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The rheology of K^+-κ-carrageenan as a weak gel

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Abstract

The pure potassium form of κ-carrageenan shows rheological behaviour which is very different to that observed for the commercial (unpurified) sample. The measured rheology at 1% concentration of the purified κ-carrageenan is consistent with weak gel behaviour. The resulting weak gel has a significantly smaller linear viscoelastic region than the unpurified commercial form. Upon large deformation, the gel flows. We report results for the viscosity versus shear rate where the sheared gel demonstrates significant structural recovery with time after shearing. The effects of temperature, polymer concentration and KCl addition on the rheology of the gel have been investigated. The role of both calcium and potassium ions is discussed. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: K^+-κ-carrageenan; rheology; sheared gel

1. Introduction

κ-Carrageenan is a sulfated galactan extracted from red algae. It is comprised of repeating disaccharide units of 3-linked β-D-galactose 4-sulfate and 4-linked 3,6-anhydro-α-D-galactose. The solution of κ-carrageenan may form strong thermally reversible gels upon cooling in the presence of specific cations such as potassium. The mechanism of gelation is reported to involve a coil–helix transition of the κ-carrageenan molecules, followed by aggregation and network formation (Morris, Rees, & Robinson, 1980).

The rheological properties of κ-carrageenan have been extensively studied using compression techniques (Cairns, Morris, Miles, & Brownsey, 1986; Rochas, Rinaudo, & Landry, 1990). These investigations assessed the effects of κ-carrageenan concentration, added salt and temperature on the mechanical properties of the gels formed.

κ-Carrageenan is known to exhibit syneresis, which results in significant slip during dynamic shear measurements (Richardson & Goycoolea, 1994). In a previous study with the binary system of κ-carrageenan and locust bean gum (Chen, Liao, Boger, & Dunstan, 2001), preformed gels glued between parallel plates were used to overcome slip.

A few dynamic studies on κ-carrageenan in a state of limited or minimal syneresis have been reported. Meunier, Nicolai, Durand, and Parker (1999) provided data on κ-carrageenan of low concentrations (0.01–0.2%) containing 0.01 M KCl plus 0.1 M NaCl. They found that at least 12 h was needed for the κ-carrageenan to approach equilibrium as measured in the rheological behaviour. Chronakis, Piculell, and Borgström (1996) studied the rheology of κ-carrageenan in mixtures of sodium and cesium iodide. Michel, Mestdagh, and Axelos (1997) found that, in the low salt concentration range (to 0.1 M), the effect of added ions on the G' of resulting κ-carrageenan gel was in the order of $K^+ > Ca^{2+} = Cu^{2+} \gg Na^+$.

When compared to other food biopolymers such as xanthan and gelatine, the number of dynamic oscillatory studies for κ-carrageenan are still limited in number. Hermansson, Eriksson, and Jordansson (1991) provided a comprehensive study with respect to the effects of potassium, sodium and calcium ions on κ-carrageenan in different ionic forms. Substantial amounts (0.02–0.5 M) of salts were added to the κ-carrageenan so that the gel formed remained in a strong gel regime.

The physical properties of κ-carrageenan gels are notoriously affected by the presence of even trace amounts of ions (Nilsson, Picullel, & Jönsson, 1989). While K^+ is very effective in promoting gelation, the rheology of pure K^+-κ-carrageenan is yet to be systematically studied. Stanley (1990) commented that κ-carrageenan in the pure potassium form is 'compliant' indicating that the pure K^+-κ-carrageenan is not brittle. Hermansson et al. (1991) tested a 1% K^+-κ-carrageenan with 25 mM added NaCl.

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The reported storage modulus, G' was only of the order of 300 Pa. By testing a pure, salt-free K^+-κ-carrageenan using a specially designed apparatus with capillary tubes, Morris and Chilvers (1983) showed that the shear modulus, G_R, was very low (<100 Pa for the K^+-κ-carrageenan at 1%). In contrast, a rigid gel with G' of approximately 10,000 Pa was observed for a commercial κ-carrageenan at 1% concentration (Chen et al., 2001). The above information indicates that pure K^+-κ-carrageenan gel is a lot weaker than that of conventional commercial products which contain mixed cations, usually with some free salts present.

The aim of this study is to characterise the rheology of purified κ-carrageenan in the potassium form without free salts, which can then provide a basis for further studies involving other variables such as KCl addition and increase in polymer concentration.

