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Structural and textural properties of calcium induced, hot-made alginate gels

Maria Papageorgiou, Stefan Kasapis* & Michelle G. Gothard

Department of Food Research and Technology, Cranfield University, Silsoe College, Silsoe, Bedford MK45 4DT, UK

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The accepted mechanism of alginate gelation at ambient temperature is by formation of egg-box junctions between pairs of polyguluronate chain sequences and an included array of site-bound calcium ions. We now present evidence (circular dichroism and low-amplitude mechanical spectroscopy data) of similar regular interactions at high temperatures where all the active ingredients (alginate, salt and sequestrant) are hydrated in a hot medium. During controlled cooling, a second transition is obtained at low temperatures, which may be attributed to the lateral interaction of dimeric chains and thus the development of an extended, three-dimensional network. A subsequent temperature increase melts the second wave of structure formation, thus producing substantial thermal hysteresis, but preserves the 'weak gel' properties of the alginate calcium reaction observed at elevated temperatures. At levels of salt higher than 40% calcium conversion aggregation can occur even at 90°C, leading to precipitation rather than a homogenous network. Prolonged refrigeration (72 h at 4–5°C) sustains a gradual molecular rearrangement to a self-supporting (visual evidence), firmer (frequency sweeps) network. Surprisingly, the strongest high guluronate alginate is not only more elastic than its low molecular weight counterpart (strain sweeps in dynamic oscillation) but it also exhibits higher yield strain on compression testing than the high molecular weight, high mannuronate sample. The distinct differences in the long-range properties of high guluronate and high mannuronate alginate samples with comparable molecular weights might serve as a guide for the development of reformed products with the desired characteristics.

INTRODUCTION

Alginate occurs as the major structural polysaccharide (up to 40% of the dry matter) of marine brown algae (Painter, 1983). It is a (1 → 4)-linked linear polymer of β-D-mannuronate and α-L-guluronate, containing homopolymeric sequences of both types (Haug *et al.*, 1966, 1967) and heteropolymeric regions in which residues occur in arrangements that can vary from almost statistically random to almost regularly alternating (Grasdalen *et al.*, 1981), depending on botanical source and the state of maturation of the plant. Pure polymannuronic acid, having (1 → 4)-diequatorial linkage geometry, adopts a flat ribbon-like two fold conformation which converts in the salt form to a similarly extended three fold ribbon (Atkins *et al.*, 1973*a*). The corresponding X-ray fibre diffraction structure for polyguluronate, by contrast, is a highly buckled two folded zig-zag, irrespective of charge or counterion (Atkins *et al.*, 1973*b*; Mackie *et al.*, 1983).

Commercially, alginate is mainly available in the sodium form. In deionised water, sodium alginate remains disordered and enhances the viscosity of solutions (thickener) for a wide variety of applications. A property of alginate that is of major importance for both its biological function and industrial applications is the formation of strong, rigid gels with divalent cations, usually Ca^{++} or the remaining cations of group II besides Mg^{++} that does not promote gelation at all (Smidsrød, 1974). Gel strength increases with increasing content of polyguluronate, and calcium ion activity in solutions of guluronate oligomers shows a sharp decrease above a critical length of about twenty residues (Kohn, 1975), indicating the onset of cooperative binding behaviour, where the binding of each ion facilitates the binding of the next. Such effects are not seen for the corresponding mannuronate residues, when circular dichroism and equilibrium dialysis techniques are

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*To whom correspondence should be addressed.

employed to study alginate gelation. Instead, the polymannuronate sequences remain soluble in the presence of Ca^{++}, and show only simple polyelectrolyte binding behaviour, indicating that they must act as solubilising interconnecting regions in alginate gels (Morris *et al.*, 1978). Investigation of molecular models of polyguluronate in the invariant two fold structure identified by X-ray shows cavities of the size required to accommodate a calcium ion, and with oxygen atoms well placed for cation chelation (Grant *et al.*, 1973). It has therefore been proposed that the interchain junctions in alginate gels, which are normally formed at room temperature, involve polyguluronate sequences locked in a two fold conformation, with arrays of site-bound cations sandwiched between them like eggs in an egg-box. In the present work we show that similar ordered structures are formed in preparations at elevated temperature, and may be of importance in understanding and controlling the textural properties of gels in industrial applications of alginate.

MATERIALS AND METHODS

Alginate samples were kindly supplied by Kelco Division of Merck & Co Inc. They are commercially available as Manugel DMB (high molecular weight, high guluronate content), Manugel DJB (low molecular weight, high guluronate content) and Manucol DH (high molecular weight, high mannuronate content). Analysis of block composition by circular dichroism (Morris *et al.*, 1980) and nuclear magnetic resonance (Grasdalen *et al.*, 1981) showed a polyguluronate content of about 58 and 32% for the Manugel and Manucol samples, respectively. Trisodium citrate dihydrate (a calcium sequestrant that controls the gelation reaction thus improves the homogeneity of the gel) and calcium chloride dihydrate were AnalaR grade from BDH. The Ca^{++} content was expressed as a fraction of the total stoichiometric requirement of alginate (i.e. as % conversion). Distilled deionised water was used throughout.

