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Physical characteristics of calcium induced κ- carrageenan networks

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Abstract

The presence of an optimum counter-ion concentration in calcium-induced κ-carrageenan gels at low polymer concentrations of 5 and 10 g/l is observed. At approximately the stoicheometric molar ratio of 1 calcium per carrageenan sulphate, a gel with high elastic modulus, high optical clarity and fine network structure is observed. On further increase of counter-ion concentration beyond this optimum, elastic modulus decreases significantly associated with sharp increase in the gels turbidity together with a network characterised with coarse and large-pore mesh.

The quite complete characterisation of the various gel networks both mechanically by ways of oscillatory and static rheology and optically by turbidimetry and cryo-SEM shows that the extensive structural charge neutralisation of the polysaccharide by divalent calcium ions is responsible for a marked aggregation of the polymer strands reminiscent of precipitation. At lower counter-ion to polymer ratios, onset of gelation might prevent such phase separation.

Keywords: κ-carrageenan; Calcium; Rheology; Turbidity; Young's modulus; Cryo-SEM; Gelation; Aggregation; Phase separation

1. Introduction

κ-Carrageenan is a linear sulphated polysaccharide extracted from marine red algae. It has a basic linear primary structure based on a repeating disaccharide of α(1-3)-D-galactose and β(1-4)-3,6-anhydro-D-galactose and contains one sulphate group per disaccharide unit at carbon 2 of the 1,3 linked galactose unit. κ-Carrageenan is a gelling agent of importance in the food industry (Glicksman, 1979) as well as having applications in the pharmaceutical (Guo, Skinner, Harcum, & Barnum, 1999) and biotechnology sectors (De Ruiter & Rudolph, 1997).

As expected for polyelectrolytes, counter-ions play a major role in the gelation process. Certain cations are found to induce conformational changes in the polymer, with initial coil to helix transition and subsequent aggregation of these helices to form a gel being the effects of greatest interest. Many initial investigations into the gelling mechanism focussed on the configuration of the initial ordered state of the helices. Morris, Rees, and Robinson (1980) found that gel formation occurred in the presence of K^+, Rb^+, Cs^+ and high concentrations of Na^+ and proposed a double helix based 'aggregated-domain' structure as the gelled structure. Rochas and Rinaudo (1984) suggested aggregation of 'helical dimers' to form a three dimensional network in the presence of potassium, sodium and rubidium. Smidsrod and Grasalden (1982) suggested as a gelling mechanism single helix formation and subsequent cation mediated aggregation in the presence of potassium, lithium and cesium. These studies on the initial conformation of the helical state in the presence of suitable monovalent counter-ions have been reviewed by Picullel (1998). The now largely accepted view is that a coil to helix transition leads to the formation of a double helix conformation in the presence of suitable monovalent counter-ions before gelation. To our knowledge the effects of divalent counter-ions on the initial coil to helix transition have yet to be studied in detail. Nevertheless, several studies have focussed on the effect of counter-ion nature on the physical aspects of the gel networks.

Michel, Mestdagh, and Axelos (1997) looked at the phase diagram of κ-carrageenan in the presence of sodium, potassium, copper and calcium. In the case of both divalent cations, they found that turbidity occurred above a low (≈0.02 M) salt concentration for all carrageenan

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concentrations studied, whether or not the polymer concentration was high enough to form a gel. In the case of relatively high polymer concentration (21.6 g/l) these authors found that the elastic modulus was not affected by divalent salt concentration above a certain threshold. They also propose that the addition of cations beyond this critical value contributes only to some localised heterogeneity within the gels without affecting the overall viscoelastic properties of the systems.

Lai, Wong, & Lii (2000) also studied the effects of increased calcium concentrations, albeit on un-purified *Eucheuma cottonii* sourced polysaccharide. A decrease in gel strength was reported for quite concentrated gels (15 g/l) above a calcium concentration ranging from 0.1 to 0.5 M. This decrease was suggested to be due to syneresis. A recent study (Takemasa, Chiba, & Date, 2001) on 20 g/l systems comparing sodium, potassium and calcium-form κ-carrageenans, showed that upon gelation the calcium-form system became the most turbid and was characterised by the highest elastic modulus even if its gelling temperature was relatively low (~10 °C). This system was also characterised by a comparatively low strain-optical coefficient, which indicates low anisotropy of polymer chain and little polymer chain reorientation caused by mechanical deformation of the gel network. These results indicate that calcium induces a high increase in branching number during gelation of κ-carrageenan, probably due to further helical aggregation upon cooling.

