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Effects of small amounts of kappa-carrageenan on the rheology of aqueous iota-carrageenan

Lennart Piculell,[a] Svante Nilsson[a] & Per Muhrbeck[b]

[a]*Department of Physical Chemistry 1,* [b]*Department of Food Technology, University of Lund, Chemical Center, Box 124, S-221 00 Lund, Sweden*

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The temperature dependence of the rheology (shear storage and loss moduli) of two ion forms (sodium and potassium) of aqueous iota-carrageenan was investigated in the presence and in the absence of small fractions (⩽10%) of added kappa-carrageenan. Both native iota-carrageenan (which already contains kappa-carrageenan as an impurity) and a sample purified by kappa-carrageenase was investigated. In either ion form, pure iota-carrageenan forms a weak gel as a consequence of its conformational transition, and the gel becomes stronger as the helical content increases. No marked ion specificity is seen in the rheology of pure iota-carrageenan. Small fractions of kappa-carrageenan, whether added or present as impurities, have no effect on the rheology of the sodium form but a large effect on the modulus of the potassium form of iota-carrageenan. The storage moduli of the potassium iota/kappa gels fall within the limits expected for a phase-separated gel structure.

INTRODUCTION

The carrageenans are linear sulfated galactans, extracted from certain marine red algae, which are widely used for their ability to thicken solutions or to form aqueous gels. The gel-forming carrageenans have a backbone which mainly consists of a repeating disaccharide of β-D-galactopyranose and 3,6-anhydro-α-D-galactopyranose, which is sulfated to varying degrees (Rees *et al.*, 1982; Painter, 1983). The most extensively studied of the gel-forming carrageenans are kappa- and iota-carrageenan, the repeating disaccharides of which are shown in Fig. 1. As extracted from the algae and purified by normal physical methods, the carrageenans obtained are never quite pure. In particular, 'native' samples of iota-carrageenan contain a small fraction (5–10% or more, depending on the algal source) of kappa-carrageenan, and vice versa (Bellion *et al.*, 1981; Rochas *et al.*, 1989). In order to improve the understanding of the molecular basis of the properties of iota- and kappa-carrageenan gels, it is therefore of interest to study, on the one hand, systems which approximate to the ideal structures as closely as possible, and, on the other hand, controlled mixtures. This is especially important for the understanding of rheological properties, since the rheology of a polymer gel may be very sensitive to the presence of small amounts of impurities.

The gross features of the gelation of kappa- and iota-carrageenan are similar, in that the network formation is preceded by a coil-to-helix transition, which may be induced by lowering the temperature and/or adding salt to the solution (Rees *et al.*, 1982). However, the ordered conformations and the networks formed by the two carrageenan types display intriguing differences. Kappa-carrageenan helices have been shown to specifically bind certain monovalent cations, notably the alkali

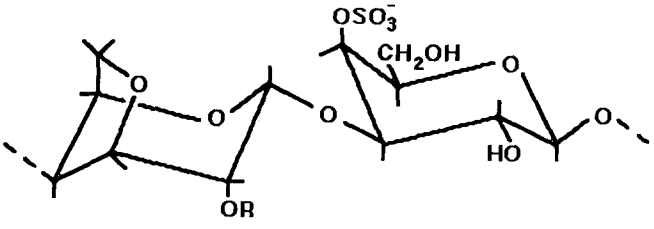

Fig. 1. Repeating disaccharide structures of kappa-carrageenan (R=H) and iota-carrageenan (R=SO_3^-).

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metal ions potassium, rubidium and cesium (Grasdalen & Smidsrød, 1981; Smidsrød & Grasdalen, 1982; Belton *et al.* 1986), whereas the interaction with other cations (sodium, lithium and tetramethylammonium) is mainly of a long-range electrostatic nature (Nilsson & Piculell, 1989; Nilsson *et al.*, 1989). The binding cations induce helix formation and gel formation at quite low concentrations (Rochas & Rinaudo 1980, Nilsson & Piculell, 1991), and they are therefore often referred to as gel-inducing ions. The network formation in kappa-carrageenan is believed to involve extensive aggregation of helices (Rees *et al.*, 1969, 1982), as is indicated, *inter alia*, by a pronounced thermal hysteresis in the conformational transition. As a consequence, kappa-carrageenan gels tend to be strong, brittle and opaque.

Iota-carrageenan gels, on the other hand, are typically clear and much weaker than the kappa-carrageenan gels (Belton *et al.*, 1984; Rochas *et al.*, 1989). The conformational transition of iota-carrageenan shows no thermal hysteresis, which indicates that there is less or no interhelical aggregation. The transition is sensitive only to the valency of the counterions, and differences among ion forms of the same valency are small (Robinson *et al.*, 1980; Piculell *et al.*, 1987; Nilsson *et al.*, 1989). Nevertheless, a cation-specificity in the rheology, as well as evidence of specific cation binding, is normally found in gels of native iota-carrageenan (Morris *et al.*, 1980; Robinson *et al.*, 1980; Belton *et al.*, 1984). Recent detailed investigations by rubidium NMR have demonstrated that the specific binding of alkali ions observed for native iota-carrageenan may be ascribed to kappa-carrageenan impurities (Piculell *et al.*, 1989; Piculell & Rochas, 1990). It is natural to assume that also the cation specificity in the rheology has the same origin, but until now, no experimental study has been presented which confirms this supposition.

