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Rheology and fracture of mixed ι- and κ-carrageenan gels: Two-step gelation

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It is shown that under certain circumstances, on cooling mixed ι- and κ-carrageenan solutions, the two forms gel separately at different temperatures, with the ι form gelling first. This 'two-step gelation' was only observed when both sodium and potassium ions were present, with a sodium/potassium mole ratio of between 1 and 100. For such mixed gels, a κ fraction as low as 2·5% of the total carrageenan has significant effects on their rheology, both at low deformation and fracture. In these systems, the κ form, gelling in the presence of an existing ι gel, produces measurable rheological effects at much lower concentrations than if it were alone. This behaviour can be used as a sensitive test of the 'rheological purity' of samples of ι-carrageenan.

INTRODUCTION

The carrageenans form a family of linear sulphated galactans (Painter, 1983) which are widely used in the food industry for their thickening (viscosity increasing) and gel-forming properties. The ι and κ forms (see Fig. 1) are the only ones which form gels, their respective rheological properties are very different. Those of the ι form are soft, weak and only fracture at large strains, whereas κ- carrageenan gels are hard, strong and brittle. Due to the weakness of pure ι-carrageenan gels, the κ form is often added to provide strength, without the mixed gel losing the desired rheological characteristics of pure ι gels.

Despite their technological importance, very little systematic study has been made of the rheology of mixed ι- and κ-carrageenan gels (Rochas *et al.*, 1989; Piculell *et al.*, 1992). Such studies are complicated by the fact that pure ι- and κ-carrageenans are difficult to obtain without careful enzymic and/or chemical purification (Bellion *et al.*, 1981; Rochas *et al.*, 1989; Piculell *et al.*, 1992). In general, samples of ι-carrageenan are found to contain significant amounts of the κ form and vice versa. In the absence of pure samples, the composition of ι- and κ-carrageenans must be carefully established before studies of their mixtures can be carried out. However, determining the purity of carrageenans is far from simple.

The use of ^{13}C nuclear magnetic resonance (NMR) has allowed the detection of small amounts of the κ form in samples of ι-carrageenan (Piculell & Rochas, 1990). Recently, Piculell and Rochas (1990) detected '< 5%' of κ-carrageenan in an impure sample of the ι form. However, to achieve this level of detection, the sample was degraded ultrasonically before measurement.

The disaccharide composition of a carrageenan sample is insufficient to predict its behaviour, even qualitatively. This is due to the fact that the contamination discussed above could, in principle, be present as: (i) separate chains, (ii) blocks in hybrid chains, or (iii) isolated groups in hybrid chains. It has been shown using enzymic degradation (Bellion *et al.*, 1981) that certain samples of native ι-carrageenan contain hybrid chains bearing blocks of the κ form, and vice versa, although their concentration was not determined. On the other hand, Rochas *et al.* (1989) found that a sample of native ι-carrageenan contained a fraction of pure κ form. The relationship between rheology and composition

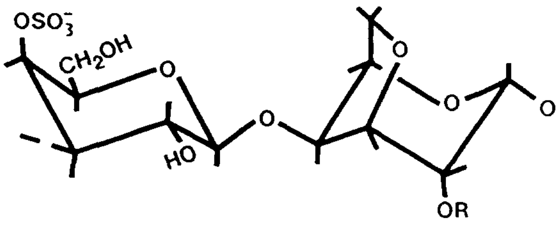

Fig. 1. Repeating disaccharides of ι- (R = SO$_3^-$) and κ- (R = H) carrageenan.

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would be expected to differ, depending on which form the contamination takes.

The conditions for gelation of both ι- and κ-carrageenans are closely related to those necessary for the helix–coil transition to occur (Rees *et al.*, 1982). In order to establish relationships between rheological measurements and composition for heterogeneous carrageenan samples, the effects of electrolyte ions on helix formation and gelation must be understood. Unfortunately, the specific and non-specific effects of cations on the behaviour of carrageenans have been subjects of controversy for many years (Piculell *et al.*, 1987). The controversy has been almost entirely due to the lack of a reliable method for the determination of the purity of carrageenan samples. In particular, the presence of small amounts of κ-carrageenan in samples thought to contain only the ι form has caused misunderstandings (Morris *et al.*, 1980*a,b*; Robinson *et al.*, 1980; Norton *et al.*, 1983), due to the strong specific binding by the κ form (Grasdalen & Smidsrød, 1981) of potassium ions (as well as those of caesium, rubidium and ammonium).

Piculell *et al.* (1987) have shown, using polarimetry, that segments of the potassium salt of ι-carrageenan showed only one order–disorder transition without thermal hysteresis, and that the addition of κ-carrageenan added a second transition which showed hysteresis. Piculell and Rochas (1990) demonstrated the effect even more clearly by showing a two-step transition in the optical rotation on cooling a solution of the rubidium of a '≥ 95% pure' native ι-carrageenan. One of these transitions disappeared after treatment with a κ-carrageenanase. It seems clear from these measurements that the two-step helix–coil transition of ι-carrageenan, observed by Robinson *et al.* (1980) and cited as evidence for their 'domain' model of ι-carrageenan gelation, was due to the presence of κ-carrageenan in their samples.

On the basis of NMR and optical rotation measurements, the current understanding of the helix–coil transition for ι- and κ-carrageenan can be summarised as follows: ι-carrageenan shows no specific ion binding (Piculell *et al.*, 1992). Its greater sensitivity to divalent ions is of the magnitude expected, due to their charge (Nilsson *et al.*, 1989). The helix–coil transition of pure ι-carrageenan does not show any thermal hysteresis (Piculell *et al.*, 1987), this is thought to be because the double helices formed do not aggregate after their formation. κ-Carrageenan in the *coil* form shows no specific binding effects (Nilsson & Piculell, 1991). However, the *helical* form binds Cs^+, Rb^+, K^+ and NH_4^+ specifically (Grasdalen & Smidsrød, 1981). The helix–coil transition shows thermal hysteresis, because the helices aggregate (Rees *et al.*, 1982).