2. Materials and methods

The sample of κ-carrageenan (type III, lot 39h1211) from '*Eucheuma cottonii*' was purchased from Sigma-Aldrich (St Louis, MO, USA), which contained 6.7% K^+, 2.3% Ca^{2+} and 0.7% Na^+. This sample contained a small amount of free salts. Notably, the seaweed species '*Eucheuma cottonii*' is presumably *Kappaphycus alvarezii*, as *Eucheuma cottonii* has never been farmed and was never a large component used for commercial extraction. Essentially, the only species farmed and currently used in industry for the production of κ-carrageenan is *Kappaphycus alvarezii* (Santos, 1989).

The sample with a concentration of 1% was passed through an ion-exchange column at 80 °C with cation exchange resin pre-regenerated to the K^+ form. The converted sample was then freeze-dried. The converted sample still contained some free salts, which were removed via 75% ethanol washing. An ICP test showed the purified sample contains 8.7% K^+ and negligible amounts of Na^+ (0.01%) and Ca^{2+} (0.07%), indicating the sample is dominantly in the potassium form with very little free salt. The potassium ion content is slightly lower than the theoretical level (9.2%) for pure potassium form κ-carrageenan comprised of idealised repeating disaccharide units.

A Carri-Med CSL 100 rheometer was used for dynamic shear measurements. A cone and plate geometry was used. The cone used was 4 cm in diameter with a 2° angle. All measurements were performed at a strain of 2%, which is well within the measured linear viscoelastic region. Evaporation was prevented by using a solvent trap in conjunction with silicon oil sealing.

Initial attempts to test the purified κ-carrageenan samples using the method of gluing preformed gels developed in an earlier study (Chen et al., 2001) were unsuccessful due to the weak gels formed at 1% polymer concentration in the purified form. In contrast, a rigid gel readily forms with the unpurified commercial sample at the same concentration. The possibility that sample degradation had occurred during purification was assessed by addition of Ca^{2+} and K^+ to the level of the commercial samples. In all the cases, the addition of ions resulted in the same rheological behaviour, within experimental error, as that observed prior to purification. Syneresis was not observed for the purified sample at concentrations up to 1.4%; so reproducible results were obtained with a normal cone and plate geometry.

3. Results and discussion

Figs. 1–3 show results for a series of measurements on purified K^+-κ-carrageenan samples at 1% concentration

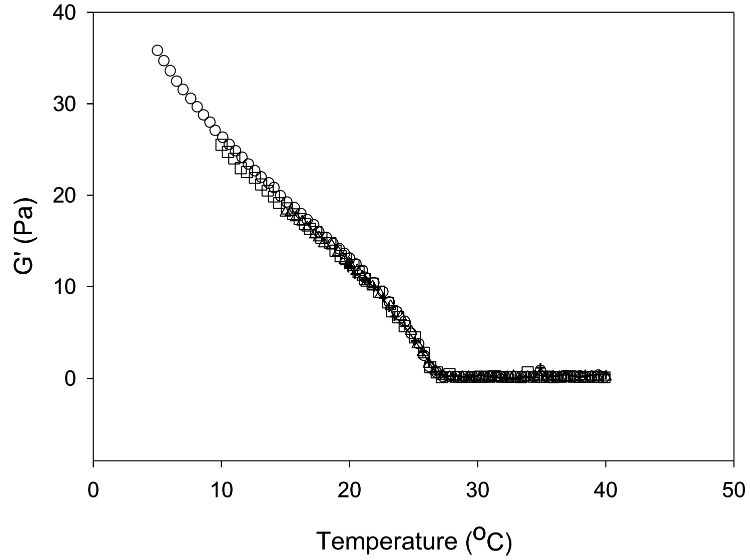

Fig. 1. G' of 1% purified K^+-κ-carrageenan for different temperature sweeps. The sample was cooled from 40 down to 20, 15, 10, and 5 °C, respectively. +, 20 °C; △, 15 °C; □, 10 °C; ○, 5 °C.

