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Gel strength of Ca-limited alginate gels made *in situ*

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Abstract

The gel strengths of Ca-alginate gels made *in situ* with different degree of cross-linking were determined by adapting three different methods: FIRA Jelly Tester, initial deformation (Youngs modulus, E) with a Stevens LFRA Texture Analyzer, and dynamic measurements with a Bohlin VOR Rheometer (dynamic storage modulus, G′). The results showed that there were relatively large differences in absolute values, but that the deviations diminished when the results were expressed as relative gel strengths. The deviations of the Youngs modulus (E) from G′ increased with decreasing gel strength. Only dynamic measurements were suitable for quantifying low gel strengths.

The gel strength and the breaking point were also measured as a function of the molecular weight of alginates isolated from stipes of *Laminaria hyperborea*. In the present Ca limited system, both the gel strength and the breaking point showed a marked increase with increasing molecular weight (Mw) up to 320–340 kD. This is considerably higher than with gels made by dialysis ('Ca saturated'), where the gel strength becomes independent on molecular weight around 100 kD. These results may have an impact on applications of alginate gels where the source of Ca crosslinking ions is limited.

Introduction

There exist basically two methods for making Ca-alginate gels. Perhaps, the best known and described one is the dialysis/diffusion method where an alginate solution is gelled by diffusion of Ca-ions from an outer reservoir. This method is applied in the alginate bead immobilization technique (Smidsrød & Skjåk-Bræk, 1990) and in the making of some restructured foods like pimiento strips. An alginate solution can also be solidified by internal setting, *i.e. in situ* gelation. By this method, a calcium salt with limited solubility, or complexed Ca^{2+} ions, are mixed with an alginate solution into which the calcium ions are released, usually by addition of a slowly acting acid such as D-glucono-δ-lactone (GDL) (Cottrell & Kovacs, 1980; Skjåk-Bræk *et al.*, 1986; Draget *et al.*, 1991). This method is chosen if the purpose is to create a homogeneous, non-syneretic alginate macrogel to fill the internal space of a given container (Draget *et al.*, 1989). The latter system with its defined supply of Ca^{2+} may be made calcium limited as opposed to the former system where Ca ions are allowed to diffuse into the alginate solution to give a calcium saturated gel. A Ca-limited gel may behave differently from a Ca saturated gel with respect to the effect of the molecular weight on the gel strength due to a lower degree of crosslinking and therefore an increased number of non-elastic chains in the network (Flory, 1953).

The rheological characterization of viscoelastic gels with physical crosslinks often becomes ambiguous if it is based on empirical rheological instruments with single point measurements such as the FIRA-test, the Bloom Gelometer, etc... These methods have been reviewed by Mitchell (1976) and are quite often used in industry. Empirical instruments have in common that they measure gel strength in parameters different from basic rheological quantities, such as the amount of water (force) required to deform a gel to a certain extent in the FIRA method, and they hence become dependent upon the measuring geometry and the instrument used.

The scope of this work was to clarify if the empirical gel strength measured in the FIRA test can be related to the fundamental rheological dynamic storage modulus (G′) as measured with a Bohlin VOR Rheometer and Youngs modulus, E, measured by Stevens LFRA Texture Analyzer. In addition, we have studied the effect of molecular weight on gel strength and breaking point of calcium limited, *in situ* made alginate gels.

Material and methods

The chemical composition, intrinsic viscosity ($[\eta]$) and molecular weight of the alginates used in this study are given in Table 1. All samples are commercial grade alginates, provided by Pronova Biopolymer A/S, Drammen, Norway, isolated from *Laminaria hyperborea*. One sample (#9) is isolated from the blade of this kelp, whereas the other 8 samples with different molecular weight are isolated from the stipe of the same kelp. The molecular weight given is the viscosity average calculated from the Mark-Houwink equation adapting values of *K* and *a* from Martinsen *et al.* (1991).

$CaCO_3$ was 'Eskal 50' (average particular size 5 μm) from KSL Staubtechnik gmbh, Lauingen, Germany, and D-glucono-δ-lactone (GDL) was purchased from Sigma Chemical Co., St. Louis, USA.

In situ alginate gels were prepared as described earlier (Draget *et al.*, 1991). Briefly, solid $CaCO_3$ was dispersed into a solution of Na-alginate followed by an addition of a freshly made GDL solution. By using equivalent amounts of carbonate and GDL, the gels had a final pH between 6 and 7. The final concentration of alginate was 1.0% (w/v).