Homogeneous preparations were made by dry blending 1 or 2% alginate with 0 to 0·45% trisodium citrate (TSC) and adding them to a range of calcium chloride solutions (20 to 80% of the alginate stoichiometric equivalent) at 90°C. In this way we avoided the problem of severe precipitation which is observed on addition of high levels of calcium chloride (in solid form or in solution) to an alginate solution (Clare, 1993), thus limiting applications to low salt conversions (inadequate gelation properties). Effectively neutral mixtures (pH values of 6·6 to 6·7) were made, suitable for the preparation of non-acidic foods such as structured vegetables (Bryden & Sanderson, 1982). At 90°C, samples were sheared for 5 min and either poured into lubricated ring moulds, covered with a lubricated glass plate and left to set at 5°C for 72 h, or used immediately for analysis at elevated temperatures. The failure properties of cylindrical gels (13 mm height; 13 mm diameter) were recorded at 5°C, using the flat, parallel plate geometry of a TA.XT2 Texture Analyser (Stable Micro Systems). Samples were compressed to 30% of the original height (i.e. 70% compression) at a rate of 0·8 mm/s (about 2 in/min). 'True' strain values were calculated using the formula $\ln(L_0/L)$, where L_0 and L are the lengths of the sample before compression (without any deformation) and during the measurement (Ross-Murphy, 1984). Small-deformation oscillation was performed on a prototype rheometer (Richardson, 1991) using cone and plate geometry of cone angle 0·02 rad and 50 mm diameter, whereas steady shear measurements were recorded on a Carri-Med CSL 500 rheometer (TA instruments) using the above geometry, with a 0·035 rad angle and 20 mm radius. The routine procedure included loading the hot preparations on the rheometer at 90°C and monitoring structural changes during cooling to 5°C at 1°C/min. At 5°C, network development was recorded for up to 72 h. At the end, gels were either reheated to 90°C (1°C/min) or subjected to an increasing oscillatory shear (strain sweep). Throughout the course of experimentation, the mechanical response as a function of frequency of oscillation (frequency sweep) was taken regularly for comparison between different samples. Finally, circular dichroism (CD) studies were carried out on a Jobin Yvon Auto Dichrograph Mark V CD spectrometer, using a thermostatted cell of 1 mm pathlength and a one-second integration period.

RESULTS AND DISCUSSION

Effect of temperature on the gelation properties of alginate

The central challenge in the practical use of calcium alginate gelling systems is to ensure that intermolecular junctions are formed slowly enough to give a homogeneous network rather than a precipitate (Sime, 1984). This is usually achieved at ambient temperature by the controlled release of a sparingly soluble calcium salt from within the alginate solution (internal or bulk setting) or by the slow diffusion of calcium ions into preparations of sodium alginate (diffusion setting). An alternative approach, however, is to introduce a soluble calcium salt at high temperature, with gelation then occurring on cooling. This method is suitable for the formation of mixed gel applications with biopolymers that require heating and cooling, e.g. gellan, gelatin and κ-carrageenan (Sanderson & Ortega, 1993; Ortega *et al.*, 1994). The normal interpretation of alginate gels prepared from hot solutions was set out clearly in a recent review article (Sime, 1990): 'Although the calcium ions are already in solution with the alginate, setting does not occur at elevated temperatures because the alginate chains have too much thermal energy to permit alignment. It is only when the solution cools that calcium-induced interchain association can occur'. Our present results, however, show that the real story is not quite so simple.

Figure 1(a) shows CD spectra reported by Morris *et al.* (1978) for alginate closely similar in composition to the Manugel samples used in the present work (about 58% polyguluronate), before and after addition of Ca^{++} (100% total stoichiometric requirement) at 25°C. The massive and almost Gaussian, change in CD over the wavelength range of the carboxyl $n \rightarrow \pi^*$ transition was attributed to specific site binding of calcium ions in polyguluronate egg-box junctions (Grant *et al.*, 1973). Figure 1(b) shows the spectra we have now obtained for alginate DJB in solution at 90°C, with and without Ca^{++} (60% stoichiometric). Closely similar spectra were recorded for the high molecular weight sample, DMB. As expected, the disordered conformations of Na^{+} alginate at low and high temperatures are directly comparable. Strikingly, a close similarity also exists between the spectrum of the calcium-induced egg-box junctions at 25°C and the CD behaviour at high temperature, suggesting specific interaction between the polymer and the cations even under conditions of high thermal energy. Although the above result suggests a primary mechanism of interchain association along the lines of the egg-box model, it could be argued that site binding of Ca^{++} ions in close proximity to the carboxyl groups of the polymer, even in the disordered state, might also give rise to very large changes in CD. In order to verify the nature of the interaction, the viscoelastic response of the hot sample was monitored under low amplitude of oscillation and unidirectional shear.