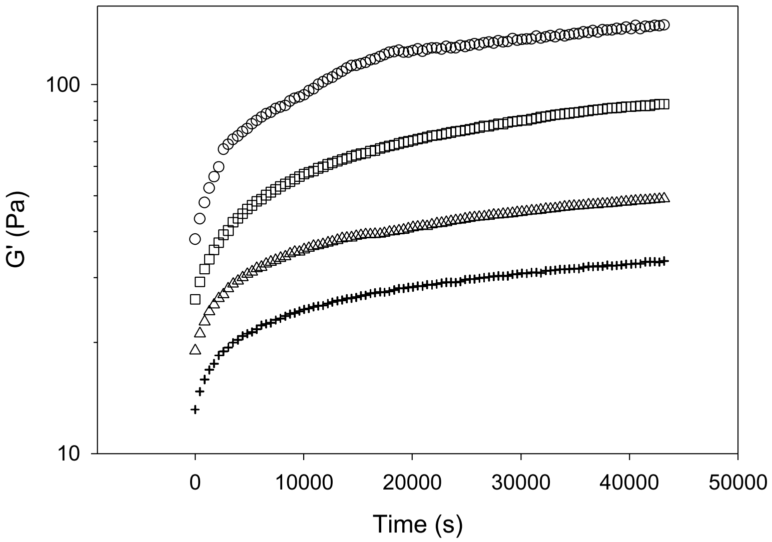

Fig. 2. G' of 1% purified K^+-κ-carrageenan gel cured at 20, 15, 10, and 5 °C. +, 20 °C; △, 15 °C; □, 10 °C; ○, 5 °C.

with no KCl added. The sample was first cooled from 40 to 20 °C, with a cooling rate of 0.5 °C/min. It was then cured for 12 h in the rheometer after which G' and G'' measurement over the frequency range 0.01–10 Hz was undertaken. Time and frequency spectra were repeated on the same sample by following the sequential procedure of heating, cooling and curing. In order to melt the sample completely, heating to 50 °C (rather than 40 °C) followed by cooling to 15, 10 and 5 °C, respectively, was performed.

Fig. 1 shows results obtained for the G' versus temperature for the cooling from 40 °C to four different temperatures, performed on the same sample. It should be noted that the last cooling run (down to 5 °C) was undertaken approximately 48 h after the sample was first loaded onto the rheometer plate. The superimposed curves indicate that the sample remained unchanged over the experimental period and possible artefacts due to evaporation were eliminated. The geometry of the experimental design used has produced reproducible results for the purified κ-carrageenan indicating that syneresis is not present in the purified samples. For unpurified commercial κ-carrageenan, the cooling curve using the same geometry would produce a G' peak, which is attributed to the occurrence of synresed water from the gel leading to slip when the true gel is formed. Such a peak can only be avoided when efficient anti-slip measures are taken (Richardson & Goycoolea, 1994). Given that slip was not present, all experiments on the purified κ-carrageenan were undertaken using the cone and plate geometry.

Fig. 2 shows results obtained during the curing of the sample at four different temperatures. The shape of these curves is typical of gel curing. The gel strength, as

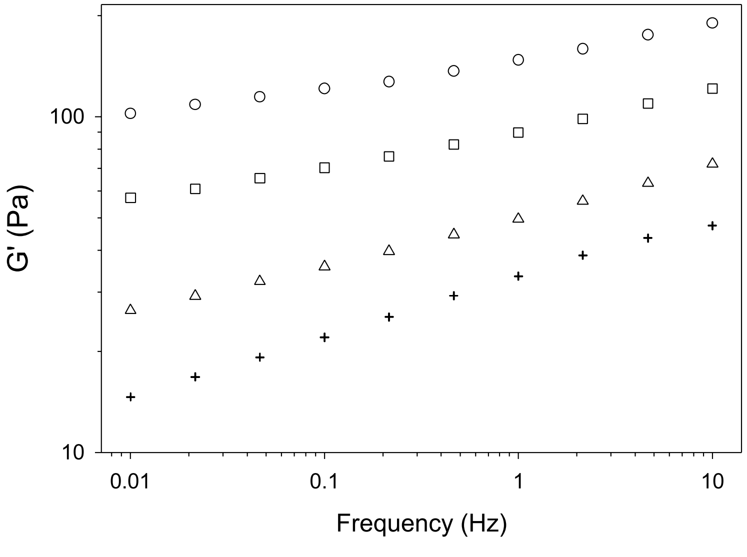

Fig. 3. Mechanical spectra comparison of G' for 1% purified K^+-κ-carrageenan at different temperatures. +, 20 °C; △, 15 °C; □, 10 °C; ○, 5 °C.