The behaviour of κ-carrageenan in solutions containing more than one cation is complex (Hermansson, 1989; Nilsson *et al.*, 1989; Hermansson *et al.*, 1991). Hermansson *et al.* (1991) showed that both the microstructure and the rheology of κ-carrageenan gels varies depending on the concentrations and ratios of sodium, potassium and calcium ions present.

Piculell *et al.* (1992) have very recently shown data for the gelation of ι- and κ-carrageenan mixtures, in sodium chloride and potassium chloride. They conclude that the ι and κ forms gel separately and that the gelation of the ι form is not affected by the presence of κ-carrageenan in the coil form. Despite concluding that the two forms gel independantly, Piculell *et al.*'s results do not systematically show gelation in two stages, although it was discernible in one set of data, with the κ form gelling first. In this paper, similar low deformation oscillatory measurements have been made, but extending over a wide range of mixed cation and carrageenan concentrations.

The fracture behaviour of gels is important both scientifically and industrially, nevertheless, it has been little studied (McEvoy *et al.*, 1985; Luyten & van Vliet, 1990; Luyten *et al.*, 1991). Since carrageenan gels are usually eaten, their large deformation and fracture properties form a much more important part of their characteristics than for gels whose function requires that they remain intact. In this paper, the temperature dependence of the cutting force, a fracture-related property which can be reproducibly and precisely measured in gels (Luyten & van Vliet, 1990), is compared with that of the storage modulus.

Finally, it is shown that, under certain ionic conditions, the rheology of samples of ι-carrageenan can be used to quantify the amount of 'rheologically active' κ-carrageenan present. The comparison of such measurements with results from ^{13}C NMR gives insight into the nature of the contamination of native ι-carrageenans.

MATERIALS AND METHODS

Most samples were industrial products manufactured by Sanofi Bio-Industries (Carentan, France). Samples of ι-carrageenan were prepared from *Eucheuma spinosum* and those of κ-carrageenan from *Eucheuma cottonii*. One sample each of ι- (from *Eucheuma spinosum*) and κ-carrageenan (from *Eucheuma cottonii*) were also obtained from Sigma Chemicals (St. Louis, MO, USA). Note that neither of these names is taxonomically correct, although they are widely used commercially. The correct names for the species in question, which are cultivated in the Philippines, are *Eucheuma denticulatum* and *Kappaphycus alvarezii* respectively (Doty, 1988).

The industrial manufacturing process consists of heating the seaweed in alkaline solution, extracting in water and finally precipitating from concentrated potassium chloride into isopropanol. No subsequent ion-exchange or other purification method was used. Samples of ι-carrageenan were prepared at a laboratory scale from carefully sorted seaweed using a procedure close to the industrial process. One of these was converted into the sodium form by precipitating several times from 100 g/litre NaCl into isopropanol.

Samples were analysed for ion content using atomic absorption spectroscopy. ^{13}C NMR measurements were carried out as follows: 2·5% w/w solutions were prepared in D_2O by heating to 80°C in a water bath, whilst stirring, for 30 min. Measurements were carried out at 60°C, using a Bruker AC 250 spectrometer operating at 62·9MHz under the following conditions: pulse width 30°, acquisition time, 1·7s, 32k data points. Data collection was carried out for between 12 and 24 h.

The apparent storage modulus, G'_{app}, was measured as a function of temperature using a Carri-Med CS100 controlled stress rheometer fitted with a 2° steel cone with a diameter of 4 cm. Since measurements were not always made in the linear viscoelastic region, the value is quoted as the apparent storage modulus, G'_{app}. Carrageenan solutions for low deformation oscillatory measurements were prepared by heating at 90°C for 30 min. Measurements were made approximately every 2·5°C between 65 and 5°C at a frequency of 1 Hz. The cooling rate was close to 1°C/min. This rate is too high to allow an equilibrium modulus to be reached at temperatures close to the gel point. Nevertheless, it was found that reproducible results could be obtained under these conditions. The gap between cone and plate was set to the correct value at 40°C. Evaporation was prevented by a solvent trap. A strain of 0·14 was used, which was found to be the lowest value giving reliable results. Hermansson (1989) has shown that such large strains are well outside the linear region for pure κ-carrageenan gels. However, the gels studied here, with ι fractions of at least 90%, are much less rigid, so that range of strains for which these gels remain linearly viscoelastic extends to higher values. In any case, as no attempt is made to interpret the results in terms of a rheological theory, the possible non-linearity of the viscoelastic response does not affect the conclusions.

In several cases, the viscoelasticity of gels was measured as a function of frequency between 0·1 and 10 Hz, at 20°C. G' was always at least 50 times greater than G''. G' increased only slightly with increasing frequency. In this work only values for G'_{app}, are quoted.

The force required to cut gels was measured using a Brookfield RVT viscometer attached to a 'Helipath' stand and fitted with a horizontal wire 37 mm long with a diameter of 0·8 mm. The viscometer rotated the wire at 0·5 rpm, whilst at the same time descending towards the sample, so that the wire always cut into intact gel. Measurements were reproducible to within 2% of full-scale deflection, unless otherwise stated. Gels were made by heating the carrageenan solution to 90°C for 30 min. The hot solution was poured into square 0·25 litre beakers, used to avoid gel slippage, and stored in a water bath for 16 h before measurement.

A few cutting force experiments were carried out at room temperature using a vertically descending wire attached to an Instron-type materials testing machine (model DY30, Adamel Lhomargy, France).

The carrageenan concentration was fixed at 15 g/litre, unless otherwise mentioned, which corresponds to a potassium concentration of approximately 40 mM, for the samples precipitated in potassium chloride, and an ι-carrageenan concentration of 28 mM disaccharide, assuming 100% ι form with the counterion composition given below. The effective concentration of counterions is less than their actual concentrations, due to the reduction of their activity coefficients caused by the presence of the carrageenan (Rochas & Rinaudo, 1980). However, at the ionic strengths used in this study, the correction is small.