Figure 2 shows the mechanical spectrum of 2% DJB alginate at 90°C and 30% stoichiometric equivalent of Ca^{++}. The frequency sweep is typical of a xanthan-like 'weak gel' (Ross-Murphy *et al.*, 1983), i.e. the storage modulus (G') is greater than the viscous response (G'') throughout the accessible frequency (ω) range (10^{-1}–10^{2} rad/s) and both show some frequency dependence. The complex dynamic viscosity (η^*) has a gradient close to −0·9 in double logarithmic plots of η^* *versus* ω and is about an order of magnitude higher than the rotational viscosity (η) at equivalent values of frequency and shear rate. For solutions of disordered coils, by contrast, $\eta(\dot{\gamma}) = \eta^*(\omega)$ and the maximum gradient of $\log \eta^*$ *versus* $\log \omega$ for shear thinning, linear entangled chains has the experimental value of −0·76 (Morris *et al.*, 1981; Morris, 1990). The above evidence proves unambiguously that alginate associates (probably at any temperature below the boiling point) to form a 'weak gel' network, stabilised by non-covalent bonding between neighbouring chains, which remains intact under small-deformation oscillatory conditions but is broken down by steady shear The 'weak gel' properties are also evident by visual inspection, since hot alginate preparations filled with small glass beads flow slowly on shaking but rapidly set again when allowed to stand, thus suspending the glass spheres.

On cooling the heated samples it was obvious (inspection by eye or finger prodding) that the gel firmness had increased. Network formation at either extremes of the temperature course (5 and 90°C) was monitored by CD spectra. Figure 3 presents the spectral differences on addition of Ca^{++} (40% conversion) to sodium Manugel DJB at each reference temperature. In the absence of additional, calcium-induced changes in

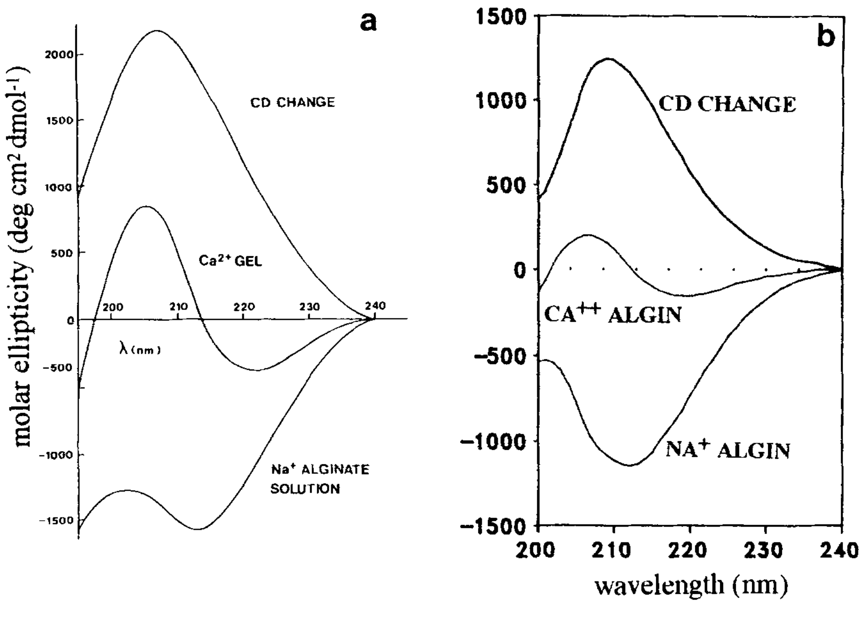

Fig. 1. (a) CD of 0·04% alginate (≈58% of polyguluronate) in the Na^{+} and Ca^{++} (100% stoichiometric requirement) forms at 25°C (Morris *et al.*, 1978). (b) CD of 2% DJB alginate (≈58% of polyguluronate) in the Na^{+} and Ca^{++} (60% conversion) forms at 90°C.

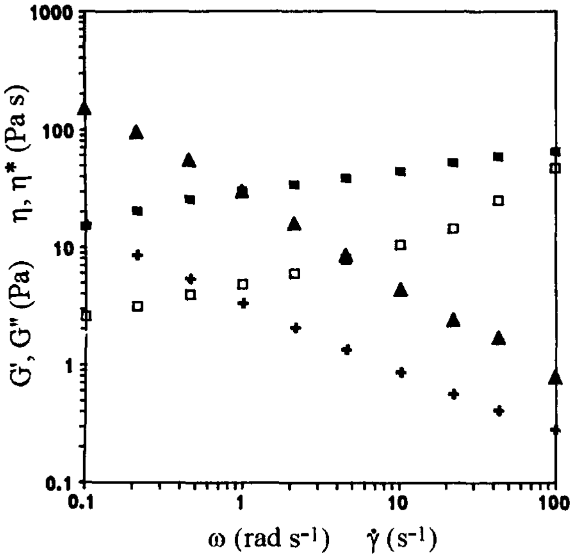

Fig. 2. Frequency (ω) dependence of G' (■), G'' (□) and η^* (▲) plus shear rate ($\dot{\gamma}$) dependence of η (+) for a 2% DJB alginate sample at 90°C and 30% stoichiometric equivalence of Ca^{++}.

the conformational arrangement of polyguluronate blocks at low temperatures, background subtractions (Na^+ alginate traces) should produce two spectra of the same magnitude. In our case, however, the controlled change of temperature (at 1°C/min) substantially alters the size (there is a 33% rise in maximum spectral intensity between the two peaks) and form (the CD spectrum at 5°C has been shifted to lower wavelength, thus crossing over the high temperature spectrum at about 215 nm) of the positive alginate band. The differences in position and CD maxima for the same polyguluronate sequences of a DJB sample, might indicate some contribution to the ordered tertiary structure from a second process at lower temperature. This interpretation was confirmed by following the network development during cooling using mechanical spectroscopy.

