Carbohydrate Polymers **19** (1992) 279–284

Temperature dependence of the elastic modulus of alginate gels

Størker T. Moe, Kurt I. Draget, Gudmund Skjåk-Bræk & Olav Smidsrød

Norwegian Biopolymer Laboratory (NOBIPOL), Laboratory of Biotechnology, Norwegian Institute of Technology, University of Trondheim, Sem Sælands vei 6–8, N-7034 Trondheim, Norway

The temperature dependence of the elastic modulus for alginate gels was studied using two different gel systems: covalently crosslinked Na-alginate gels and in-situ prepared Ca-alginate gels. The modulus of physically crosslinked gels showed a complex behaviour. The temperature coefficient of the modulus of covalently crosslink gels changed from positive for the lowest crosslinked gels to negative for the highest crosslinked gels. This suggests a change from rubberlike to enthalpy-driven elasticity with an increasing degree of crosslinking for these gel networks.

INTRODUCTION

Alginates are linear copolymers of α-L-guluronate and β-D-mannuronate, and their ability to form gels with multivalent cations is well known. Alginate gels are in general strong, and the modulus of stiffness is known to depend on the molecular weight of the alginate (Mitchell, 1980), the composition and sequence of the monomers in the alginate chain (Smidsrød & Haug, 1972; Martinsen *et al.*, 1989) and the type of gelling cation (Haug, 1964; Stokke *et al.*, 1991). The nature of the elasticity of alginate gels has, however, not been unambiguously determined (Segeren *et al.*, 1974; Andresen & Smidsrød, 1977).

Many synthetic polymer networks behave according to the theory of rubber elasticity (Flory, 1953; Treloar, 1975). According to this theory, the elasticity of a polymer network originates from the decrease in the entropy of each elastic chain due to a perturbation from the statistically most probable end-to-end distance. The entropic nature of the rubber elasticity leads to an increase in elasticity modulus upon an increase in temperature (Treloar, 1975):

$$\frac{\sigma}{\gamma} = G = NkT = \frac{cRT}{M_c} \tag{1}$$

or

$$G \propto T \tag{2}$$

where σ is the shear stress in the sample, γ the shear strain, G the shear modulus of the sample, N the number of elastic chains per unit volume, k Boltzmann's constant, T absolute temperature, c the concentration of polymer in the gel, R the gas constant and M_c the molecular weight of an elastic chain.

The main assumptions in the theory of rubber elasticity are the random-coil behaviour of the elastic chains, and that the network chains are linked together by point-like crosslinks. The alginate gel structure does not conform to any of these assumptions. The alginate gel consists of long junction zones (Stokke *et al.*, 1991) where homopolymeric guluronate sequences — termed 'G-blocks' — dimerize, bound by multivalent cations (Grant *et al.*, 1973). This leads to a gel network with chains bound together in long junction zones rather than in point-like crosslinks, a gel structure which has been theoretically treated by Higgs and Ball (1989). The behaviour of the network chains in the alginate gel also suggests that the application of rubber elasticity theory on alginate gels is questionable. The network chains between junction zones consist partly of homopolymeric mannuronate sequences ('M-blocks'), partly of copolymeric regions ('MG-blocks') and partly of non-crosslinked G-blocks. Calculations suggest that alginate chains do not exhibit Gaussian (random-coil) behaviour before the degree of polymerization is above approximately 1000 monomer units, the number depending on the composition of the chain (Bailey *et al.*, 1977). It has

Carbohydrate Polymers 0144-8617/92/$05.00

been shown that the elastic modulus of some polysaccharide networks is 3–10 times that predicted using the theory of rubber elasticity (Clark *et al.*, 1983), which may support the view of polysaccharide gels as polymer networks that do not conform to rubber elasticity theory.