The term 'κ fraction', given in percent, is used here to express the fraction of the total carrageenan by weight which is in the κ form, and not the concentration of κ-carrageenan.

RESULTS AND DISCUSSION

Sample characteristics

The cation analysis and ^{13}C NMR results for the various samples of carrageenan are shown in Table 1. It can be seen that the industrial carrageenans are mainly in the potassium form, with a ratio close to 70 mol% K^+/ 30mol% Na^+. Samples having this cation composition are referred to as potassium salts in the text that follows. Two of the samples, ι1 and ι2, contained significant amounts of calcium and magnesium, due to minor differences in the manufacturing process.

The NMR results are worth commenting on, since it was found that ≥ 98% pure samples of both ι- and κ-carrageenan can be prepared, without the need for special purification. The detection limit was estimated to be 2%. Previous studies of native samples have invariably shown (Bellion *et al.*, 1981; Piculell *et al.*, 1987; Rochas *et al.*, 1989; Piculell & Rochas, 1990) contamination of ι-carrageenan by the κ form and vice versa. The present study, together with other unpublished results, suggests that the correct point of view is that such contamination, whilst widespread, is not universal. Lastly, it was found that both the carrageenan samples purchased from Sigma Chemicals were contaminated. The conclusion from these results is that the only guarantee of pure carrageenans is careful chemical analysis, preferably including ^{13}C NMR.

Storage modulus as a function of temperature

G'_{app} was measured as a function of temperature for a series of mixtures of pure ι- and κ-carrageenan (samples ι1 and κ1, respectively), both as the potassium salt, dissolved in 200 mM NaCl. Figure 2 shows the results of

Table 1. Cation content and ^{13}C NMR analysis of carrageenans

Sample	Seaweed	Source[a]	Purity[b] (^{13}C NMR)	Cation[c]			
				Na^+	K^+	Ca^{2+}	Mg^{2+}
ι1	*E. denticulatum*	Ind.	≤ 2	23	57	17	3
ι2	*E. denticulatum*	Ind.	6	18	65	14	3
ι3	*E. denticulatum*	Ind.	≤ 2	27	70	2	1
ι4	*E. denticulatum*	Ind.	4	32	68	0·1	0·4
ι5	*E. denticulatum*	Lab.	≤ 2	97	3	—	—
ι6	*E. denticulatum*	Lab.	≤ 2	—	—	—	—
ι7	*'E. spinosum'*	Sigma	5	—	—	—	—
κ1	*K. alvarezii*	Ind.	≥ 98	27	69	4	0·5
κ2	*'E. cottonii'*	Sigma	94	—	—	—	—

[a] Ind. — industrial product, Lab. — produced at laboratory scale, Sigma — obtained from Sigma chemicals.
[b] Purity of carrageenan is expressed in mol% κ-carrageenan of total carrageenan.
[c] Cation contents are expressed in mol% of total cations.

substituting small amounts of κ-carrageenan for the ι at a constant total carrageenan concentration of 15 g/litre.

All of the mixtures start to gel at a temperature close to 65°C. This figure is close to the value found by Nilsson and Piculell (1991), who found using polarimetry that helix formation started at 61°C for a 25 g/litre solution of the sodium salt of ι-carrageenan in 200 mM NaCl. The gel temperature found here is a few degrees higher than for a 100% sodium system, because the sample of iota carrageenan used (ι1) contains significant amounts of divalent ions. A correspondence between the gel temperature and the onset of helix formation is expected (Piculell *et al.*, 1992), since only a small amount of helices are required to form a gel.

For the pure ι-carrageenan, G'_{app} rises steadily, reaching a plateau value of about 180 Pa at 30°C, but when the κ fraction is as low as 2·5%, G'_{app} no longer rises smoothly, there is an abrupt change in slope close to 37°C. With larger additions of the κ form, this increase becomes more marked. It is clear that the two forms of carrageenan are gelling at different temperatures. In this paper, this phenomenon is referred to as two-step gelation.

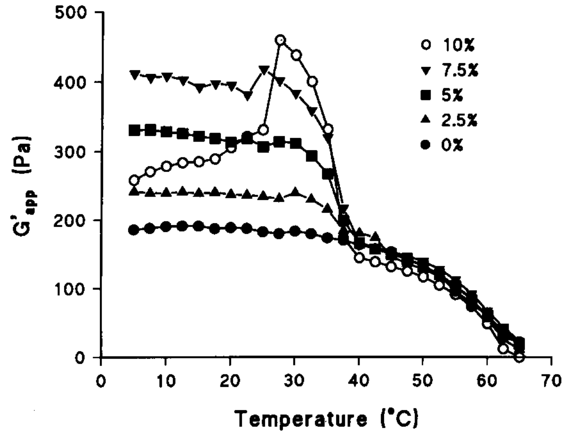

Fig. 2. Apparent storage modulus as a function of temperature for mixtures of ι- (ι1) and κ-carrageenan. Total carrageenan concentration —15 g/litre. Solvent 200 mM NaCl. κ fraction identified on the graph.

When more than 5% of the κ form is present, there is a sharp drop in G'_{app} at some temperature below 30°C. The temperature at which this drop occurred was not reproducible. This drop in G'_{app} is ascribed to the syneresis of these gels, rather than to their brittleness. Careful examination of gel samples after tests showing the drop in G'_{app} did not reveal any sign of fracture, which might otherwise be thought to be a reasonable explanation of this phenomenon. Hermansson *et al.* (1991) found that it was necessary to take special precautions in order to follow the storage modulus of pure κ-carrageenan gels as a function of temperature. The rheometer cup was made of a low expansion alloy, and the strains used were up to 100 times smaller than those used here. It is planned to repeat these measurements at lower strains using a porous plate to prevent the formation of a solvent layer. Moldenaers *et al.* (1988) found that this was a successful way to avoid the problems observed here.