In this study our objectives were to investigate the physico-chemical characteristics of κ-carrageenan gels in the semi-dilute regime as affected by calcium concentrations, and to study the relationship between thermal stability, microstructure and rheological properties.

2. Materials and methods

2.1. Samples

κ-Carrageenan was obtained from Quest International (batch no. S9904186) under the trade name Deltagel 379. The pure sodium form was obtained according to a procedure derived from Ramzi et al. (1999). 1.5 g Of Deltagel 379 was dissolved in 300 ml of a hot 500 mM NaCl, 50 mM NaOH and 50 mM EDTA solution to remove calcium and potassium ions and balance to pH7. This solution was twice redissolved in NaCl solution with precipitation in 2-propanol followed by washing in 2-propanol-water mix and final drying with pure 2-propanol. The resulting κ-carrageenan had 0.16 wt% K^+, 8.98 wt% Na^+ and 0.13 Ca^{+2} wt% by atomic absorption spectroscopy.

The specific viscosity of the polymer was assessed a 25 °C in 0.1 M NaCl at 672 ml/g which corresponds to a Mw of 415000 according to Rochas, Rinaudo, and Landry (1990).

Samples were prepared by adding volumes of desired salt solution to samples of carrageenan completely dissolved in water and the solution were mixed under heating.

2.2. Apparatus

Dynamic rheology measurements were carried out on a Rheometric Scientific SR2000 stress controlled rheometer with parallel plate geometry. Plate diameter was 40 mm and the gap was set to 0.2 mm. Measurements at small amplitude oscillation were carried out at 1 rad s^{-1} frequency and 1 or 3 Pa stress, for 5 and 10 g/l gels, respectively. Samples were gelled to 5 °C for 30 min before testing. Dynamic frequency sweeps and dynamic stress sweeps were carried out to ensure that the frequencies and stresses used were within the linear viscoelastic domain. G' values were ascertained from small amplitude oscillation measurements of gels by frequency sweeps in the plateau region at 5 °C. Dynamic temperature ramp tests were used to ascertain the melting and setting temperatures of the gels. The melting and setting points were defined as the crossover points of G' and G'' curves. The temperature ramps were run at 1 °C per minute.

Compressive stress experiments were carried out on an Instron 5544 (Instron Corp., Canton, MA, USA) fitted with a 500 N load cell. The plunger was made of aluminium and had a contact surface diameter of 3.5 cm. Samples for test were moulded in inverted 3.5 cm polystyrene petri dishes and were kept refrigerated at 5 °C for 18 h before testing. The samples were allowed to equilibrate to room temperature (23 °C) for 1 h before being compressed at a crosshead speed of 2 mm/min. Young's Modulus calculations were carried out on the force and compression results at low deformation ($<5\%$).

Turbidity measurements were made on a temperature controlled Pharmacia LKB (NJ, USA) Ultrospec 3 UV-Visible spectrometer at 450 nm maintained at 8 °C by a circulating water bath.

Cryogenic Scanning Electron Microscopy was carried out on a Jeol 5410 (Akishima, Japan) scanning electron microscope with and Oxford Instruments (Oxford, UK) cryo-preparation chamber. The samples were loaded as gels and then frozen in liquid nitrogen slush, fractured under vacuum and sublimed at −80 °C for up to 45 min before sputter coating with gold and examined under an acceleration voltage of 10 kV.