Table 1. Chemical composition and sequence of the alginate samples used in this study as determined by 500 MHz ^{1}H NMR spectroscopy (Grasdalen *et al.*, 1979, Grasdalen, 1983). F_G = fraction of guluronic acid residues, F_{GG} = fraction of guluronic acid residues in neighbour to another guluronic acid residue, F_{GGG} = fraction of guluronic acid residues between two other guluronic acid residues, $N_{G>1}$ = average block length of guluronic acid blocks larger than 1. $[\eta]$ = intrinsic viscosity. M_W = viscosity average molecular weight.

Alginate sample	F_G	F_{GG}	F_{GGG}	$N_{G>1}$	$[\eta]$ (dl/g)	M_W (g/mole)
1	0.69	0.58	0.54	14.1	1.7	60000
2	0.69	0.59	0.54	13.1	3.0	100000
3	0.68	0.52	0.47	11.2	5.2	160000
4	0.69	0.57	0.52	13.2	7.2	210000
5	0.66	0.57	0.52	13.1	10.1	280000
6	0.68	0.58	0.52	12.0	11.6	320000
7	0.69	0.59	0.55	14.9	13.8	380000
8	0.66	0.54	0.49	11.8	14.8	400000
9	0.48	0.31	0.27	9.7	13.1	430000

The instrumental parameters of the dynamic Bohlin VOR measurements were as follows: Measuring system: SP30, Frequency: 1 Hz, Amplitude: 20%, Temperature: 25 °C and Torsion Bar: 12.15 g cm. A 1.30 ml sample of a freshly made gelling mixture was applied, and the gap between the plates was set to 1.00 mm. In order to avoid evaporation during the extended time measurements, the gelling system was covered with a low viscosity silicone oil (4.6 mPas) in a 'guard ring'. The dynamic measurements were run until an apparent equilibrium in the dynamic storage modulus (G′) was obtained.

Young's modulus (E) for the different gels was calculated by drawing the initial tangent of a force-deformation curve obtained in a Stevens LFRA Texture Analyzer with the probe TA11 as described earlier (Smidsrød *et al.*, 1972). The gel cylinders had a height of 17 mm and a diameter of 15 mm. The rate of compression was 0.2 mm s^{-1}.

The FIRA Jelly Test experiments were carried out as follows: A freshly made gelling solution was poured into 5.5 cm (diameter) FIRA steel containers and covered to avoid evaporation. The water filling speed of the instrument was calibrated to 100 ml $min^{-1} \pm 5$ ml min^{-1}. After the time necessary to obtain apparent equilibrium in gel strength determined in the dynamic measurements, the gel strength was measured as the water necessary to turn the blade 30° within the gel. By adapting the correlation given by Campbell (1938), the amount of water was converted to force per unit area. Breaking strengths were determined by using a bead probe (TA8, 0.63 cm in diameter) with the Stevens Analyzer, and recording the load necessary to create rupture. The gel cylinders were 35 mm in diameter and had a height of 41 mm. The rate of compression was 0.2 mm s^{-1}.

Results and discussion

The initial chemical reaction in the present gelling system is that the D-glucono-δ-lactone, GDL, hydrolyses in water with ring-opening to produce a carboxyl group. Upon this slow acidification, the insoluble $CaCO_3$ converts to HCO_3^- and free calcium ions which can create a continuous gel network by interacting with the alginate molecules and form junction zones. Further acidification converts the bicarbonate ions to CO_2. In order to work with homogeneous and non-syneretic gels, we chose to study different degrees of crosslinking between the lower Ca^{2+} limit given by the incipient sol-gel transition and the upper limit given by visible syneresis after 24 hours. These limits are 5 to 10 and 7.5 to 15 mM liberated calcium ions for a 1.0% solution of alginates isolated from *L. hyperborea* blade and stipe, respectively. This difference is related to the different chemical composition of the two alginates (see Table 1). In the case of molecular weight dependence, which was studied for *L. hyperborea* stipe alginates only, 15 mM $CaCO_3$ and 30 mM GDL was used throughout.

An amplitude (strain) sweep of a highly crosslinked alginate gel is presented in Fig. 1. It can be seen that a deviation from linearity does not occur before 50–60% of maximum amplitude is reached in a Bohlin VOR Rheometer (1 × sensitivity). The chosen 20% amplitude is well within this limit.