Figure 4 shows the time–temperature profile of gelation for a 2% DJB alginate at 30% stoichiometric equivalence of Ca^{++}. As expected from Fig. 2, the gel-like character of the sample ($G' > G''$) can be seen even at 90°C (zero time). During cooling, an initial monotonic increase in the shear moduli was recorded until a second wave of structure formation was observed at about 20°C, marking a sharp increase in G'. On holding at 5°C for about 2·5 h, the network strength progressively increased, giving overall a distinct sigmoidal time–temperature dependence of G'. The onset of a second transition at 20°C is also apparent on the $\tan\delta$ (a ratio of the viscous to elastic component for the network at a particular frequency; $\tan\delta = G''/G'$) trace as a result of reduction in phase difference between the imposed stress and resultant strain. Although the gel increases its structural coherence during keeping at 5°C, the $\tan\delta$ value ($G''/G = 0{\cdot}25$) at the end of the time course is reminiscent of a 'pseudoplastic' rather than a 'true' gel (Richardson & Ross-Murphy, 1987). Therefore, we decided to extend the time course of gelation for selected samples at 5°C.

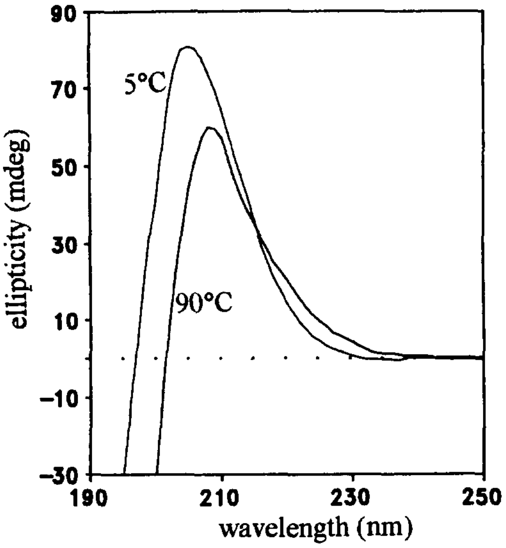

Fig. 3. CD spectral changes (40% Ca^{++} conversion – Na^+ alginate trace) for DJB alginate at the two extremes of the experimental temperature range.

As shown in Fig. 5, the gelation curves of G' and G'' for the Manugel sample of Fig. 4 creep slowly to equilibrium (values of shear moduli become gradually less time-dependent) and G' is almost an order of magnitude higher than G'' towards the end of the three-day period. The increased structural integrity of alginate samples, given suitable storage conditions (low temperature and long time), is reflected on the gel-like response of G', G'' and η^* as a function of frequency of oscillation (Fig. 6). In

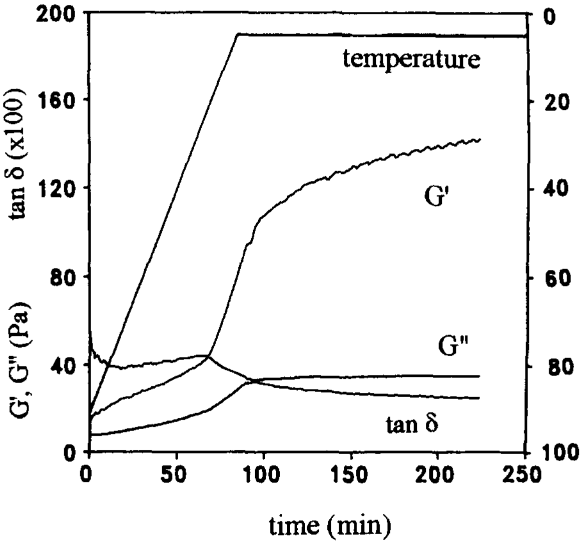

Fig. 4. Combined temperature–time sweep of three oscillatory parameters (G', G'' and $\tan\delta$) for 2% DJB alginate sample at 30% Ca^{++} conversion.