3. Results and discussion

3.1. Thermal stability

Thermal stability was assessed at the point of crossover of G' and G'' in dynamic temperature ramps with stress control. The results of inverse of melting temperature, T_M, and gelling temperature, T_G, are plotted against the log C_T in

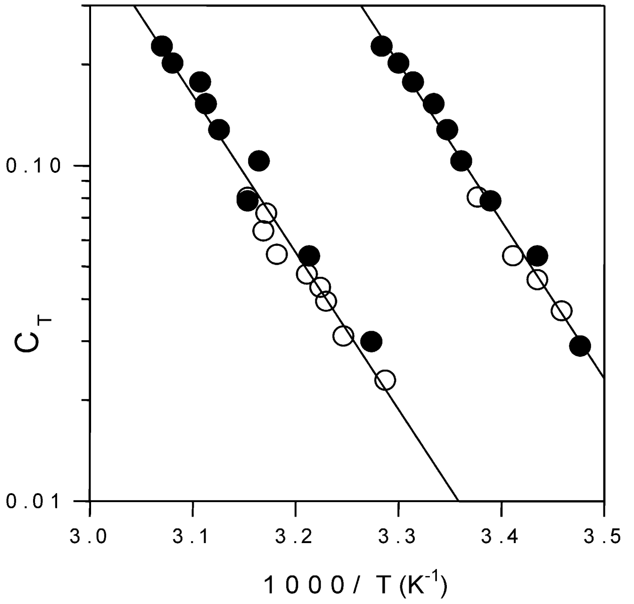

Fig. 1. Variation of T_M (solid line) and T_G (dashed line) with total ionic concentration C_T (molar) for 5 g/l (○) and 10 g/l (●) κ-carrageenan concentrations.

Fig. 1. C_T, the total ion concentration has been calculated according to Rochas and Rinaudo (1980), taking into account the ionic strength generated by the added calcium chloride solution instead of the concentration of added counter-ion.

This phase diagram shows a linear variation of the inverse of temperatures against the log of C_T indicating a constant enthalpy associated with both the melting and gelling processes over the entire range of salt and polymer concentrations studied. Both slope values are approximately −4500 K, in reasonable agreement with data from Rochas and Rinaudo (1980). However, the slopes of the variations appear to be equal and there is no indication of a tricritical point in the range studied, as opposed to the phase diagram established by Rochas and Rinaudo (1980) in the case of potassium κ-carrageenan systems.

3.2. Mechanical properties

3.2.1. Dynamic rheology

Dynamic frequency sweeps of the various samples at 5 °C show a peaking of the elastic modulus, G', as a function of the molar calcium to carrageenan ratio, as shown in Fig. 2.

This peaking appears at the stoicheometric ratio of one calcium per carrageenan sulphate for both the 5 and 10 g/l samples. Starting from low calcium-carrageenan ratios the pronounced increase in G' is followed by a marked decrease in elastic modulus values with excess calcium. This had been observed before by Lai et al. (2000) and had been explained as an artefact arising from inappropriate contact between the sample and the measuring plates due to syneresis. Syneresis effects are frequently cited as being a limiting factor in the measurement of the mechanical properties of carrageenans. Many solutions to the problem have been sought such as affixing the gels for measurement to the parallel plates themselves as in Chen, Liao, Boger, and Dunstan (2001). In our system these measures were not necessary as there was no loss of contact with the plates over time as illustrated in Fig. 3. This type of system was also studied by Hermansson, Eriksson, & Jordansson (1991)) who obtained similar gelling curves but at higher temperatures. However, additional clarification on the nature of this apparent weakening of the network structure was sought in compressive modulus measurements of the gels.

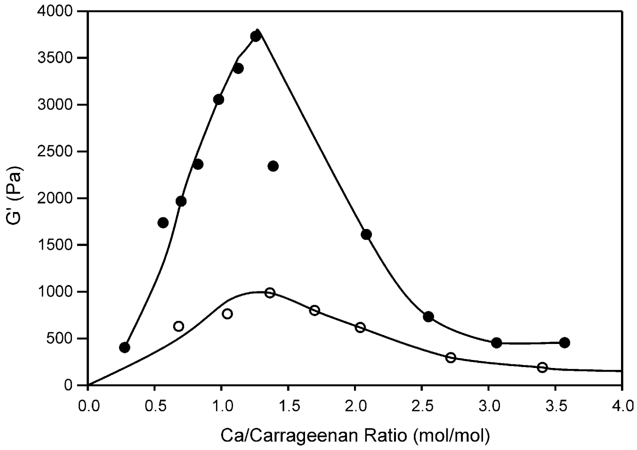

Fig. 2. G' variation with changing calcium to carrageenan ratio for 5 g/l (○) and 10 g/l (●) κ-carrageenan concentrations.