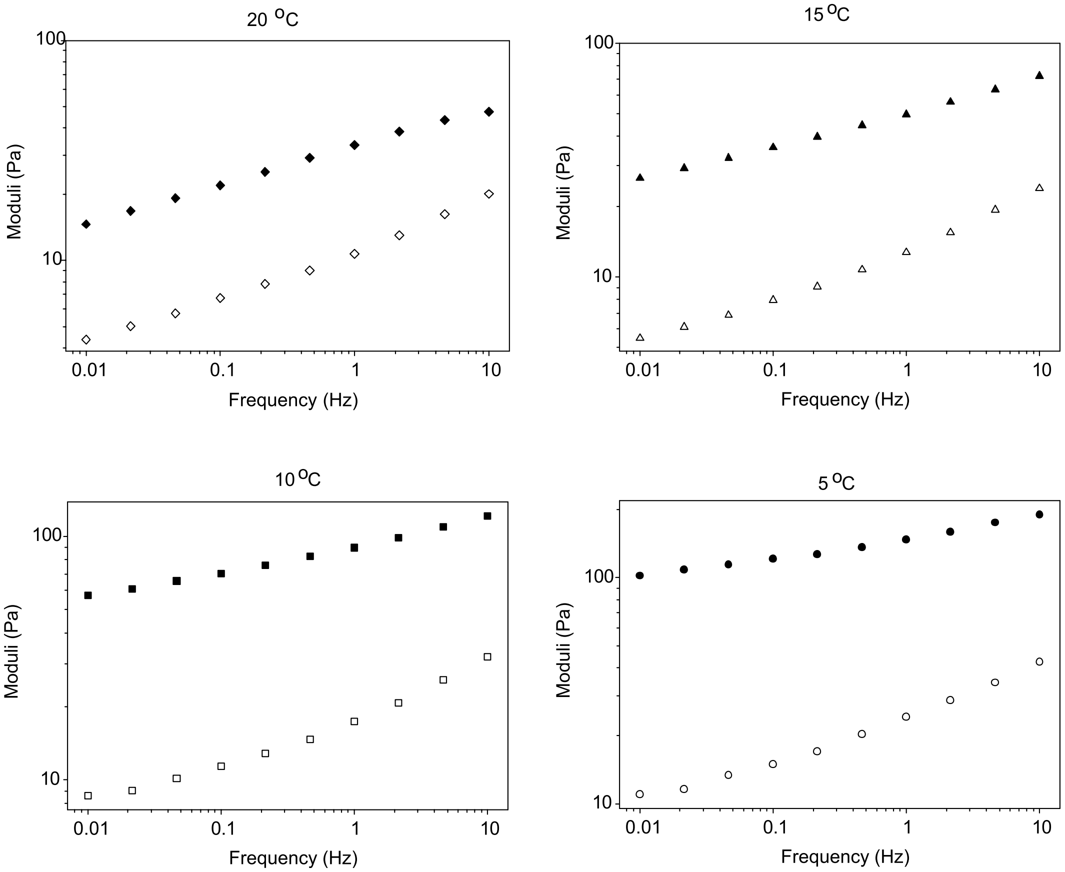

Fig. 4. Mechanical spectra of 1% purified K^+-κ-carrageenan at different temperatures. Closed symbols, G'; Open symbols, G''.

monitored by G', is surprisingly low, however. At 20 °C, the G' value after 12 h of curing is 30 Pa. In contrast, the original unpurified commercial sample yielded a gel strength of around 10,000 Pa (Chen et al., 2001). However, the cured sample of purified κ-carrageenan is able to support its own weight in a container when placed upside down in a 15 mm diameter tube. Such behaviour suggests a weak gel structure is formed in the purified sample. To examine more closely the properties of the purified sample, the mechanical spectra are displayed in Fig. 3. The frequency dependence of G' reduces as the temperature is reduced. This is expected, as the gel becomes more solid-like at lower temperatures. At 5 °C, G' shows significantly lower frequency dependence, an attribute of a stronger gel.

The mechanical spectra, G' and G'' versus frequency for the different temperatures are compared in Fig. 4. It is readily seen that G' has remained higher than G'' for all frequencies at all temperatures. This indicates that the sample is indeed in a gel state. However, the difference between G' and G'' at higher frequencies is greatly reduced at higher temperatures.