A stiff coil can, upon heating, increase its flexibility because of the increased number of allowed torsion angles in the chain with increasing temperature. Stiff chain networks can thus be expected to show a decrease in storage modulus with increasing temperature. Storage moduli decreasing with increasing temperature have been observed for gels made from globular proteins (Richardson & Ross-Murphy, 1981), which has been interpreted as enthalpic elasticity for this network (Clark & Ross-Murphy, 1987). Other biopolymer (gelatin) gels have, however, shown behaviour compatible with the theory of rubber elasticity (Preston & Meyer, 1971).

The discrepancies in the literature concerning the applicability of rubber elasticity theory on alginate gels originate mainly from two works: Segeren *et al.* (1974) showed that for alginate gels prepared using D-glucono-δ-lactone (GDL) and dicalcium orthophosphate, the storage modulus was proportional to temperature in the range 22–49°C. This has generally been taken as an indication of rubberlike behaviour of the alginate gel, although the authors themselves found from swelling experiments that the number of statistical segments in the average elastic chain was less than two, a number which clearly is far too small to allow the use of rubber elasticity theory (Flory, 1953). Andresen and Smidsrød (1977) used alginate gels prepared by dialysing alginate solutions extensively against $CaCl_2$ solutions, and found that the elastic moduli of the gels decreased with increasing temperature. The decrease in elastic modulus was not attributed to the behaviour of the alginate chains themselves, but rather to the behaviour of the nonpermanent crosslinks of the gel. The decrease was interpreted as a breaking and rearrangement of junction zones when force was applied to the dense network of stiff chains. The junction zones were in a way regarded as the 'weak points' in the alginate gel, and different ions could give different gel strengths depending on their ability to bind and give 'strength' to the junction zones.

Even though the two groups have used similar alginates with approximately 70% guluronate residues, a direct comparison is not possible, partly because of the large differences in methods. An alginate gel prepared using GDL and an inactive form of Ca^{2+} ions has often a lower degree of crosslinking than gels prepared by dialysis, something which can be seen by comparing the elastic moduli of the two gels (Skjåk-Bræk *et al.*, 1986; Smidsrød *et al.*, 1992). Additionally, no data can be found concerning the final pH of the gel in the case of Segeren *et al.* (1974). This is especially important when GDL is used because the resulting D-gluconic acid inevitably causes a pH lowering in the system. It is well known that alginates form acid gels. When the pH of the alginate gel is in the range of the pK_a of the uronic acids or lower, additional crosslinks will be present due to acid gel formation. Also, when a sequestering agent like orthophosphate or EDTA is used, great care must be taken to investigate the proportion between calcium bound to the alginate and calcium bound to the sequestering agent.

The scope of this work is to investigate the temperature properties of alginate gels and to clarify the question of the applicability of rubber elasticity theory on alginate gels. Two different approaches have been chosen: (1) In-situ prepared gels made by the method of Draget *et al.* (1991) using GDL and calcium carbonate and (2) covalently crosslinked gels prepared by the method of Moe *et al.* (1991). By using calcium carbonate as a calcium source for the ionically crosslinked gels, one has a much easier system to analyse than by using other forms of calcium such as Ca-EDTA or Ca_2-orthophosphate because of the pH of the resulting gel. The use of covalently crosslinked alginate gels should make it possible to study the behaviour of the alginate chains themselves without interference from the Ca^{2+}-oligoguluronate interactions.

MATERIALS AND METHODS

Chemicals

The alginate samples were provided by Protan Biopolymer A/S. The alginate was extracted from *L. hyperborea* and had an intrinsic viscosity of 6·3 dl/g, corresponding to a viscosity-average molecular weight of 190 000 g/mole. The content of guluronic acid was 65% and the average number of guluronate residues in homopolymeric G-blocks was 15·5, determined by ^{1}H-NMR (Grasdalen, 1983). Epichlorohydrin was Merck p.s. grade. Calcium chloride, sodium chloride, sodium hydroxide and EDTA was Merck p.a. grade. $CaCO_3$ was 'Eskal 50' from KSL Staubtechnik GMBH, Lauingen, Germany. D-glucono-δ-lactone (GDL) was obtained from Sigma Chemical Co., St Louis, USA.