3.2.2. Uniaxial rheology

Compressive stress studies on gels at the same polymer concentrations and similar amounts of added calcium show that the gels were indeed weakened with the addition of calcium as seen in Fig. 4.

Young's modulus, E, was calculated at 5% deformation. This type of experiment is not affected by slippage due to syneresis, and therefore confirms the results obtained from dynamic rheology where the same peaking is observed for a molar ratio of 1 calcium per carrageenan sulphate. Young's

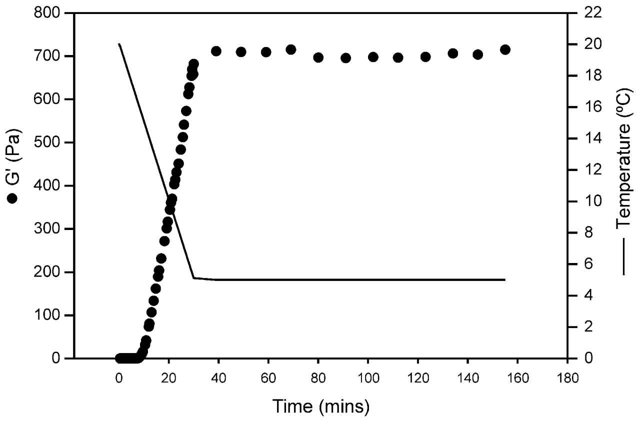

Fig. 3. Setting curve for calcium induced κ-carrageenan gel. ●: G', —: temperature. Carrageenan concentration; 10 g/l, [Ca^{2+}] = 14 mM.

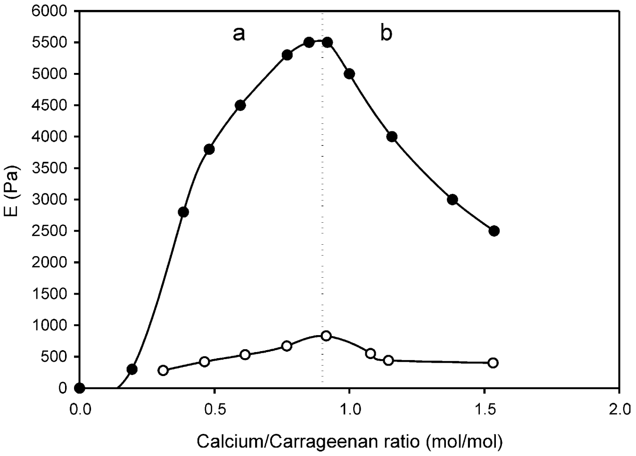

Fig. 4. Variation of Young's Modulus, *E*, with changing calcium to carrageenan ratio for 5 g/l (○) and 10 g/l (●) κ-carrageenan concentrations. For explanation of regions 'a' and 'b', see text.

modulus values are approximately equivalent to those of G' where a threefold increase should be expected in the case of incompressible gels. This inconsistency in results cannot result from slip under shear which result in lower G' values, but is attributed to lack of sensitivity from the compression equipment due to the very fragile nature of the gels studied, a known problem (Chen et al., 2001), and also the difference in temperature between the two experiments (5 °C for shear, 23 °C for compression) which would yield stronger gels for oscillatory measurements.

Also extracted from the Young's modulus experiments was the fracture properties of the gel. In a markedly similar system to that found in gellan experiments by Milas and Rinaudo (1996), the presence of divalent counter-ions causes a change in the nature of the gels from weak and pliable to strong but brittle gels. In Fig. 5, the first region, labelled 'a', corresponds to low counter-ion concentration and clear gels. In region 'b' the high salt gels are becoming weaker, and more and more brittle.

3.3. Optical properties

Calcium induced κ-carrageenan gels have been described as turbid in the literature. (Michel et al., 1997) In the case of our more dilute gel systems we proceeded to the quantitative evaluation of the turbidity and its relation to the network microstructure.

3.3.1. Turbidity

Fig. 6 shows the percentage transmittance of the samples as a function of the calcium to carrageenan molar ratio.