One might expect a G'/G'' crossover at higher

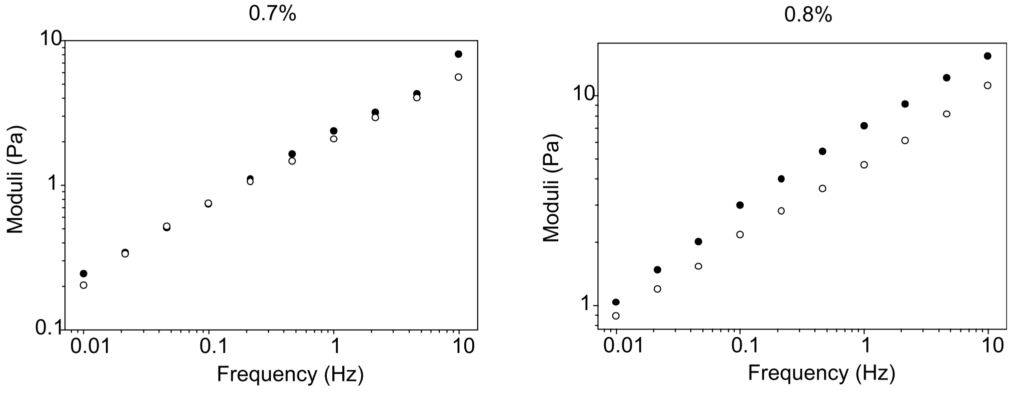

Fig. 5. Mechanical spectra of 0.7 and 0.8% purified K^+-κ-carrageenan samples at 20 °C. Closed symbols, G'; Open symbols, G''.

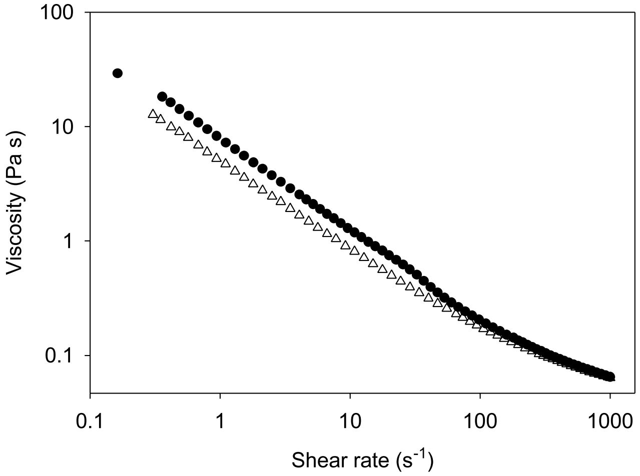

Fig. 6. Flow behaviour of 1% purified K^+-κ-carrageenan fluid–gel at 20 °C. ●, viscosity, ascending shear rate; △, viscosity, descending shear rate.

temperatures or lower concentrations, however, this was not observed for the range of concentrations measured. Fig. 5 shows the mechanical spectra of samples at concentrations of 0.7 and 0.8%. Both G' and G'' are below 10 Pa at frequency of 1 Hz after 12 h of curing. Moreover, G' remains no higher than G'' over the frequency range measured. At lower concentrations, such as 0.6%, no gelation was observed and G' remained lower than G'' after 12 h of curing. All these evidences suggest that the testing sample at concentrations 0.7–1% is a weak gel at 20 °C.

A subsequent flow test on the sample (1% concentration and 20 °C) which had been cured for 12 h shows shear thinning behaviour (Fig. 6). The shear rate was first increased from 0.3 to 1000 s^{-1} and reduced immediately back to 0.3 s^{-1}. The difference between the two curves indicates an initial structure that could not be recovered in a short period of time at low shear rates. The sample was then left in the rheometer for 12 h to monitor G' recovery. Fig. 7 shows G' plateaus off at around 10 h of recovery. A subsequent mechanical spectra was measured and is compared with that measured prior to the flow test (Fig. 8). The two show very similar behaviour although the G' and G'' values are slightly smaller for the sample after shear. This result suggests that the structure disturbed by the shear can be recovered to a large extent with appropriate equilibration time.

Although the difference in rheology measured before and after flow is barely distinguishable, the resulting liquid-like material after flow was unable to form a self-supporting gel in a container even after two weeks of curing. A self-supporting gel in a container formed, however, after 2 months of curing.