Preparation of Na-alginate gels

Homogeneous Ca-alginate gel cylinders (14 mm diameter, 15 mm height) were made by the method of Skjåk-Bræk *et al.* (1989). The cylinders were washed and dialysed against 96% (v/v) ethanol until equilibrium. The Ca-alginate–ethanol gels were crosslinked using a mixture of 3 parts by volume alginate gel, 2 parts epichlorohydrin, 1 part 0·1 M $CaCl_2$ in distilled water and 1 part 1 M NaOH in distilled water. The temperature was held at 25°C with a water bath, and the concentration of NaOH was held constant with a Radiometer automatic titration system using a PHM 84 pH-meter equipped with a G202B pH-electrode and a K711 double salt bridge reference electrode with concentrated Li-acetate, a TTT 60 titration unit and a ABU 80 autobyrette. The reaction was allowed to proceed for 2–5 h, after which the reaction was stopped by addition of 4 M HCl and subsequent washing with water. The calcium ions were removed by dialysis against 50 mM EDTA/3 M NaCl, and the EDTA was removed by dialysis against 3 M NaCl.

Temperature dependence of Na-alginate gel volume

The temperature dependence of the gel volume was determined by measuring the diameter of gel beads (prepared together with the gel cylinders) when exposed to temperatures ranging from 3°C to 50°C. There was no detectable volume change (data not shown).

Distribution of crosslink density in Na-alginate gel

The crosslink homogeneity was monitored by cutting gel cylinders into cubes measuring approx. 1 mm. The gel cubes were immersed in 3 M NaCl and in distilled water. Cubes prepared from the edges of the gel should show the same volume change as cubes prepared from the centre of the gel if the crosslink density and polymer concentration of the gel was constant throughout the gel (Flory, 1953). Since the gel cylinders were originally prepared to have a constant alginate concentration throughout the gel, any difference in swelling would indicate an inhomogeneity in crosslink density.

Preparation of Ca-alginate gels

To obtain Ca-alginate gels a gelling system based on $CaCO_3$ and the slowly hydrolysing D-glucono-δ-lactone (GDL) was applied as described earlier (Draget *et al.*, 1991). The gels were made at 1·0% alginate concentration and the content of Ca^{2+} was 7·5 mM. An equivalent amount of GDL was added to ensure complete dissolution of the Ca-carbonate and at the same time preserving a final pH around neutrality (pH 6·5–7·0).

Rheological measurements

(a) Covalently crosslinked sodium alginate gels

The elastic moduli of the gels were determined in a Bohlin VOR rheometer equipped with a PP30 (30 mm diameter plate/plate geometry) sensor and a 0·235 g cm torsion bar. The Na-alginate gels were fixed to the plates with cyanoacrylate glue ('superglue'). Because the gels did not cover the whole plate, the moduli were recalculated using the relationship:

$$G'_{\mathrm{corr}} = G'_{\mathrm{measured}}\left(\frac{R_{\mathrm{plate}}}{R_{\mathrm{gel}}}\right)^4 \tag{3}$$

where G' is the dynamic storage modulus and R denotes the radii of the gel and measuring plate, respectively. The shear wave length of the gel was calculated to be approximately 10 cm, which allowed the use of this simple scaling model. Both frequency scans (0·01–2 Hz) and temperature scans (10–50°C, 1°C/30 s) were performed.

(b) Ca^{2+}-crosslinked alginate gels

$CaCO_3$ was dispersed in a solution of Na-alginate, and a freshly made GDL solution was added. After a rapid mixing of the components, a 900 μl sample was placed within the PP30 sensor system of the Bohlin VOR rheometer equipped with a 4·0 g cm torsion bar and the gap between the plates was set to 1·00 mm. When the gel had reached apparent equilibrium (approx. 18 h), a frequency scan (0·01–2 Hz, 20°C) and several temperature scans (10–85°C, 1 Hz) were performed (strain: 0·022). The sample was submerged in low viscosity (4·6 mPa s) silicone oil to prevent evaporation.