These results are the clearest to date showing two-step *gelation,* as opposed to helix formation, in mixtures of ι- and κ-carrageenan. They are also the first to show two-step gelation with the ι form gelling at higher temperatures. Piculell *et al.* (1992) showed results for mixtures of ι- and κ-carrageenans in a 100% potassium system, with the κ form gelling at higher temperatures. However, the differentiation of the two gelation steps was much less marked than in the present work.

In electrostatic terms, the observation of Piculell *et al.* (1992) that the κ form gels alone at higher temperatures than the ι form from their mixtures is not surprising, since the former contains only one charged group per disaccharide, as opposed to two for the ι form. A consequence of this difference in charge density is that the formation of κ–κ double helices is electrostatically more favourable than that of mixed κ–ι double helices. On the other hand, the finding here that, under different ionic conditions, the ι form gels alone at higher temperatures is unexpected from an electrostatic point of view, since in this case ι–ι double helices are formed rather than the electrostatically favourable mixed ι–κ helices. The conclusion from this finding is that the thermodynamic incompatibility of the two forms (Flory, 1983) predominates over the favourable electrostatic contribution to prevent the formation of mixed helices.

The gelation of the ι form in the presence of the coil form of κ-carrageenan is unaffected by the presence of the latter, which shows that no mixed ι/κ junctions are formed. In 200 mM NaCl, the variation of G'_{app} above 40° can be entirely explained, to within experimental error, by the gelation of the ι form. As the κ form is substituted, the value of G'_{app} in this temperature range is reduced slightly. This finding agrees with the conclusions of Piculell *et al.* (1992) for 100% sodium systems, in which κ-carrageenan was in the coil form at all accessible temperatures.

Measurements of κ-carrageenan alone, in conditions otherwise identical to those used to obtain the data in Fig. 2, showed no gelation at a concentration corresponding to a κ fraction of 5%. For concentrations corresponding to κ fractions of 7·5% and 10%, G'_{app} rose to a few Pa at 37°C, but dropped again at temperatures below 32°C. Thus, κ-carrageenan can form gels in mixtures with ι-carrageenan at concentrations below its gel point when alone, which shows that not only do the two forms gel separately, but also that they gel in separate phases. If this were not so, the elastic moduli of the two forms would be additive (Zhang & Rochas, 1990), and the presence of κ-carrageenan would not affect the total modulus unless its concentration were above that required for its gelation alone. Piculell *et al.* (1992) came to the same conclusion.

Lastly, the difference in the sharpness of the gelation between the two forms should be noted. Piculell (1993) has pointed out that the helix–coil transition, measured by polarimetry, is broader for ι-carrageenan than for the κ form. The same phenomenon is visible in Fig. 2, so that the close parallels between the sol–gel and coil–helix transitions extend to the form of the transitions, not only to their temperatures of onset.

The origin of two-step gelation was clarified by measuring firstly the effect of substituting potassium for sodium in a system containing 15 g/litre of pure ι-carrageenan and initially free from potassium. Secondly, the measurements were repeated with 5% of the ι-carrageenan replaced by the κ form.

In Fig. 3, the effect of increasing amounts of potassium on the sodium salt of a pure sample of ι-carrageenan (ι5) is shown. The solvent always contained 200 mM electrolyte. It is clear that, to within experimental error, the replacement of sodium by potassium does not modify the storage modulus/temperature behaviour of pure ι-carrageenan. This result is not in agreement with that of Piculell *et al.* (1992), who found that ι-carrageenan in 100 mM KCl gelled at a temperature 5°C higher than in 100 mM NaCl. Rinaudo *et al.* (1979) found that the helix–coil transition of ι-carrageenan was at higher temperatures in potassium chloride than in sodium chloride. The reason for this difference is not clear, especially in the case of Piculell *et al.*'s results.

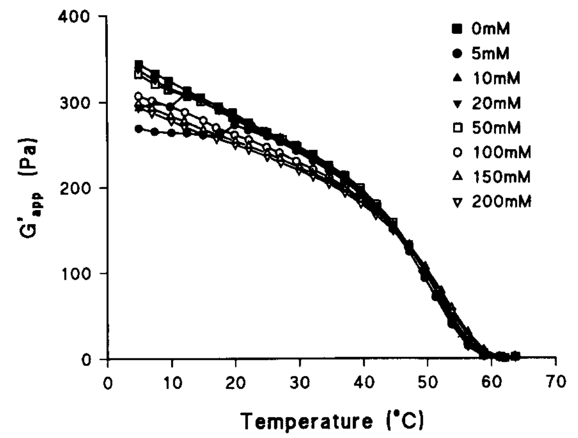

Fig. 3. Apparent storage modulus as a function of temperature for sodium salt of ι-carrageenan (ι5), in various mixtures of sodium chloride and potassium chloride. Carrageenan concentration — 15 g/litre. Solvent 200 mM total electrolyte. Concentrations of potassium are identified on the graph.

It is worth noting that for this sample (ι5), there is no plateau value for the modulus at low temperatures, as was seen in Fig. 2. The absence of a plateau value is thought to be due to the presence of small amounts of helix-blocking precursor disaccharides, further studies are under way to elucidate this point.