Previously described phase diagrams (Michel et al., 1997) show an abrupt transition from clear to turbid systems at 0.02 M added calcium. As can be seen from Fig. 6 our systems show a gradual decrease in percentage transmittance with added calcium and no clear transition from clear to opaque systems can be detected. This discrepancy between the two studies may be due to the fact that our samples were examined at low temperature in the gelled state. At high temperature in the sol state, all samples have a transmittance close to 100%. Therefore percentage transmittance of the cooled samples in the gel state is a good measure of polymer aggregation upon

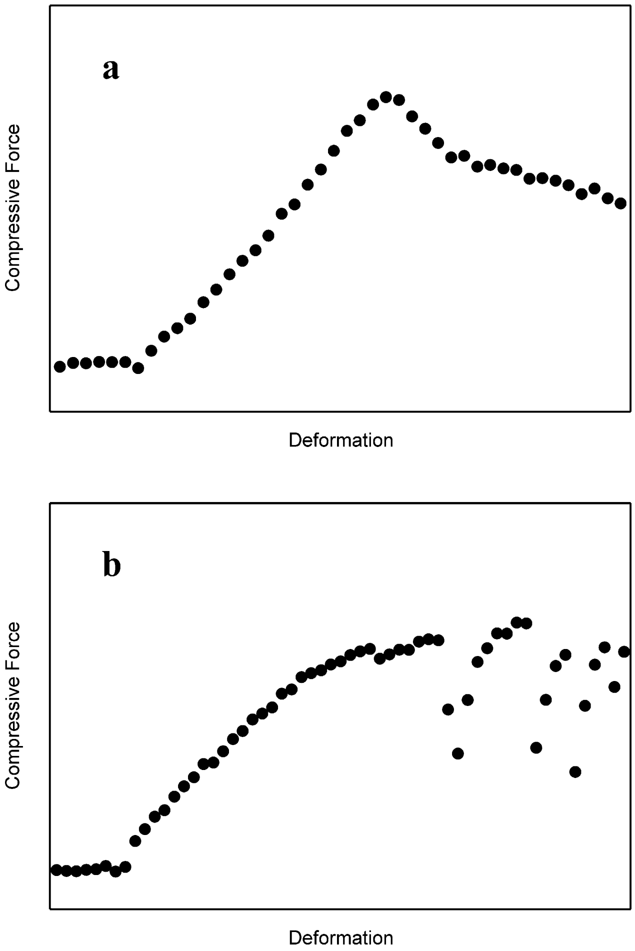

Fig. 5. Breaking profile by compressive rheology of gels in; 'a', optimum calcium region and 'b', excess added calcium region.

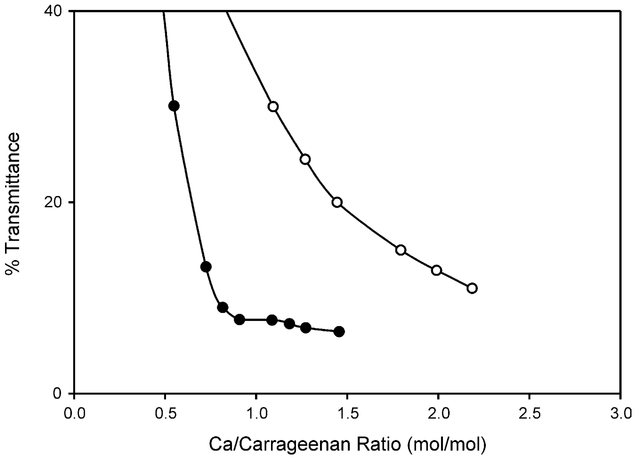

Fig. 6. Turbidity profiles of 5 g/l (○) and 10 g/l (●) gels with changing calcium to carrageenan ratios.