RESULTS AND DISCUSSION

Monitoring crosslink density and crosslink homogeneity of Na-alginate gels

The results of the swelling of cubes cut from Na-alginate gel cylinders is given in Fig. 1. The figure shows that cubes prepared from the centre of the gel swell significantly more than cubes prepared from the edges of the gel cylinders. Since the swelling of gels is supposed to increase with decreasing crosslink density (Flory, 1953), this result suggests an inhomogeneous distribution of crosslinks through the gel, probably a direct result of the fact that the crosslinker had to be introduced by diffusion into the gel from the outer reagent mixture. As we have no methods of producing a more homogeneous distribution, we have chosen to consider the rheological data obtained as an average for the gel.

The absolute degree of crosslinking is not determined for the Na-alginate gels. Several approaches have been considered, but none has given reliable results so far. The best suited methods are probably an analysis involving acid degradation of the gel followed by GC-MS (Holmberg, 1983) or computer simulations of fully swollen networks. The latter approach is presently being pursued in our laboratory.

The storage modulus increases with reaction time, as shown in Fig. 2, confirming that the average crosslink density increases with reaction time as should be expected.

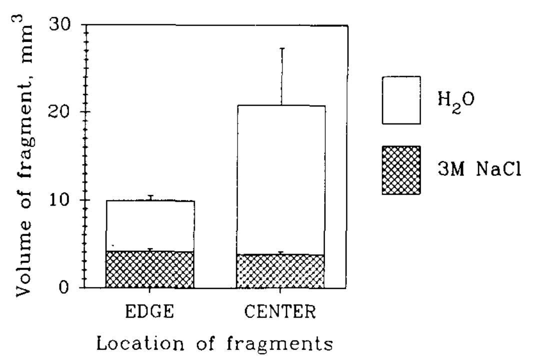

Fig. 1. Swelling of gel cubes in 3 M NaCl and in distilled water. The cubes were cut from Na-alginate gel cylinders cross-linked for 5 h. Volumes are given for gel sections cut at the edge and at the centre of the gel cylinders.

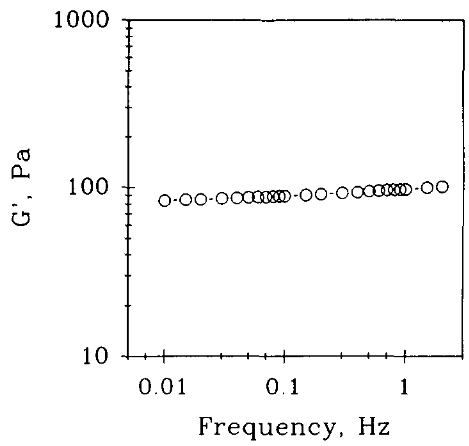

Fig. 3. Frequency dependence of storage modulus (G') for *in situ* prepared Ca-alginate gels, 7·5 mM Ca^{2+}.

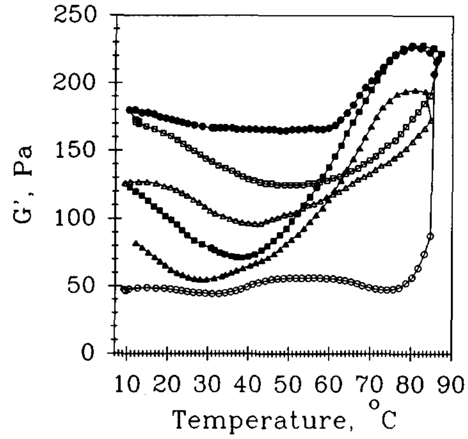

Fig. 4. Temperature dependence of storage modulus (G') for Ca-alginate gels. ○, scan No. 1 (up); ●, No. 1 (down); □, No. 2 (up); ■, No. 2 (down); △, No. 3 (up); ▲, No. 3 (down).