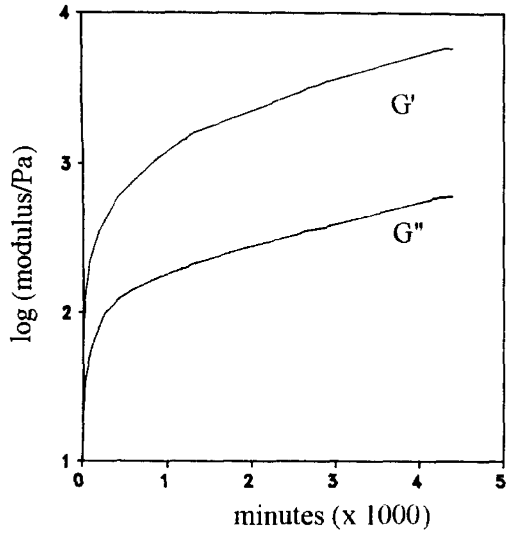

Fig. 5. The effect of prolonged storage (72 h at 5 C) on the mechanical properties of 2% DJB Manugel sample (30% stoichiometric requirement of Ca^{++})

comparison with the frequency sweep of Fig. 2 ('weak gel' conditions), there is at least a 100-fold increase in gel strength and an appreciably more gel-like spectrum (Morris, 1984), i.e. both moduli are effectively frequency-independent, the gradient of the log η^* *versus* log ω plot approaches the value of -1 expected when G' and G'' are constant and the tan δ remains constant throughout the experimentally accessible frequency range at $\approx 0{\cdot}1$. The development of substantial rigidity in refrigerated alginate samples (at 4–5°C for 72 h) was also manifest by eye, since samples did not flow on shaking and were able to support their shape against gravity on overturning their plastic containers. Consequently, the three-day storage period was chosen for the preparation of demouldable cylindrical rings used in compression analysis of the failure properties of alginate gels (see below).

Having investigated the behaviour of alginate systems on cooling, we then looked at the melting properties of gels during controlled heating. In general terms, it is accepted in the art that unlike gelatin gels (a popular choice for structuring water dessert products), alginates set by cooling remain thermally irreversible when the temperature is increased (Sime, 1990). Figure 7 shows a detailed representation of modulus variation for the DMB alginate gels as a result of a temperature change between 5 and 90°C. During heating, the increased thermal energy destabilises the structure of the second transition observed on cooling (thermally reversible process), albeit at higher temperatures, thus producing an extensive thermal hysteresis for G'. At the same time the melted material augments the viscous fraction of the gel, causing a progressive increase in the values of loss modulus. However, there is no indication of complete melting at temperatures near the boiling point, with both the elastic and viscous moduli paralleling the structure ($G' > G''$) of a hot-made (90°C), high guluronate alginate sample before the beginning of cooling (thermally irreversible transition). The overall picture of combined temperature profiles with frequency sweeps for the Manugel samples (DMB and DJB) is that of an alike qualitative response. High molecular weight preparations (DMB alginates) merely shift the rigidity modulus of long-chain ensembles to higher values keeping the same pattern of behaviour throughout the time–temperature operation. However, the effect of long, polyguluronate chains on the elasticity of gels is manifest on their structural breakdown under increasing shear. Figure 8 shows the variation of G' and G'' for Manugel alginates with increasing amplitude of oscillation at 5 C. The amount of salt

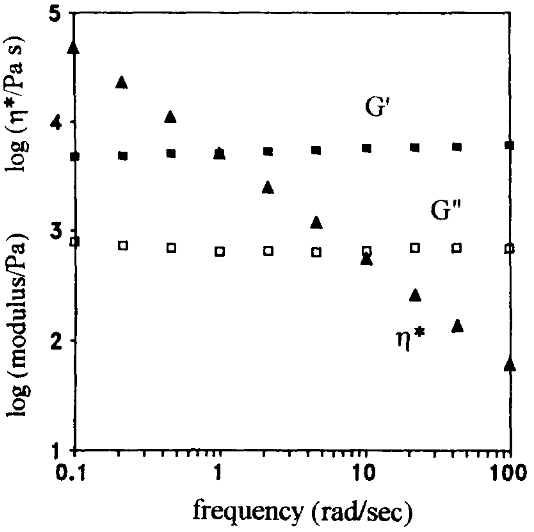

Fig. 6. Frequency sweep at 5 C of the alginate sample in Fig. 5 at the end of the 72 h time run.

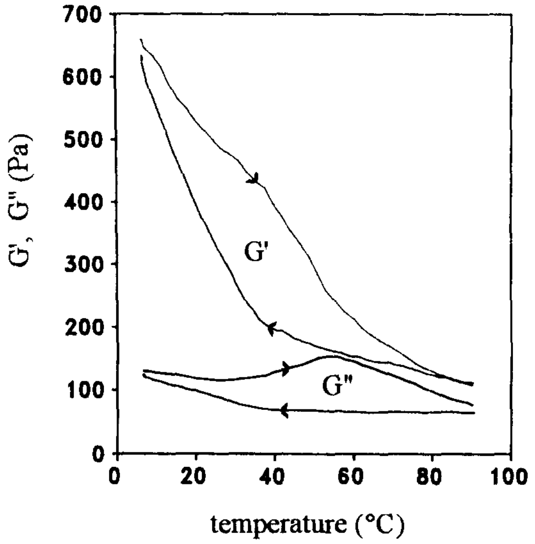

Fig. 7. Temperature dependence of G' and G'' on cooling and heating at 1°C/min for 2% DMB alginate gel (40% Ca^{++} conversion).