Rheological measurements

The observed gel strengths of *L. hyperborea* blade (sample 9) and stipe (sample 7) alginate gels are given in Figs 2 and 3, respectively. Figures 2A and 3A show the measured values determined for G′ (the dynamic storage modulus measured in the Bohlin VOR), E (Young's modulus, longitudinal deformation from Stevens Texture Analyzer) and the FIRA test in force per unit area (Campbell, 1938). Figures 2B and 3B show the relative gel strength by setting the highest strength observed (at the highest degree of crosslinking) in each of the three instruments to 100%. Due to very low gel strength at the lowest degree of crosslinking of both leaf and stipe alginate gels (25 and 75 Pa, respectively), only dynamic measurements in the Bohlin VOR Rheometer were

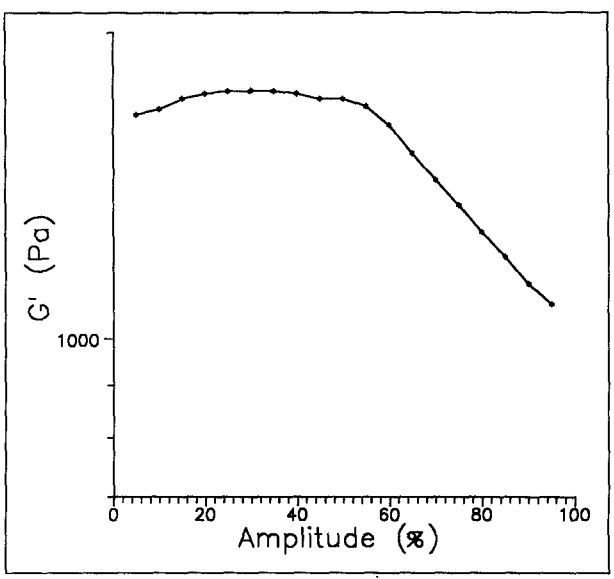

Fig. 1. Dynamic storage modulus as function of amplitude (strain) in a gel made of sample #8 crosslinked with 15 mM Ca^{2+}.

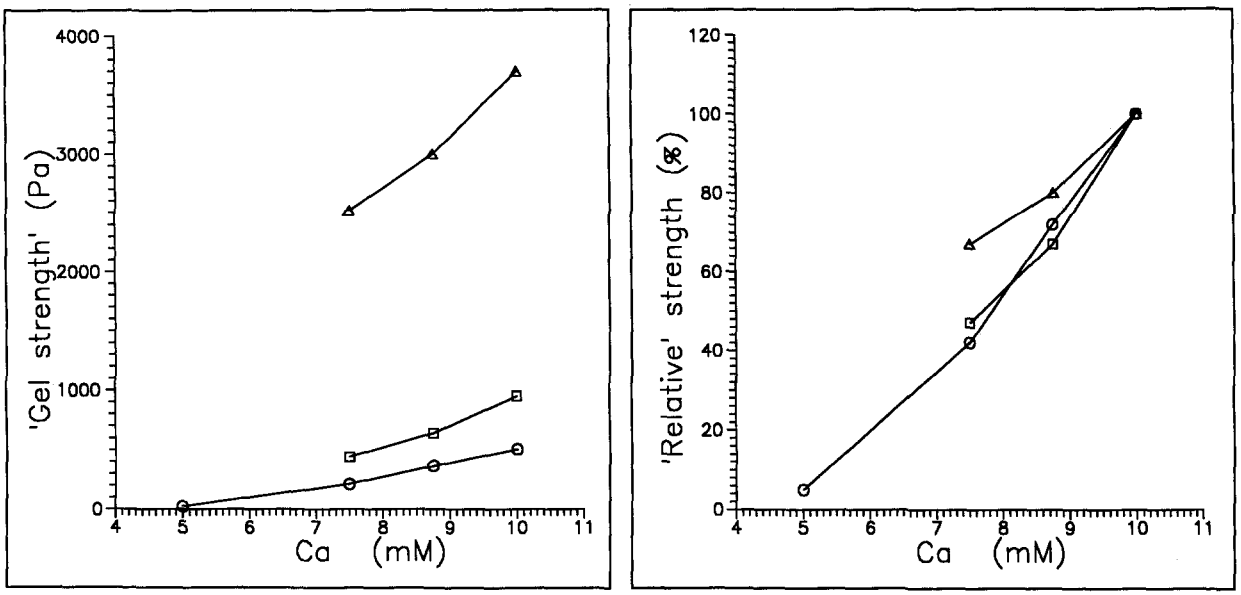

Fig. 2. Strength of gels made from alginate sample #9 (isolated from blades of *L. hyperborea*) crosslinked with different amounts of Ca^{2+}. A = measured gel strength, B = relative gel strength calculated by setting the highest value = 100%. ○ = dynamic storage modulus (G′), □ = FIRA test, △ = Young's modulus (E).

able to give reproducible results. Neither compression in the Stevens Texture Analyzer nor the FIRA test were suited to measure strength in these low regions.