Figure 4 shows the results for the same system as for the data in Fig. 3, except that 5% by weight of the ι-carrageenan was replaced by the κ form, thus the κ-carrageenan concentration was 750 mg/litre. Temperature sweeps down to 5°C were carried out for all the samples, but points after the drop in G'_{app}, which

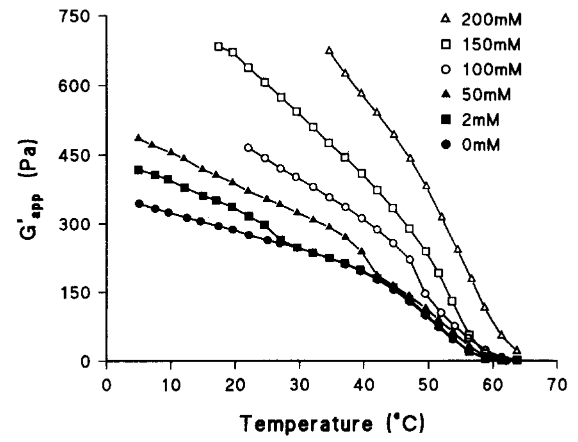

Fig. 4. Apparent storage modulus as a function of temperature for a mixture of 95% ι- (ι5)/5% κ-carrageenan in various mixtures of potassium chloride and sodium chloride. Carrageenan concentration — 15 g/litre. Total electrolyte concentration 200 mM. Concentrations of potassium are identified on the graph.

occurred in the presence of more than 50 mM K^+, are not shown, to improve the clarity of presentation. Two-step gelation is observed for the samples containing from 2 to 100 mM K^+. However, when the concentration of potassium equalled or exceeded 150 mM, the gelation temperatures of the two forms are so close to each other that they can no longer be distinguished.

The effect of increasing the potassium/sodium ratio, shown in Fig. 4, is to increase the gelation temperature of the κ form, but not that of the ι form, at least up to 150 mM potassium. It is interesting to note that the gelation temperature of the ι form in the mixtures does not vary up to 150 mM potassium, but its apparent value is increased in 200 mM K^+, due to the presence of κ–carrageenan. This result shows that especial attention must be paid to purity when studying the rheology of ι-carrageenan in K^+-rich solvents, as very low amounts of contamination by the κ form can lead to completely erroneous results.

As well as increasing the gel temperature of the κ form, increasing the ratio of K^+ to Na^+ reinforces the κ gel, so that when K^+ represents 75% or 100% of the total cations, the contribution of the ι gel is masked.

It is clear from these measurements that two-step gelation can be observed in systems which have a Na^+ to K^+ mole ratio of between 1 and 100, but not in systems containing almost entirely sodium or almost entirely potassium.

The two-step gelation shown in Fig. 2 and 4 is not confined to these particular electrolyte and carrageenan concentrations. Figure 5 shows the effect of the sodium chloride concentration in the solvent on G'_{app} as a function of temperature for a sample containing 15 g/litre of the potassium salt of a sample of ι-carrageenan (ι2), which was contaminated by 6% of the κ form. As expected, the apparent gelation temperatures of both the κ and ι fractions decrease with decreasing sodium chloride concentration. Two-step gelation is visible at all salt concentrations.

Figure 6 shows the effect of varying the total ι-carrageenan concentration, for the same contaminated sample, ι2. The solvent is 200 mM NaCl. Note that the ordinate is logarithmic in this case, so that the gelation is clearly visible at the lowest concentrations. The gel temperatures of both the ι and κ forms decrease with decreasing total carrageenan concentration, due to the effect of the latter on the total ionic concentration. Even at a total carrageenan concentration of 5 g/litre, corresponding to a concentration of the κ form of only 300 mg/litre, two-step gelation is still visible. This finding is surprising, since it was concluded from the data presented in Fig. 2 that the mixtures formed a phase-separated network, in which case, it would be expected that a critical concentration exists below which phase separation, and thus two-step gelation, does not occur. In the κ/ι system, the critical concentration must be very low, which is unexpected given the similarity of their structures. For comparison, Nilsson and Muhrbeck (1992) found that a mixture containing 10 g/litre of κ carrageenan and 5 g/litre of the synthetic polymer sodium polystyrenesulphonate in 100 mM KCl was not phase separated.

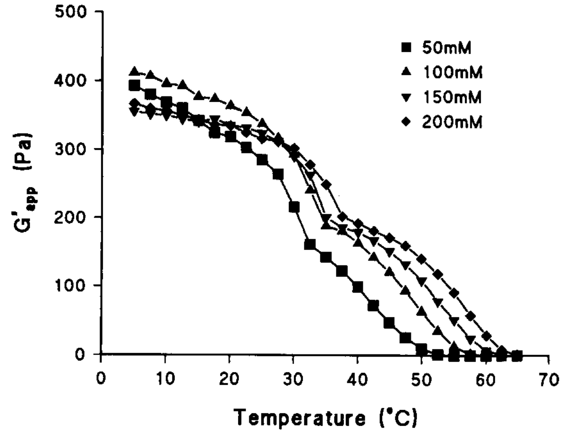

Fig. 5. Apparent storage modulus as a function of temperature for the potassium salt of a contaminated native ι-carrageenan (ι2) in various concentrations of sodium chloride. Carrageenan concentration — 15 g/litre. Sodium chloride concentrations are identified on the graph.

The data in Fig. 6 can be used to determine the dependence of G'_{app} on the total concentration of carrageenan at various temperatures. At 10, 20 and 40°C, i.e. at temperatures not too close to the gelation temperatures, log/log plots of G'_{app} against the total carrageenan concentration were fitted well by straight lines of slope $2{\cdot}0 \pm 0{\cdot}1$. A value close to 2 has been frequently observed (Clark & Ross-Murphy, 1985) for polysaccharides.

Figure 7 shows the effect of varying the potassium chloride concentration in the solvent between 20 and 70 mM, in the absence of added sodium on the same contaminated ι-carrageenan (ι2). The carrageenan concentration is 15 g/litre. No two-step gelation is visible, as

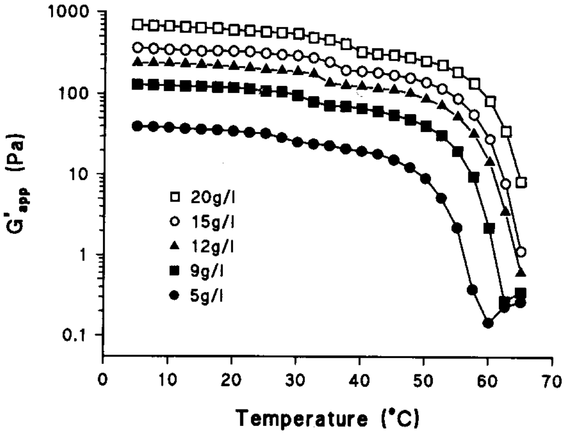

Fig. 6. Apparent storage modulus as a function of temperature for the potassium salt of a contaminated native ι-carrageenan (ι2) at various carrageenan concentrations (identified on the graph) in 200 mM NaCl.