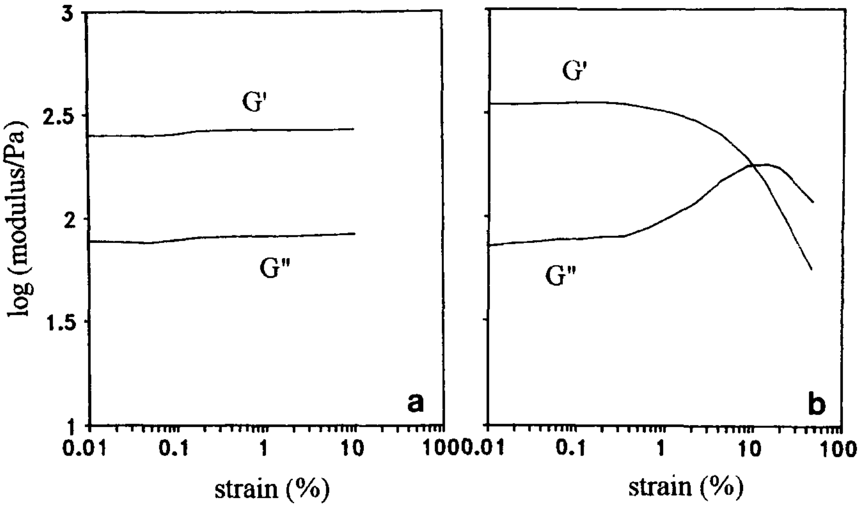

Fig. 8. Strain sweeps at 5°C of 2% DMB (a) and DJB (b) alginate samples at 30 and 40% calcium ions stoichiometric requirement, respectively.

was adjusted (30 and 40% conversion for the DMB and DJB samples, respectively) to produce gels of comparable initial strength (values of $G' \approx 300$ Pa at 0·01% strain). Over the experimentally accessible strain range, the rheological properties of high molecular weight Manugel gels remain essentially constant with no evidence of disturbance in the gel-like character. In sharp contrast to the elastic response of DMB gels, the rigidity modulus of DJB samples shows an early deviation from the linear viscoelastic region ($\approx$0·5% strain), in the way seen for the 'weak gel' preparations of xanthan polysaccharide (Richardson & Ross-Murphy, 1987). At levels of deformation higher than 0·5%, the viscous-like character (G'') rises gradually and crosses over the G' trace, finally declining sharply at about 20% strain. The initial increase and subsequent fall in G'' values have been also observed in the failure properties of acid pigskin gelatin (Papageorgiou *et al.*, 1994) and high-methoxy pectin (Ptitchkina *et al.*, 1994) gels, and they might be attributed to fragments, disconnected from the continuous matrix, that add to the viscous element of the network. Eventually, the increased amplitude of oscillation shears down the loose aggregates further, thus decreasing the resistance of the liquid-like part of the system to flow.

The effect of trisodium citrate and calcium chloride on the gelation properties of alginate

In practical applications of alginate gelation at ambient temperature, calcium sequestrants are frequently employed to reduce the concentration of free calcium ions during mixing of the ingredients in the system, thus allowing controlled gelation (Sneath, 1977). The same competing reaction will, of course, take place during mixing of trisodium citrate and alginate in a calcium chloride solution at high temperature (90°C). At the end, the gelling reaction reaches an equilibrium that can be depicted as follows:

$$\begin{tikzcd} \text{Alginate} \arrow[r, leftrightarrow] & Ca^{++} \arrow[r, leftrightarrow] & \text{Citrate} \\ & CaCl_2 \arrow[u, Rightarrow] & \end{tikzcd}$$

One possible interpretation of the second transition observed at low temperatures (Fig. 4) might be the formation of additional egg-box junctions by alginate stripping calcium ions from the citrate sequestrant. This idea was tested by monitoring the gel-like character of several alginate samples in the presence and absence of TSC. As shown in Fig. 9, the change in setting rate at low temperatures is also observed on cooling of alginate

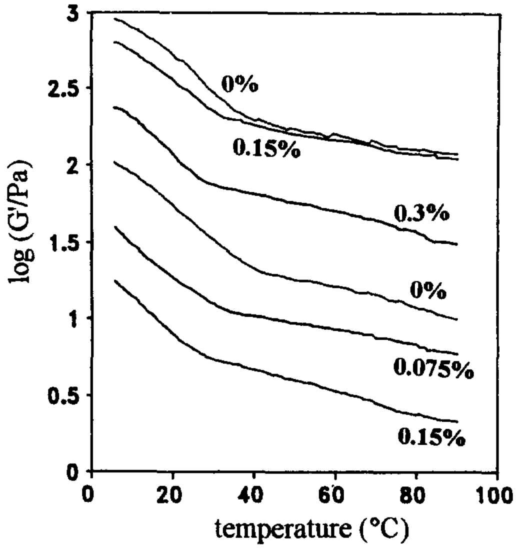

Fig. 9. Cooling profiles of 1 and 2% (bottom 3 traces and top 3 traces, respectively) high molecular weight, high polyguluronate content alginate samples at levels of TSC shown beside the individual traces (40 Ca^{++} conversion).