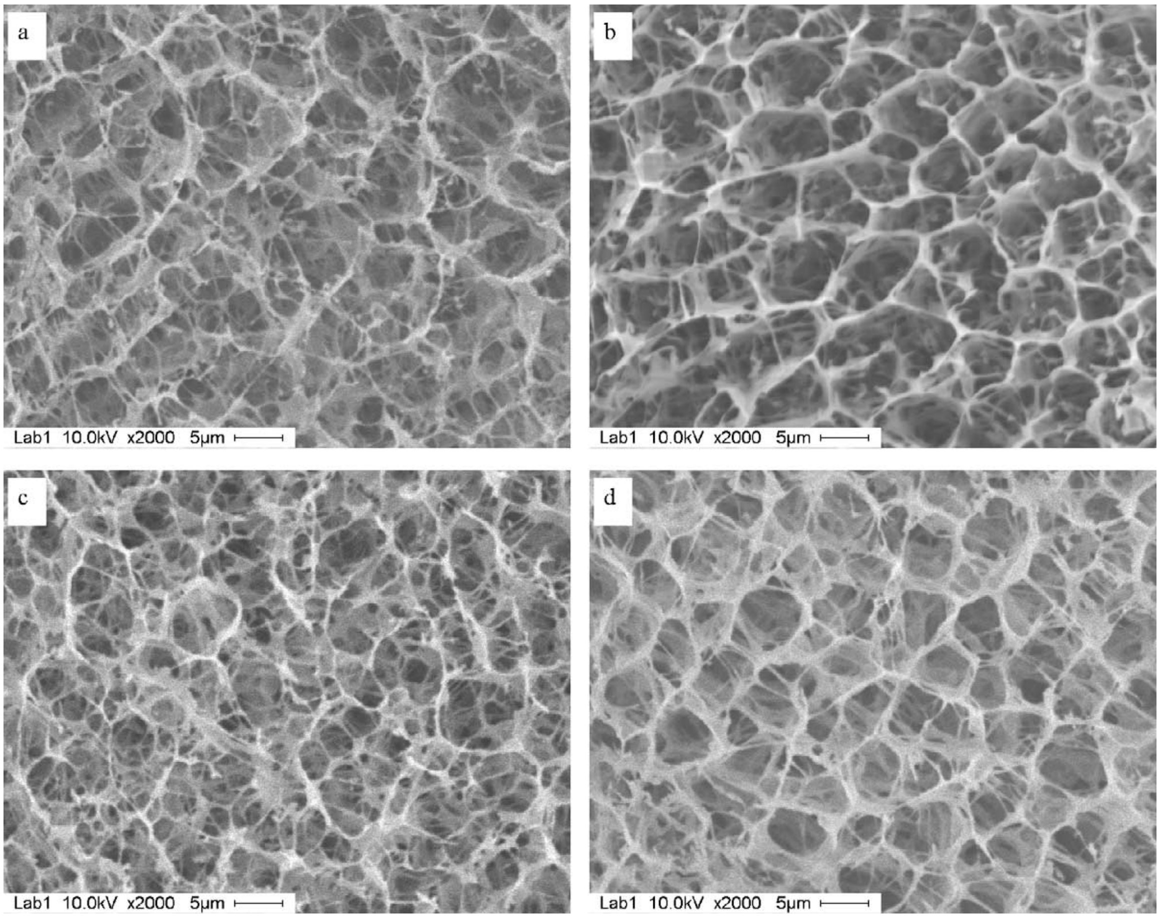

Fig. 7. Scanning Electron Microscopy images of carrageenan networks at: (a) 5 g/l, 1:1 ratio added Ca^{2+}; (b) 5 g/l, 4:1 ratio added Ca^{2+}; (c) 10 g/l, 1:1 ratio added Ca^{2+}; (d) 10 g/l, 2:1 ratio added Ca^{2+}.

cooling. The increasing opacity of the samples with added calcium is a good reflection of additional polymer aggregation due to excess divalent cation.

3.3.2. Scanning electron microscopy

Further information about the microstructure of the gels was gained from scanning electron microscopy. Cryogenic preparation of samples with sublimation of water from the network at optimum and high calcium concentrations gave images as shown in Fig. 7.

It can be seen that the 5 g/l sample (Fig. 7a) at 1:1 calcium:carrageenan ratio has a fine network within the larger pores. The resulting pore sizes are small. This scheme is mirrored at higher polymer concentrations of 10 g/l (Fig. 7c). At higher calcium/carrageenan ratios, a more open network with thicker strands and wider pores corresponds to gels that are weak and turbid. The network at 4:1 calcium:carrageenan ratio (Fig. 7b) at 5 g/l has visible and quite defined strands compared with the fine strands seen in the low calcium ratio sample. The calcium ratio of 2:1 at 10 g/l polymer concentration shown in Fig. 7d again exhibits larger pores when compared to the optimum network in Fig. 7c. The results presented here are similar in trend to those found in the gellan system by Mao, Tang and Swanson (2001). The formation of coarser strands and consequent more open nature of the network at higher counter-ion concentrations support the view that added cations add to the localised aggregation of the strands. We can therefore draw a very strong parallel between our κ-carrageenan system and gellan. At low added divalent cation concentration gellan samples show high gel strength, fine network structure and high water holding capacity, whereas excess divalent cation is responsible for polymer aggregation resulting in low gel strength and coarse networks with syneresis (Mao et al., 2001; Milas & Rinaudo, 1996).