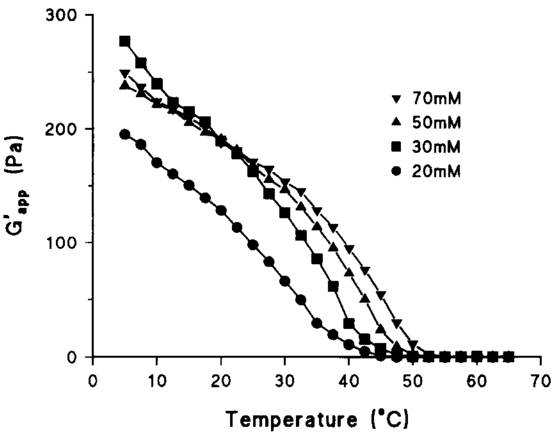

Fig. 7. Apparent storage modulus as a function of temperature for the potassium salt of a contaminated native ι-carrageenan (ι2) in various concentrations of potassium chloride, no sodium added. Concentrations of potassium are identified on the graph.

was found in 200 mM KCl, shown in Fig. 4. In the absence of Na^+, the gel temperature of κ-carrageenan is always above that of the ι form, so that the gelation of the latter is masked.

Figure 8 shows the effect of varying the concentration of contaminated ι-carrageenan (ι-2) when 50 mM KCl is the solvent. In this case too, no two-step gelation is visible.

Cutting force measurements

Measurement of the cutting force is of interest because it is a fracture-related property which is not affected by small imperfections in the sample, as is the case for the force at rupture measured during deformation of the whole sample. The cutting force contains contributions from the following (Luyten *et al.*, 1991): (i) elastic deformation of the gel, (ii) viscous dissipation, and (iii) creation of a new surface.

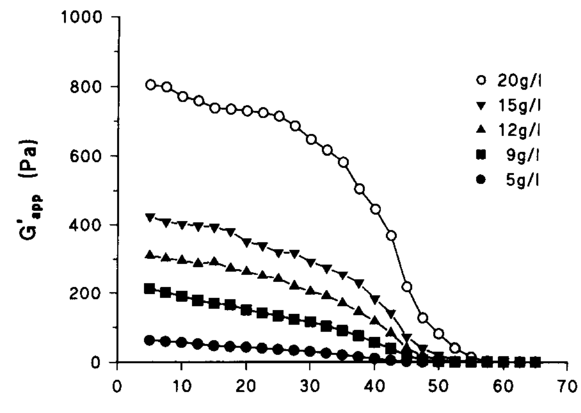

Fig. 8. Apparent storage modulus as a function of temperature for various concentrations of the potassium salt of a contaminated native ι-carrageenan (ι2) in 50 mM KCl. Concentrations of carrageenan are identified on the graph.

Luyten *et al.* (1991) have shown that the force required to cut a soft viscoelastic solid, cheese, depends on both the speed of cutting and the diameter of the wire used. The technique adopted here of measuring the force on a rotating wire obviously imposes a range of cutting speeds from zero to the speed at the tips of the wire; thus, the values obtained are averaged over a range of cutting speeds. However, trial experiments using a non-rotating wire attached to a compression–traction apparatus showed that, qualitatively, the same phenomena are observed using the two methods. The technique used here was found to be more convenient experimentally.

Cutting force measurements were carried out on gels with the same composition and in the same solvent as those used to obtain the data shown in Fig. 2. Figure 9 shows the relative force required to cut gels at 45°C and 25°C, that is at temperatures above and below the gel point of the κ form in this electrolyte solution, 37°C. The effect of two-step gelation is the same as that observed by measurements of the storage modulus: the force required at 45° is almost independent of the amount of the κ form present, whereas at 25° in this solvent, the cutting force increases significantly in the presence of a κ fraction as low as 2·5%.

Substituting the κ form for the ι contained to increase the cutting force of the mixed gels at 25°C up to a κ fraction of 10%. Up to this point, the reading was always steady and reproducible, the gel remained supple and elastic. However, when the κ fraction increased above 10%, the texture of the cut gel changed, it became friable and broke up into lumps, rather than fracturing smoothly. In consequence, the reading of the viscometer oscillated randomly and reproducible results could no longer be obtained. This result was not due to any limitation of the apparatus, similar results were obtained in cutting force measurements using a non-rotating wire, provided that the cutting speed was not too low.

Examination of gels after cutting showed that if the cutting force was steady, the fracture surfaces were smooth, whereas unsteady cutting force measurements corresponded to rough fracture surfaces.

The data shown in Fig. 10 confirm the conclusions drawn on the basis of the measurements of the storage modulus, shown in Fig. 2: κ- and ι-carrageenan gel separately, and the presence of an existing ι-carrageenan network greatly enhances the contribution of low concentrations of the κ form to the total modulus/cutting force.