The initial observation from Figs 2 and 3 is that by increasing the degree of crosslinking, one does not obtain a linear relationship but rather a positive curvature. This result may be attributed to the cooperative binding of Ca^{2+} ions into junction zones (Smidsrød & Haug, 1972a). Cooperative binding increases with increasing length of the guluronic acid blocks up to a degree of polymerization of 20 (Kohn & Larsen, 1972). This effect could imply that when Ca^{2+} ions are liberated from the carbonate, they will enter and fill up the longest junctions before they start to create junctions given by shorter guluronic acid blocks. If it is assumed that the number of crosslinks per unit volume determines the gel strength (Stokke *et al.*, 1991) rather than the strength (energy) of each crosslink, a result like this is expected. However, since the crosslinking is a multimolecular reaction in a two-phase system, other explanations for this curvature can not be ruled out.

The obtained gel strengths in the FIRA test are approximately 2 times higher than the dynamic storage moduli (Figs 2A, 3A). There is, however, a surprisingly good correlation between the FIRA test and G′, considering the rapid gel deformation of the FIRA method, when compared as relative gel strengths (Figs 2B, 3B). The FIRA test thus seems to be consistent with the basic rheological G′ in the range between 500 and 2000 Pa.

Small longitudinal deformations obtained in the Stevens LFRA Texture Analyzer give Young's moduli which are 4.5–12 times greater than G′. This is well above the ideal factor of 3 resulting from pure geometrical considerations (G′ being the shear modulus). This result gives reason to believe that there exist some degree of non-linearity in the stress-strain relationship even at small deformations. One reason for this could be that the rate of compression is too high to allow maintenance of equilibrium so that the elastic seg-

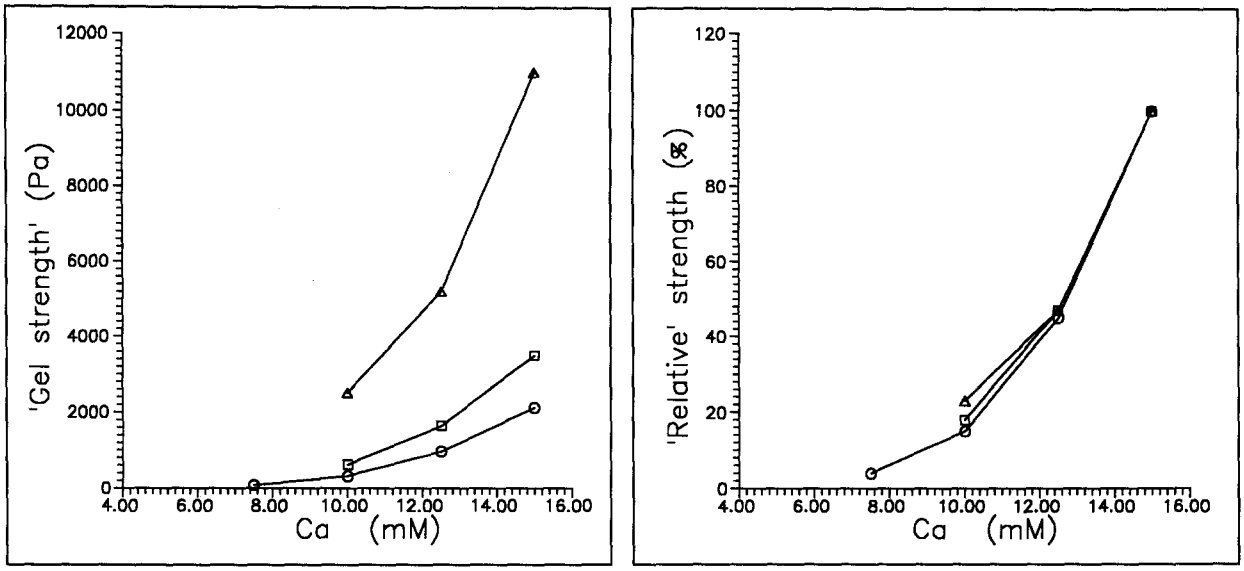

Fig. 3. Strength of gels made from alginate sample #7 (isolated from stipes of *L. hyperborea*) crosslinked with different amounts of Ca^{2+}. A = measured gel strength, B = relative gel strength calculated by setting the highest value = 100%. ○ = dynamic storage modulus (G'), □ = FIRA test, △ = Young's modulus (E).

ments of the alginate molecules (*i.e.* between junctions) do not reach their energy minima during compression. Additionally, non-elastic chains in the form of loose ends or entrapped entangled chains could contribute to the measured modulus for the same reason (incomplete relaxation).