The difference between a strong gel and a weak gel may also be determined by the large strain behaviour. Strong gels tend to break while weak gels flow (Ross-Murphy, 1995). The linear viscoelastic limit of our purified 1% K^+-κ-carrageenan gel was determined to be around 45% strain. This is significantly smaller than that of most strong gels, which is normally beyond 100%. However, the linear region observed for our gel is significantly larger than that of the 'weak gel' defined in Ross-Murphy's context (<5%). Although our K^+-κ-carrageenan gel's linear region falls within the 'strong gel' regime in the Ross-Murphy terminology, it does show flow and recovery behaviour typical of a 'weak gel'. The measured recovery as observed in the rise of G' with time, however, is significantly different from that of the original weak gel. With the original gel

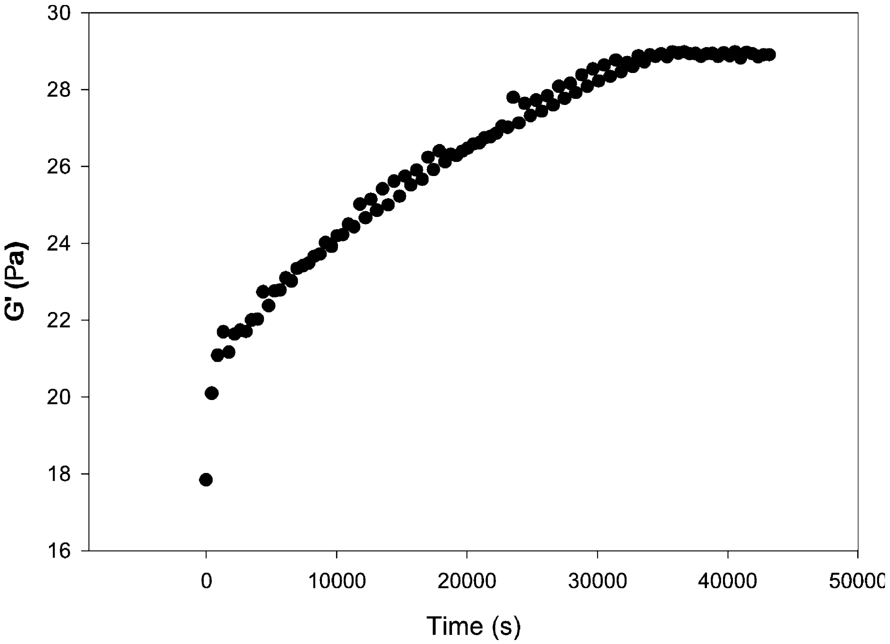

Fig. 7. Recovery of the sheared sample for 1% purified K^+-κ-carrageenan at 20 °C.

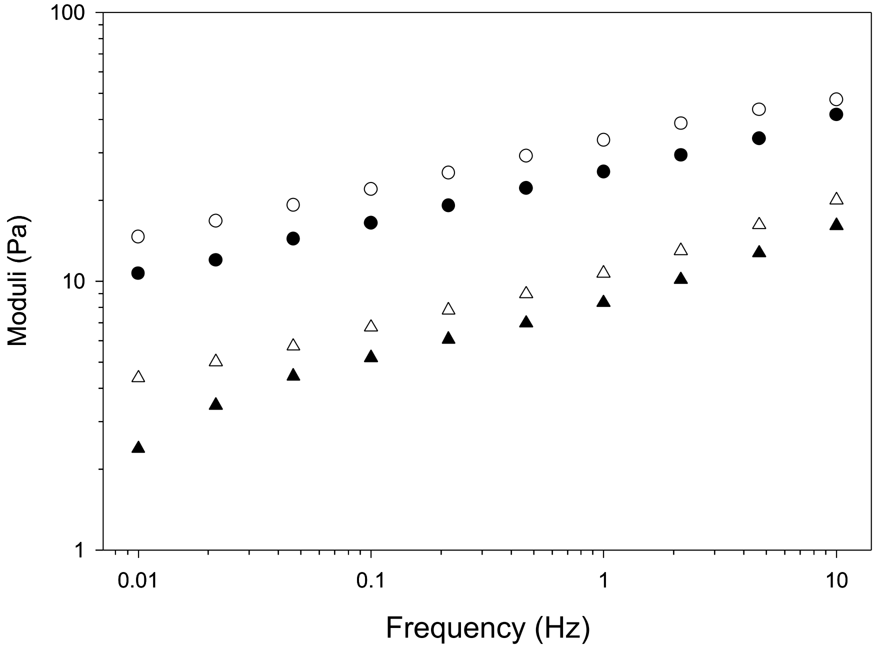

Fig. 8. Frequency sweep comparison before and after shear for a 1% purified K^+-κ-carrageenan sample at 20 °C. Circles, G'; Triangles, G''; Open symbols, before shear; Closed symbols, after shear.

curing curve up to 12 h (Fig. 2), there is no sign of completion in gel structure development. In contrast, the sheared gel reaches a plateau after approximately 10 h recovery time (Fig. 7). It is possible that part of the original structure was permanently broken by the shear, and only partial recovery was, therefore, able to be achieved. Thus the purified K^+-κ-carrageenan gel is in a true physical gel state, although the gel strength is much weaker than a conventional strong gel. To avoid confusion, the term 'weak gel' is used in this study with reference only to a gel with low gel strength, and not the 'weak gel' as defined by Ross-Murphy.