As well as confirming the disproportionate effect of low concentrations of the κ form on the rheology of mixed gels, the cutting force measurements show a qualitative change in the fracture properties of mixed gels. For the gels studied here, there is a critical κ fraction, between 10 and 15%, above which the fracture properties of mixed gels change from 'ι-like', with steady values of the cutting force, to 'κ-like', oscillating values of the cutting force. This change coincides with a change

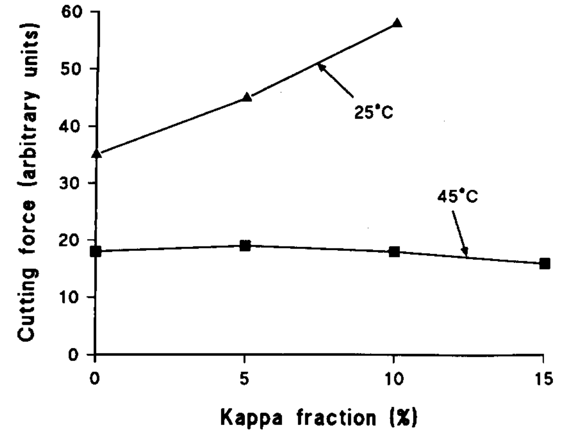

Fig. 9. Cutting force of gels at 25°C and 45°C as a function of the fraction of κ substituted. Same conditions as for Fig. 2.

in appearance of the fracture surfaces from smooth to rough. This change in the nature of the fracture has not been observed previously. Rochas *et al.* (1989) measured the stress and strain at fracture of mixed ι/κ-carrageenan gels under uniaxial compression, but did not observe any dislocation in variation of these properties as a function of composition. However, uniaxial compression measurements are not well adapted to observing changes in the nature of the fracture.

The fracture properties of ι- and κ-carrageenan gels can be rationalised in terms of their respective structures:

ι-Carrageenan gels consist of double helices with no or low degrees of aggregation, so that the network strands are of low modulus and flexible. Such a structure will not transmit strain efficiently. In the region close to a fracture, each strand will first stretch before affecting its neighbours and, being weak, will break soon afterwards. Fractures cannot propagate through such a structure, since the energy fed into the system is dissipated viscously in moving the strands, so that only the zone close to the cut will be affected. In this zone, strands are stretched and broken, without affecting those further from the fracture. Under these conditions, the cutting force is steady.

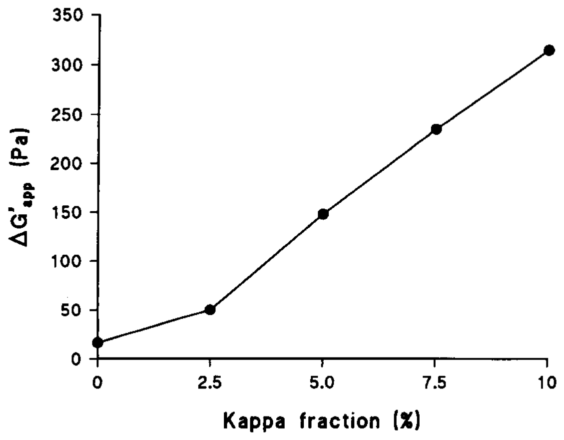

Fig. 10. Effect of the substitution of κ-carrageenan for ι-carrageenan on the difference between G'_{app} at 27°C and 45°C. Data taken from Fig. 2.

κ-Carrageenan gels consist of aggregated helices, which are of higher modulus and more rigid than a single double helix. Rigid strands cannot dissipate energy viscously. Close to the fracture, each strand only stretches a little before affecting its neighbours, so that the zone affected by the fracture is much larger. Because the strands are brittle, fracture is very cooperative: as soon as one strand breaks, the stress is concentrated on its neighbours, which break too, until the fracture has propagated far enough to dissipate the stress. In this structure, the cutting force is unsteady. This type of fracture is typical of brittle solids (Gordon, 1976).

Although the above explanation reasonably rationalises the difference in the nature of fracture for pure, ι- and κ-carrageenan gels, it does not help to understand why in their mixtures an abrupt change occurs from one type of fracture to the other. It would be tempting to suggest that this change is evidence of a percolation threshold (de Gennes, 1979), if the dependence of the storage modulus on composition, discussed in the previous section, did not imply that a bicontinuous phase-separated structure is already present when the κ-fraction is 2·5%. It may simply be that the mixed ι/κ gel takes on the fracture characteristics of the stronger, brittler κ gel over a narrow concentration range naturally and there is no need to invoke critical phenomena to explain this result. More detailed measurements are currently being made.

Measurement of the purity of ι-carrageenans

From Fig. 2, it seems possible to establish a calibration curve to give at least a qualitative idea of the purity of samples of ι-carrageenan. The difference between G'_{app} at 27·5° and G'_{app} at 45° was chosen as a measure of the fraction of the κ form. Figure 10 shows such a calibration curve established for the κ fraction in a mixture of ι- and κ-carrageenan, using the data in Fig. 2. The increase in G'_{app} between 345° and 27·5°C is an almost linear function of the concentration of the κ form over this rather narrow range.

A comparison of the fraction of the κ form in the first six samples of carrageenan shown in Table 1 estimated (i) by rheology, using the calibration curve in Fig. 10, and (ii) by ^{13}C NMR gave agreement to within ±1%, which is considered to be excellent. However, it is obvious that the agreement between the two methods cannot be general, since NMR directly measures the quantity of the ι and κ forms present in a sample, whereas G'_{app} only measures the quantity of each form present in a particular sample in so far as the calibration curve, Fig. 9, is correct.

The value of G'_{app} found for mixed ι/κ gels will depend on several factors, other than the ratio of κ to ι (Rochas *et al.*, 1989): their molecular weights (Rochas *et al.*, 1989), the total cation composition of the solvent, and the amount of 'kink'-forming non-transformed precursor sugars (Robinson *et al.*, 1980). These sugars are not helix forming.

The agreement between NMR and rheology in this case shows that all these parameters are practically constant across the range of samples. This is not surprising, since they are all produced by the same manufacturing process.

The rheological data shown here, together with the ^{13}C NMR data, clarify a number of points concerning the purity of carrageenans.

(1) Native samples of both ι- and κ-carrageenan exist which are pure, or only a few percent contaminated by the other form, as measured by ^{13}C NMR.

(2) The rheological behaviour of artificially contaminated ι-carrageenans is the same as that of the native samples measured in this study. The samples of ι-carrageenan tested here were not analysed to check whether the contamination was due to separate κ chains, κ blocks, or isolated dissaccharides inserted into ι chains. However, given the good correlation between the κ fraction measured by NMR and that obtained by a calibration curve from rheological measurements, it is safe to assert that the κ-carrageenan in the samples studied here acts as though it were in the form of separate chains. Piculell *et al.* (1992) came to the same conclusion. The ι/κ block copolymers observed by Bellion *et al.* (1981), do not seem to have an effect on the rheology of native ι-carrageenans distinct from that of separate κ chains.