4. Conclusion

In this study of the structure of calcium induced κ-carrageenan gels a clear optimum calcium to carrageenan ratio is established in terms of elastic modulus, G', and Young's modulus, E. Increasing this optimum concentration decreases both G' and Young's modulus, an increase in gel turbidity and in a coarsening of the network structure. For this system it appears that the extensive structural charge neutralisation of the polysaccharide by excess divalent calcium ions is responsible for a marked aggregation of the polymer strands reminiscent of precipitation. At lower counter-ion to polymer ratios, onset of gelation might prevent such phase separation.

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References

Chen, Y., Liao, M.-L., Boger, D. V., & Dunstan, D. E. (2001). Rheological characterisation of kappa-carrageenan/locust bean gum mixtures. *Carbohydrate Polymers*, *46*, 117–124.

De Ruiter, G. A., & Rudolph, B. (1997). Carrageenan biotechnology. *Trends in Food Science and Technology*, *8*(12), 389–395.

Glicksman, M. (1979). Gelling hydrocolloids in food product applications. In J. M. V. Blanshard, & J. R. Mitchell (Eds.), *Polysaccharides in food* (pp. 185–204). London: Butterworths.

Guo, J.-H., Skinner, G. W., Harcum, W. W., & Barnum, P. E. (1999). Investigating the fundamental effects of binders on pharmaceutical tablet performance. *Drug Development and Industrial Pharmacy*, *25*(10), 1129–1135.

Hermansson, A.-M., Eriksson, E., & Jordansson, E. (1991). Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. *Carbohydrate Polymers*, *16*, 297–320.

Lai, V. M. F., Wong, P. A.-L., & Lii, C.-Y. (2000). Effects of cation properties on sol-gel transition and gel properties of kappa carrageenan. *Journal of Food Science*, *65*(8), 1332–1337.

Mao, R., Tang, J., & Swanson, B. G. (2001). Water holding capacity and microstructure of gellan gels. *Carbohydrate Polymers*, *46*, 365–371.

Michel, A. S., Mestdagh, M. M., & Axelos, M. A. V. (1997). Physico-chemical properties of carrageenan gels in presence of various cations. *International Journal of Biological Macromolecules*, *21*, 195–200.

Milas, M., & Rinaudo, M. (1996). The gellan sol–gel transition. *Carbohydrate Polymers*, *30*, 177–184.

Morris, E. R., Rees, D. A., & Robinson, G. (1980). Cation-specific aggregation of carrageenan helices: Domain model of polymer gel structure. *Journal of Molecular Biology*, *138*, 349–362.

Picullel, L. (1998). Gelling Polysaccharides. *Current Opinion in Colloid and Interface Science*, *3*(6), 643–650.

Ramzi, M., Borgstrom, J., & Piculell, L. (1999). Effects of added polysaccharides on the isotropic/nematic phase equilibrium of kappa-carrageenan. *Macromolecules*, *32*, 2250–2255.

Rochas, C., & Rinaudo, M. (1980). Activity coefficients of counter-ions and conformation in kappa carrageenan systems. *Biopolymers*, *19*, 1675–1687.

Rochas, C., & Rinaudo, M. (1984). Mechanism of gel formation in kappa carrageenan. *Biopolymers*, *23*, 735–745.

Rochas, C., Rinaudo, M., & Landry, S. (1990). Role of molecular weight on the mechanical properties of kappa carrageenan gels. *Carbohydrate Polymers*, *12*, 255–266.

Smidsrod, O., & Grasalden, H. (1982). Some physical properties of carrageenan in solution and gel state. *Carbohydrate Polymers*, *2*, 270–272.

Takemasa, M., Chiba, A., & Date, M. (2001). Gelation mechanism of kappa and iota carrageenan investigated by correlation between the strain-optical coefficient and the dynamic shear modulus. *Macromolecules*, *34*, 7427–7434.