Rheological measurements

As seen in Fig. 2, the storage moduli (G') for the Na-alginate gels were frequency-independent within experimental errors in the range from 0·01 Hz to 2 Hz. The Ca-alginate gel showed a slight increase in storage modulus with increasing frequency (Fig. 3).

Temperature scans of the calcium limited alginate gel are shown in Fig. 4. These scans clearly show that the observed temperature dependencies are of a complex nature with large hysteresis. Although the hysteresis decreases with the number of scans, the system is still uninterpretable. The non-equilibrium nature of the Ca-alginate gel apparently gives rise to large kinetic effects which totally overshadow the effect of temperature on the alginate elastic chains. It should be stressed that these experiments were carried out at low strains (0·022), which is well within the limit of linear viscoelastic response for such systems in general (Mitchell, 1980) and the observed viscoelastic linearity for this particular system (results not included). The pronounced hysteresis between scans 1 and 2 (increased G') has also been described in earlier works (Andresen & Smidsrød, 1977; Lapasin *et al.*, 1988). Andresen & Smidsrød, (1977) overcame the kinetic effects observed by studying the time dependence of the moduli at different temperatures and extrapolating to zero time at each temperature to obtain meaningful data. In general, great care should be taken using such systems to obtain information about the behaviour of the alginate gel, and this non-linear behaviour of physical gel shows that covalently crosslinked systems are necessary to determine the question of entropic or enthalpic elasticity of alginate gels.

An up and down temperature scan of the covalent crosslinked Na-alginate gels gives the impression of a linear relationship and shows very little hysteresis (Fig. 5). This result is fundamentally different from the Ca-alginate gel (Fig. 4), and gives the impression of non-complex behaviour in a system at equilibrium. We therefore believe that the results from the temperature scans on the Na-alginate gels are more reliable in the sense that they may be related to the behaviour of the

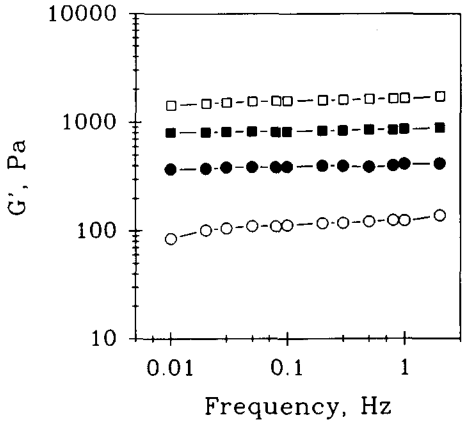

Fig. 2. Frequency dependence of storage modulus (G') for some Na-alginate gel cylinders. ○, gel crosslinked 2 h; ●, 3 h; ■, 4 h; □, 5 h.

alginate molecule itself, rather than to the more complex Ca/guluronate-block interactions of the Ca-alginate gels. Some of the temperature scans of the Na-alginate gels are given in Fig. 6.

The temperature coefficient of the storage modulus (dG'/dT) was calculated using linear regression. The data have been fitted to a simple first-order proportionality. The temperature coefficient of the Na-alginate gels versus their storage modulus is given in Fig. 7. It is evident that the temperature coefficient for the Na-alginate gels is changing from positive in the least crosslinked gels to negative in the most crosslinked gels. This suggests a change from rubber-like elasticity to an enthalpy-driven elasticity. The negative temperature coefficient for the dynamic storage modulus of the highest crosslinked gels suggest a network of stiff chains whose flexibility increases with increasing temperature.