The polymer concentration and KCl dependence of the purified κ-carrageenan sample was also examined. All tests were subject to the same procedure at only one temperature of 20 °C. The results are shown in Figs. 9 and 10.

The effect of polymer concentration is as shown in Fig. 9, where the gel strength increases exponentially with increasing concentration.

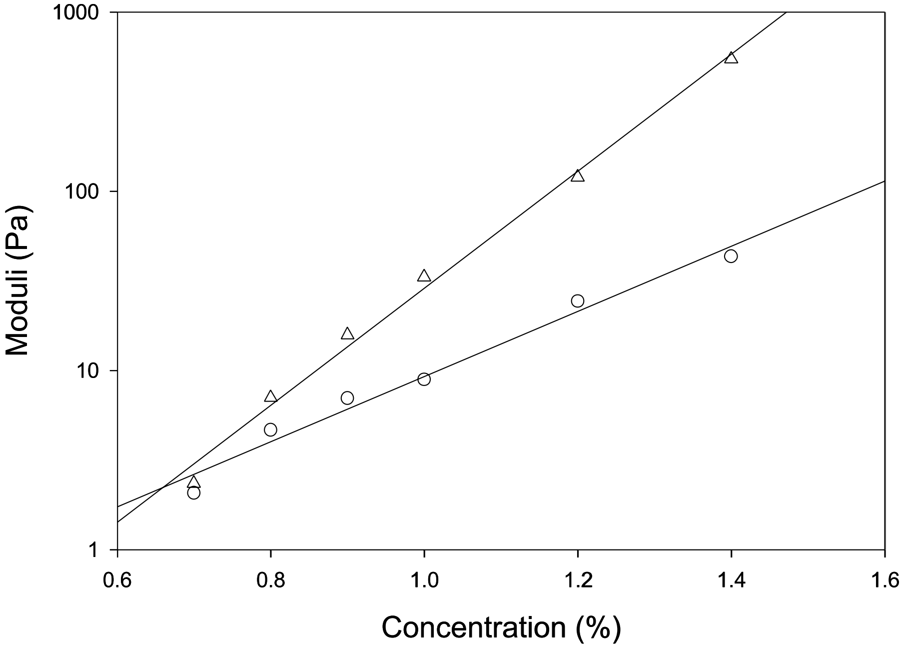

Fig. 9. G' and G'' for purified K^+-κ-carrageenan of different concentrations at 20 °C. G'; ○, G''.

