حسن، صلاح الناجي محمد وسليمان طاهر بوسلوم
قسم علوم وتقنية ألأغذية – كلية الزراعة – جامعة عمر المختار- البيضاء- ليبيا

تم دراسة ظاهرة عملية تكوين الجل لالجينات الصوديوم بواسطة عملية التحميض (خفض pH) عن طريق تحلل دلتا جلوكونو ألفا لاكتون GDL . عملية تكوين الشبكة الجلية لخليط من الالجينات و GDL على درجة حرارة 25 مئوي تم مراقبتها عن طريق جهاز ريولوجي باستخدام اهتزازات منخفضة وتم من خلالها قياس معدل اللزوجة 'G ومعدل الصلابة "G١٠ \دج٩٨ح٥٦ وكان معدل الذبذبات 1 راد / ث ومعدل التشوه 0.5 %. لقد بينت الدراسة أن العينات التي تم خفظ درجة pH الي 3 والتي تم تسخينها الي درجة حرارة 85 مئوي ومن ثم تبريدها الي درجة حرارة 25مئوي أدت الي زيادة معدل اللزوجة 'G عشر أضعاف عند تركيز 0.5 من ألألجينات والسبب يرجع الي اعادة تركيب وتكوين الروابط الكيمائية أثناء عملية التبريد والتي قد تم تكسيرها أو تحليلها أثناء عملية التسخين. لوحظ من الدراسة ان ارتفاع معدل اللزوجة G أثناء التبريد كان بطيئا بارتفاع درجة تركيز ألألجينات الي 1 % بينما كان الارتفاع في معدل اللزوجة 'G بطئي جدا عند استخدام درجة تركيز1.5 % من ألألجينات بسبب حدوث عملية تجمع عشوائي للالجينات مما أدى في النهاية الي حدوث عملية الترسيب.

قام بتحكيم البحث

أ.د / محمد طة شلبى	**كلية الزراعة – جامعة المنصورة**
أ.د / احمد عبد العزيز الرفاعى	**كلية الزراعة – جامعة المنصورة**