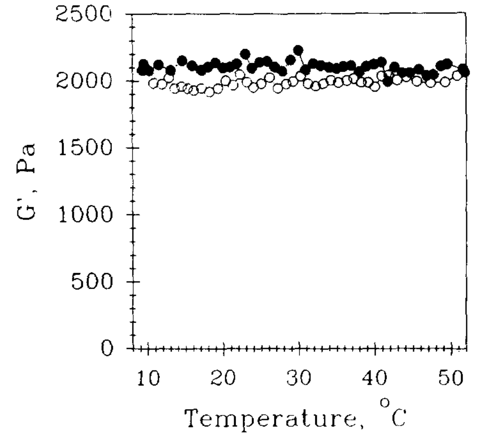

Fig. 5. Hysteresis curve for temperature scan of Na-alginate gel cylinders, crosslinked 3 h. ●, up gradient; ○, down gradient.

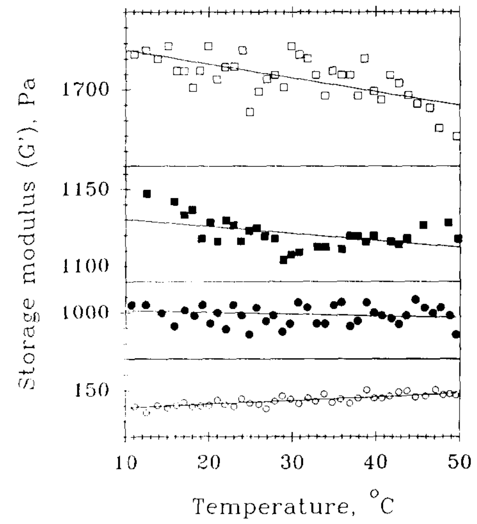

Fig. 6. Temperature dependence of the storage modulus (G') for some Na-alginate gel cylinders. ○, gel crosslinked 2 h; ●, 3 h; ■, 4 h; □, 5 h.

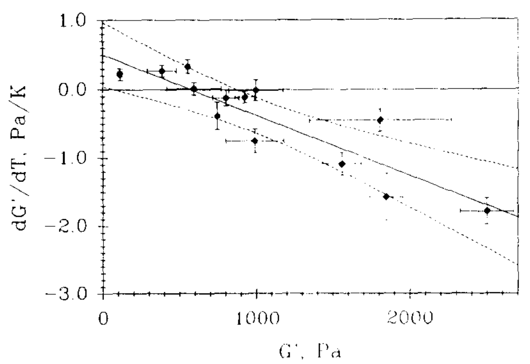

Fig. 7. Temperature coefficient of the storage modulus (dG'/dT) versus the storage modulus (G') for Na-alginate gel cylinders. Straight line shows first-order regression, dotted lines show 99% confidence interval for regression.

CONCLUSION

The storage moduli of Ca-limited *in situ* made alginate gel show a complex dependence upon temperature. There are reasons to believe that kinetic effects totally overshadow the thermal behaviour of the alginate molecule itself. One should therefore take great care when the thermal effects on the storage modulus in such systems are to be attributed to entropic or enthalpic behaviour of the alginate molecule.

The temperature coefficient of the storage modulus for covalently crosslinked Na-alginate gels changes from positive for low-crosslinked gels to negative for high-crosslinked gels. This suggests a change from rubber-like (entropic) elasticity for the low-crosslinked gels to an enthalpic elasticity for the high-crosslinked gels. In these equilibrium systems, we therefore conclude that it may be possible to prepare low-crosslinked gels showing an increase in elastic modulus upon an increase in temperature in accordance with the theory of rubber elasticity. It would also be possible to prepare alginate gels with higher crosslink densities showing a decrease in elastic modulus upon an increase in temperature. This then, corresponds to an enthalpic elasticity behaviour even in a system of covalently crosslinked alginate gels. It is therefore not necessary to postulate rupture of junctions to explain the enthalpic elasticity of calcium alginate gels (Andresen & Smidsrød, 1977). Our data are thus not in disagreement with a new gel model where the physical junctions in ionically crosslinked alginate gels are regarded as permanent crosslinks when the number of guluronate residues involved in the junction zones exceeds a certain, ion dependent, critical number (Stokke *et al.* 1991).

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