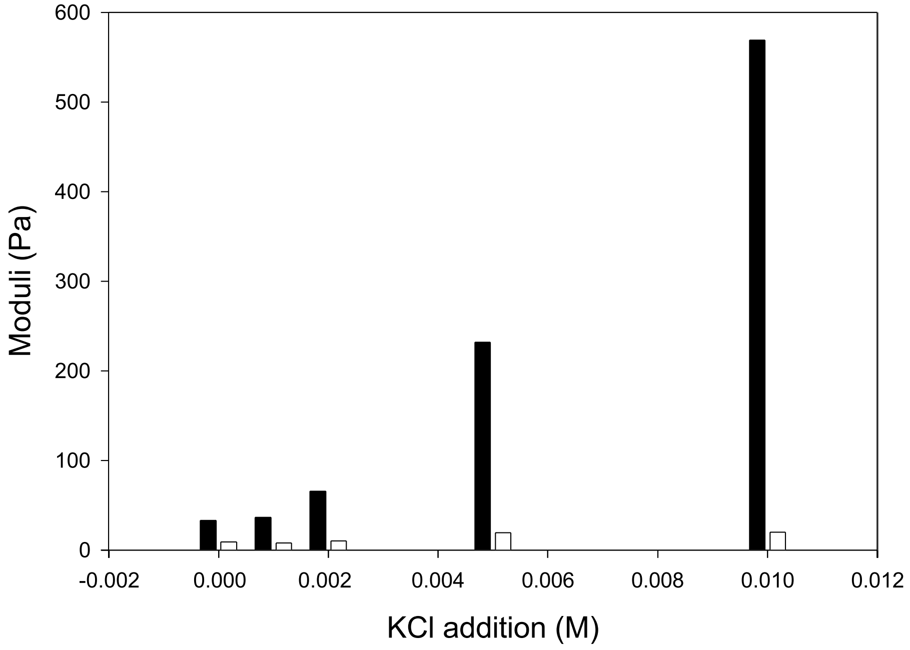

Fig. 10. G' and G'' for 1% purified K^+-κ-carrageenan under different added KCl concentrations at 20 °C. Closed column, G'; Open column, G''.

Small amounts of KCl (0.001, 0.002, 0.005 and 0.01 M) were introduced to monitor the effect of K^+ on the rheology of κ-carrageenan. The level of addition is small compared to the free salt levels normally found in commercial samples, yet a significant impact on the gel strength is observed as seen in Fig. 10. The results indicate that any rheological comparison of κ-carrageenan samples must consider the precise ionic concentration and composition.

The G' value of the unpurified sample (1%), from which our purified K^+-κ-carrageenan was obtained, was determined to be of the order of 10,000 Pa (Chen et al., 2001). The total molar concentration of cations for a 1% solution of that unpurified sample is estimated to be 0.03 M. The total molar concentration of cations for a 1% sample of the purified κ-carrageenan with 0.01 M KCl added is almost the same, but the gel strength observed is considerably lower, at only 569 Pa (Fig. 10). It was also demonstrated that κ-carrageenan in the pure calcium form was able to form gels only in the presence of significant levels (0.03–0.1 M) of $CaCl_2$ and the gel formed was also weaker than the comparable mixed ionic forms (Hermansson et al., 1991). These findings lead to the conclusion that a mixed ionic presence (potassium and calcium) is crucial to the gel formation of κ-carrageenan, especially at relatively low ionic strengths.

The evidence from detailed studies suggests most carrageenans obtained from red algae are hybrid polymers, containing different proportions of heterounits within the primary chain of the dominant repeating disaccharide (See for example, van de Velde, Peppelman, Rollema, & Tromp, 2001). Even the highest purity κ-carrageenan, readily available from marine-farmed *Kappaphycus alvarezii* (as '*Eucheuma cottonii*' in commerce), contains a significant portion (~8 mol% or more) of ι-carrageenan repeating disaccharide units, as revealed by linkage analysis of the biopolymer (Stevenson & Furneaux, 1991) and confirmed in our routine structural analysis using the κ-carrageenan as a standard. As has been emphasised by Piculell (1995), the presence of minor structural variations may have a large influence on the properties of carrageenans closely approaching the ideal structure. It is, therefore, crucial to elucidate the role of the ι-carrageenan repeating units in the gelation of κ-carrageenan. However, no technical breakthrough has emerged to enable the removal (e.g. through the approach of using carrageenase enzymes) or modification of the minor units without adversely affecting the molecular weight of carrageenan sample. Thus the contribution of the ι-carrageenan units to the gelation of κ-carrageenan remains to be determined.

Although information about the effects of Ca^{2+} on κ-carrageenan gelation is still confusing and contradictory, it has been established (for example, Michel et al., 1997) that divalent cations (e.g. Ca^{2+}) are far more effective than monovalent cations in increasing the elastic modulus (G') of ι-carrageenan. Thus the question remains as whether the K^+ and Ca^{2+} ions would still exhibit any synergistic effects on the gelation of κ-carrageenan in the absence of low levels of ι-carrageenan repeating units.

Higher κ-carrageenan concentrations and KCl levels result in stronger gels with G' values beyond the range observed in the current experiments. The presence of syneresis has prevented them from being measured using the original cone and plate geometry. Our investigation at this stage is thus limited to the above-mentioned concentrations due to the presence of syneresis and moduli beyond the range experimentally accessible using the current system.

4. Conclusions

Purified κ-carrageenan in the potassium form with essentially no free salts forms a weak gel at moderate concentrations (0.7–1.4%). The gels formed flow under large strains and their structure is able to recover significantly when re-cured. The addition of small amounts of potassium salts and increasing the polymer concentration greatly effects the measured rheological behaviour of the gels. Strong synergy is observed for the gel system in the presence of both potassium and calcium ions.

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