(3) Defining the algal source, as suggested by Piculell *et al.* (1992), is insufficient to define the purity of samples of ι-carrageenan.

(4) Measuring the storage modulus of samples of ι-carrageenan as a function of temperature in electrolyte solutions containing both Na^+ and K^+ provides a simple way to verify their 'rheological purity'.

CONCLUSIONS

The observation of two-step gelation in mixtures of ι- and κ-carrageenan confirms that these two polysaccharides gel independently, without the formation of mixed junctions, to form a phase-separated interpenetrating network.

Despite the unfavourable electrostatics, two-step gelation with the ι form gelling at a higher temperature than the κ form is observed over a wide range of conditions.

In summary, the simple rule that two-step gelation is never observed for pure sodium and or pure potassium systems always holds for the results shown here. On the other hand, in systems containing both sodium and potassium where at least half the cations are sodium, two-step gelation is always observed. In pure sodium systems, the κ form gels at inaccessibly low temperatures. In pure potassium systems, the much greater strength of the κ gel, which forms first, masks the contribution of the ι gel.

The quantitative changes due to two-step gelation can be observed both at low deformation and fracture. In addition, fracture measurements show a qualitative as well as a quantitative difference if sufficient κ-carrageenan is present.

Samples of native ι- and κ-carrageenan which are pure, as measured by NMR and rheology, exist. However, three of the seven samples of native ι-carrageenan analysed by NMR were contaminated by the κ form. Comparison of results from NMR and rheology suggested that the contamination was in the form of separate κ chains.

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REFERENCES

Bellion, C., Hamer, G.K. & Yaphe, W. (1981). *Proc. Int. Seaweed Symp.*, **10**, 379–84.

Clark, A.H. & Ross-Murphy, S.B. (1985). *Adv. Polym. Sci.*, **83**, 57–92.

de Gennes, P.-G. (1979). *Scaling Concepts in Polymer Physics*. Cornell University Press, Ithaca, NY, USA.

Doty, M.S. (1988). In *Taxonomy of Economic Seaweeds*, ed. I.A. Abbott. California sea grant college program report No. CSGCP-018, CA, USA, pp. 159–207.

Flory, P.J. (1983). In *Principles of Polymer Chemistry* (12th edn). Cornell University Press, Ithaca, NY, USA.

Gordon, J.E. (1976). *The New Science of Strong Materials* (2nd edn). Penguin Books, Middlesex, UK.

Grasdalen, H. & Smidsrød, O. (1981). *Macromolecules*, **22**, 3919.

Hermansson, A.M. (1989). *Carbohydr. Polym.*, **10**, 163.

Hermansson, A.-M., Eriksson, E. & Jordansson, E. (1991). *Carbohydr. Polym.*, **16**, 297–320.

Luyten, H. & van Vliet, T. (1990). In *Gums and stabilisers in the food industry 5*, eds G.O. Phillips, D.J. Wedlock & P.A. Williams. Oxford University Press, Oxford, UK, p. 117.

Luyten, H., van Vliet, T. & Walstra, P. (1991). *Neth. Milk Dairy J.*, **45**, 55–80.

McEvoy, H., Ross-Murphy, S.B. & Clarke, A.H. (1985). *Polymer*, **26**, 1483–92.

Moldenaers, P., Mewis, J. & Berghmans, H. (1988). In *Progress and Trends in Rheology II*, eds H. Gieskus & F.F. Hibberd. Steinkopff Verlag, Darmstadt, Germany, p. 420.

Morris, E.R., Rees, D.A., Norton, I.T. & Goodall, D.M. (1980*a*). *Carbohydr. Res.*, **80**, 317–23.

Morris, E.R., Rees, D.A. & Robinson, G. (1980*b*). *J. Mol. Biol.*, **138**, 349–62.

Nilsson, S. & Muhrbeck, P. (1992). *J. Polym. Sci. B*, **30**, 681.

Nilsson, S. & Piculell, L. (1991). *Macromolecules*, **24**, 3804.

Nilsson, S., Piculell, L. & Jönsson, B. (1989). *Macromolecules*, **22**, 2367.

Norton, I.T., Goodall, D.M., Morris, E.R. & Rees, D.A. (1983). *J. Chem. Soc., Faraday Trans. 1.*, **79**, 2475–88.

Painter, T. (1983). In *The Polysaccharides 2*, ed. G.O. Aspinall. Academic press, New York, USA. pp. 195–285.

Piculell, L. (1993). In *Food Polysaccharides*, ed. A. Stevens. Marcel Dekker, New York, USA.

Piculell, L. & Rochas, C. (1990). *Carbohydr. Res.*, **208**, 127.

Piculell, L., Håkansson, C. & Nilsson, S. (1987). *Int. J. Biol. Macromol.*, **9**, 297.

Piculell, L., Nilsson, S. & Muhrbeck, P. (1992). *Carbohydr. Polym.*, **18**, 199–208.

Rees, D.A., Morris, E.R., Thom, D. & Madden, J.K. (1982). In *The Polysaccharides 1*, ed. G.O. Aspinall. Academic Press, New York, USA, pp. 195–285.

Rinaudo, M., Karimian, A. & Milas, M. (1979). *Biopolymers*, **18**, 1673–83.

Robinson, G., Morris, E.R. & Rees, D.A. (1980). *J. Chem. Soc., Chem. Commun.*, **152**, 152–3.

Rochas, C. & Rinaudo, M. (1980). *Biopolymers*, **19**, 1675.

Rochas, C., Rinaudo, M. & Landry, S. (1989). *Carbohydr. Polym.*, **10**, 115–27.

Zhang, J. & Rochas, C. (1990). *Carbohydr. Polym.*, **13**, 257–71.