From relative gel strengths (Figs 2B, 3B) it can be seen that Young's modulus deviates increasingly from G' with decreasing degree of crosslinking caused by low calcium content and low content of guluronic acid residues. This is a result which also supports the proposed effect of the non-elastic chains, which will increase in number as the degree of crosslinking is reduced.

Gel and breaking strength as function of molecular weight

Figure 4 shows the development of the dynamic storage modulus G' (Bohlin VOR) and breaking strength (Stevens LFRA Texture Analyzer, bead probe) for gels at apparent equilibrium and with identical degree of crosslinking as a function of the molecular weight of the alginate samples

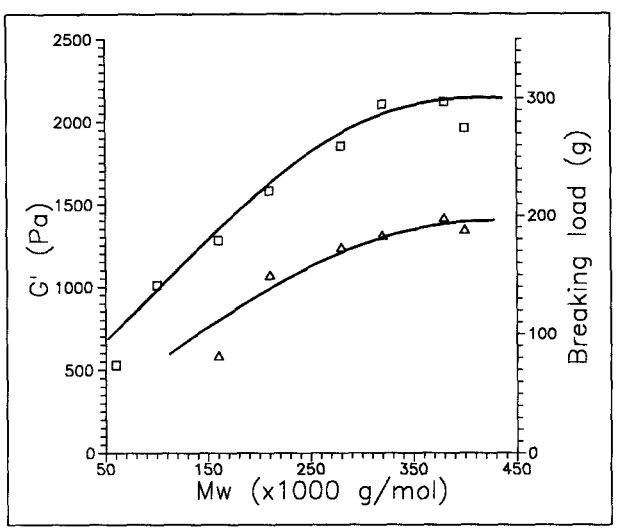

Fig. 4. Measured dynamic storage modulus (G') and breaking strength of gels made from alginate samples #1 to 8 (isolated from stipes of *L. hyperborea*) crosslinked with 15 mM Ca^{2+}. □ = G' (Pa), △ = breaking load (g).

(samples 1 to 8). G′ seems to increase with molecular weight up to between 300 and 350 kD. This is 3 to 4 times the molecular weight giving a modulus independence of molecular weight for alginate gels made by dialysis (Smidsrød & Haug, 1972b). As pointed out earlier, the *in situ* made alginate gels must be regarded as calcium limited compared to gels crosslinked by dialysis and may thus have a larger fraction of non-elastic chains (loose ends and entrapped chains). The average molecular weight will determine the size of these fractions (Stokke *et al.*, 1991). As the molecular weight increases, the fraction of non-elastic chains will decrease. Thus, a molecular weight regime will be reached where the fraction of non-elastic chains will be so low that the gel strength becomes independent of molecular weight, but at higher values of molecular weight than for gels made by dialysis. It should be added that a molecular weight regime in which the gel strength becomes independent of molecular weight could not be found when the Young's modulus was calculated from the initial deformation curve (results not included). This may suggest that the moduli obtained by this method have a contribution from incomplete relaxing non-elastic chains as discussed earlier, making the molecular weight independent regime more difficult to observe.

The breaking strength of these gels also shows an increase with increasing molecular weight. Although the breaking strength becomes less dependent upon molecular weight in the higher molecular weight regime, it is hard to judge from the data if a full molecular weight independence occurs. Both theoretical considerations and experimental results (Mitchell, 1976, 1980) point against a molecular weight independent regime in breaking point measurements.

Conclusions

Gel strengths measured in the FIRA test seem to be consistent with the basic rheological dynamic storage modulus (G′) when converted from amount of water used to force per unit area as long as the moduli are between 500 to 2000 Pa. For the calcium limited *in situ* made alginate gels studied, Young's moduli calculated from initial deformation curves do not seem to be consistent with G′. This is most probably due to a non-equilibrium state during compression giving contribution from both elastic and non-elastic chains with long relaxation times.

The effect of the molecular weight on the gel strength for calcium limited alginate gels is somewhat different from results obtained earlier on calcium saturated, dialysed gels, in that the molecular weight independent regime is moved to 3 to 4 times the molecular weight independence of the saturated gels. This is most probably due to a higher content of non-elastic chains in the form of loose ends and entrapped chains, requiring a higher molecular weight to be reduced to a negligible quantity compared to the elastic chain fraction. The breaking strength increases with molecular weight in the regime studied (160–400 kD) as expected.

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