samples (1 or 2% at 40% conversion) without sequestrant. Therefore, the cause of the sharp increase in G' below ≈30 C is not the release of calcium from the sequestrant to the alginate that would change the equilibrium in favour of the latter. Instead, the origin of the second wave of structure formation should be traced in the mode of intermolecular association of alginate chains in calcium-induced gels. Earlier investigations (Morris *et al.*, 1978) using swamping concentrations of sodium ions have shown that alginate gelation involves an equilibrium between extensive dimerisation of polyguluronate chains and limited aggregation of the polyguluronate dimers. Pure electrostatic interactions are claimed for calcium's preferential binding of isolated polyguluronate sequences, since their charge density is substantially higher than for the dimeric complexes. Consequently, a feasible scenario would dictate strong selectivity of calcium for individual molecules at high temperatures, dropping off sharply at lower temperature where reduction in the entropic forces allows stabilisation of dimer–dimer interactions, thus having a pronounced effect on the setting rate. Dissociation of aggregated dimers could also account for thermal hysteresis observed between the gelling and 'melting' profiles of alginate samples (Fig. 7). Rearrangement of molecular chains and subsequent reduction in the size of ordered segments, as a result of increasing thermal energy, is a phenomenon commonly observed during heating and before the dissolution of aggregated biopolymer gels (Watase *et al.*, 1989).

Besides temperature, the reaction parameter important for attainment of equilibrium gel strength is the amount of calcium chloride in the system. For a given level of alginate and sequestrant, an increasing concentration of calcium ions will shift the balance between dimers and aggregates in favour of the latter. As shown in Fig. 10, for up to 40% of calcium conversion, the values of the rigidity modulus increase systematically with the amount of salt in the system and the onset of the second process moves orderly to higher temperatures (from about 18 to 35 C). However, in the presence of higher levels of $CaCl_2$ the cooling scans bear evidence of the difficulties a practical food technologist may encounter in his effort to design a suitable formulation for a particular application. Excessive quantities of salt fail to meet expectations for higher gel strength, so that network development follows a progressive, monotonic increase on cooling with no signs of cooperative enhancement of shear modulus at lower temperatures. As a result, at a Ca^{++} level of 60% of the theoretical requirement for alginate, the ultimate modulus of the system remains below the corresponding gel strength at 40% conversion and the 80% stoichiometric equivalence forces the network to fall short of the final modulus attained at 30% conversion (below about 20°C). This major drawback in alginate gel technology may be attributed to the saturated levels of salt (e.g. the 60% conversion exceeds the requirement of total calcium binding at both surfaces of each chain for the 58% polyguluronate samples) promoting complete aggregation and considerable dehydration of the ordered segments even at temperatures near the boiling point. The formation of precipitated complexes instead of homogeneous preparations is also apparent by eye and serves as a reminder that the structural weakening of alginate gels at high levels of salt is accompanied by complex textural changes in the polymeric assemblage. Changes in the magnitude of the shear modulus as a function of the $CaCl_2$ content invite analogy with differences in network strength for systems where the amount of sequestrant is allowed to vary at constant ionic strength (Fig. 9). In terms of the alginate–Ca^{++}–sequestrant equilibrium reaction, higher concentrations of TSC will reduce the amount of salt available for the alginate, thus resulting in progressively weaker gels. Experimentally, it is a straightforward task to show that the real molarity of calcium ions (effective salt concentration for gel formation) accessible to alginate is always a function of the quantity of sequestrant. For example, we have noticed that the 2% alginate series produces an identical mechanical response during cooling for the gels of 40% conversion plus 0·3% TSC (Fig. 9) and 30% conversion plus 0·15% TSC (Fig. 10).

Failure properties of alginate gels

Large deformation analysis of alginate samples was carried out with a view to facilitating technological progress in the development of reformed products (Bryden & Sanderson, 1982). In this respect, the high mannuronate content (high-M) alginate samples (Manucol DH) are of considerable interest since they are believed to form 'weaker and more elastic gels' than the high guluronate (high-G) counterparts (Sime, 1990).

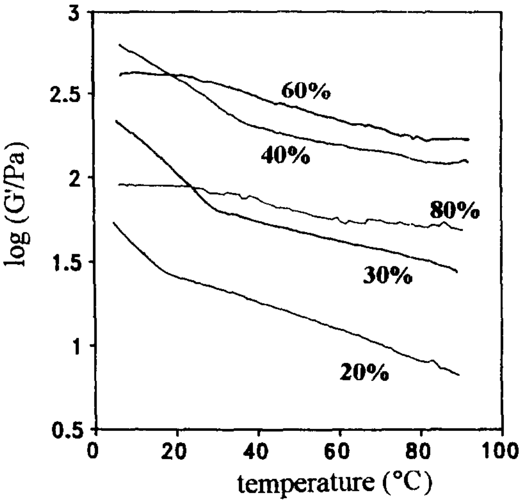

Fig. 10. Development of shear modulus during cooling of 2% DMB Manugel samples (0·15% citrate) in the presence of increasing amounts of calcium ions (% conversion).

Thus the ultimate properties (yield stress and yield strain) of both types of alginate were characterised under compression testing at 5°C. Only preparations containing between 30 and 80% of the calcium stoichiometric requirement were capable of forming demouldable gels upon ageing (72 h and 5°C). During the refrigerated storage, gels became progressively firmer with minimum loss of water from the network. This is a significant advantage over samples prepared by diffusion or internal setting which cause greater shrinkage and syneresis due to the technique-inherited instability of the resulting gel network (Clare, 1993).

Figure 11 illustrates the texture profiles obtained on compression of 2% Manugel and Manucol gel rings at 40% Ca^{++} conversion. As anticipated, the maximum force (hardness) necessary to break the high-M gels remains well below the yield stress of the high molecular weight, high polyguluronate samples. In fact, the compression curve of the Manucol samples is rather similar to the force–deformation profile of DJB alginates, albeit shifted to slightly higher strain values, e.g. the strain at yield point (brittleness) is about 38 and 35%, respectively. Surprisingly, the high molecular weight, high-G DMB samples remain intact up to 54% compression level, thus forming more elastic gels than the high-M alginates. Similarly, characterisation of hardness and brittleness for gels prepared with $CaCl_2$ concentrations between 30 and 80% of alginate conversion results in the strongest and most elastic DMB gels (Fig. 12). For both Manugel and Manucol alginates, the maximum gel strength occurs at a calcium ion concentration of about 40%. In accordance with the small deformation studies (Fig. 10), reduction in hardness values at lower amounts of salt is an expected consequence of formation of fewer intermolecular junction zones, whereas reduction at higher concentrations can be attributed to excessive, calcium-induced aggregation of egg-box dimers, driving the network towards a precipitate. As shown in Fig. 12(b), the decrease in yield stress with increasing salt is accompanied by a progressive reduction in the amount of deformation that the gels will withstand before they break, with the sample of shorter chainlength (DJB) fracturing at lower strain throughout and the Manucol gels exhibiting elastic properties intermediate to the two Manugel samples.

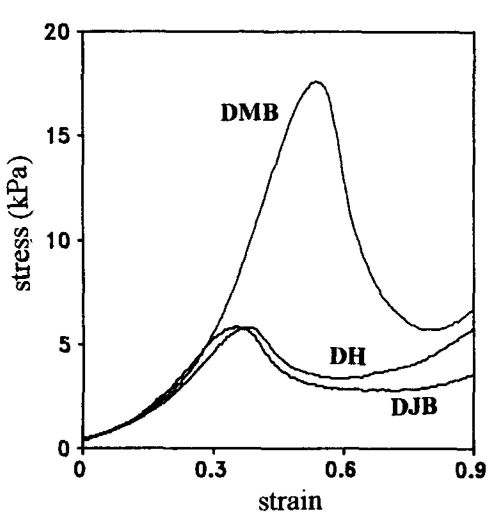

Fig. 11. Force–deformation curves for 2% DMB, DJB and DH alginate gels stored at 5°C for 72 h and then compressed at a rate of 0·8 mm/s to 30% of the original height (0·15% TSC; 40% conversion).

In conclusion, we hope that the insights gained on the mechanism of the alginate–calcium reaction from hot-made preparations, will provide a guide towards a deeper understanding of the factors involved in alginate gelation on its own or in a composite mixture with cold-setting agents such as gelatin, κ-carrageenan and gellan gum. Furthermore, the spectacular performance (combining elasticity with strength) of high guluronate DMB samples might contribute to the development of new, appealing structured or water-based products.

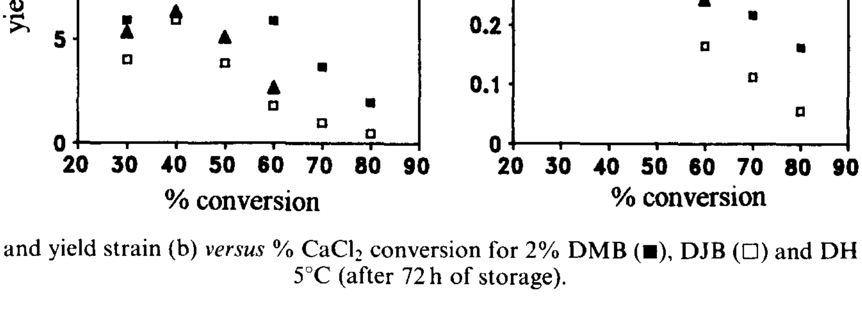

Fig. 12. Yield stress (a) and yield strain (b) *versus* % $CaCl_2$ conversion for 2% DMB (■), DJB (□) and DH (▲) alginate samples at 5°C (after 72 h of storage).

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