In the present work, the authors have studied the rheology of aqueous iota-carrageenan in two ionic environments: systems containing a gel-inducing cation (potassium) have been compared with those containing a non-specific cation (sodium). The authors also compared gels from native iota-carrageenan with gels from a sample which had been treated with kappa-carrageenase (McLean & Williamson, 1979), which specifically removes sequences of kappa-carrageenan structure. Furthermore, the effects of small amounts of added kappa-carrageenan on the two ion forms of iota-carrageenan have been studied. In order to facilitate the molecular interpretation of the rheological results, and to obtain information on the sequence of events in the gel formation, the authors have studied the temperature dependence of the rheology of the samples and combined these studies with studies of the conformational state of the carrageenans using optical rotation.

EXPERIMENTAL

Materials

Commercial samples of iota-carrageenan (type V, from *Eucheuma spinosa*) and kappa-carrageenan (Type III, from *Eucheuma cottonii*) were obtained from Sigma (St Louis, Missouri, USA) and were purified by physical methods (salt precipitation followed by washing with ethanol) as described previously (Piculell *et al.*, 1989). The iota-carrageenan thus obtained will be referred to as 'native' iota-carrageenan. An aliquot of iota-carrageenan from the same lot (No. 115F-0664) was purified by enzyme treatment according to the following procedure. A 5 g liter^{-1} solution was hydrolyzed with kappa-carrageenase for 5 days at 45 °C, followed by heating at 100 °C (to destroy the enzyme), addition of hot NaCl to a final concentration of 1 M, precipitation with isopropanol, centrifugation, washing with isopropanol and drying. The iota-carrageenan thus obtained will be referred to as 'purified' iota-carrageenan. Dry stock samples of different ion forms were obtained by ion exchange at elevated temperatures followed by freeze-drying and storage in a desiccator over P_4O_{10}. Samples for measurements were prepared by dissolving dry carrageenan in hot solutions with the appropriate salt content. When preparing different ion forms of the same concentration, the amount of carrageenan was adjusted so as to give the same molar concentration of both ion forms, assuming ideal disaccharide molecular weights of the dry samples. The concentrations of comparable samples of the two ion forms thus prepared were checked by optical rotation and were found to differ by less than 5%. For convenience, carrageenan concentrations are given as a percentage (w/v) throughout.

Methods

A Bohlin (Lund, Sweden) VOR rheometer was used for the small-deformation oscillatory studies. Measurements of storage (G') and loss (G'') moduli were performed at four frequencies (0·2, 0·5, 1 and 2 Hz) at a strain of approximately 0·02. The following protocol was followed in all measurements. A hot solution of the sample to be studied was placed between the two coaxial cylinders of the measuring cell. The solution was covered with a thin layer of a low-viscosity silicon fluid, in order to prevent evaporation and/or formation of a surface film during the measurements. The temperature dependence was obtained by lowering the temperature stepwise, with measurements at each temperature, down to the lowest chosen temperature, after which a similar heating procedure followed. At each temperature, 10 minutes were allowed for equilibrium before measurements. All temperatures given are corrected for the small difference (1·5–3 °C, depending

on the temperature) between the temperature of the circulating water bath and the temperature measured in the cell in a control experiment. Occasional replicated measurements on freshly prepared samples showed that the reproducibility of the rheological data obtained for gel samples was within 10% for G' and within 2° for the phase angle (δ). The temperature dependence of the optical rotation was measured with a Jasco (Tokyo, Japan) DIP-360 polarimeter, where the temperature, which was controlled by the circulation of thermostatically regulated water through the jacketed 5-cm cell, was varied continuously at a rate of 0·33 °C min^{-1}.

RESULTS

General features

Figure 2 illustrates the typical features of the temperature dependences of G' and δ in aqueous iota-carrageenan. At high temperatures, the measured values are erratic (especially δ), a feature which is particularly evident when recordings at different frequencies are compared. This behavior we take as characteristic of solution conditions, where no accurate determination of elastic behavior could be made at the strain employed. At a certain temperature, however, the results from different frequencies merge, and below this temperature consistent trends in G' and δ are observed, with the elasticity of the system progressively increasing with decreasing temperature. This temperature, which separates the high-temperature solution region from the low-temperature region, will here be denoted as T_e, where the subscript signifies an onset of observable (under the conditions of the measurements) elastic behavior. At temperatures below T_e, the frequency dependence of G' or G'' is quite small and will be neglected in the following. The mechanical spectrum in this temperature region is characteristic of a gel (Fig. 3), and as the temperature is lowered, the gel-like character clearly increases. (While G' increases, G'' decreases with decreasing temperature.) For the majority of the samples (exceptions are given below), no significant difference between results obtained on cooling or on heating could be detected (cf. Fig. 2).

1% systems without added kappa-carrageenan

Figure 4 compares the rheology of the potassium and sodium forms of native iota-carrageenan, dissolved as 1% solutions in 0·1M of the respective chloride salts. The results for the two ion forms are quite similar. The only difference that can be detected is that the potassium curves are slightly displaced (by roughly 5 °C) towards higher temperatures. This result would be expected, however, since the temperature of onset of

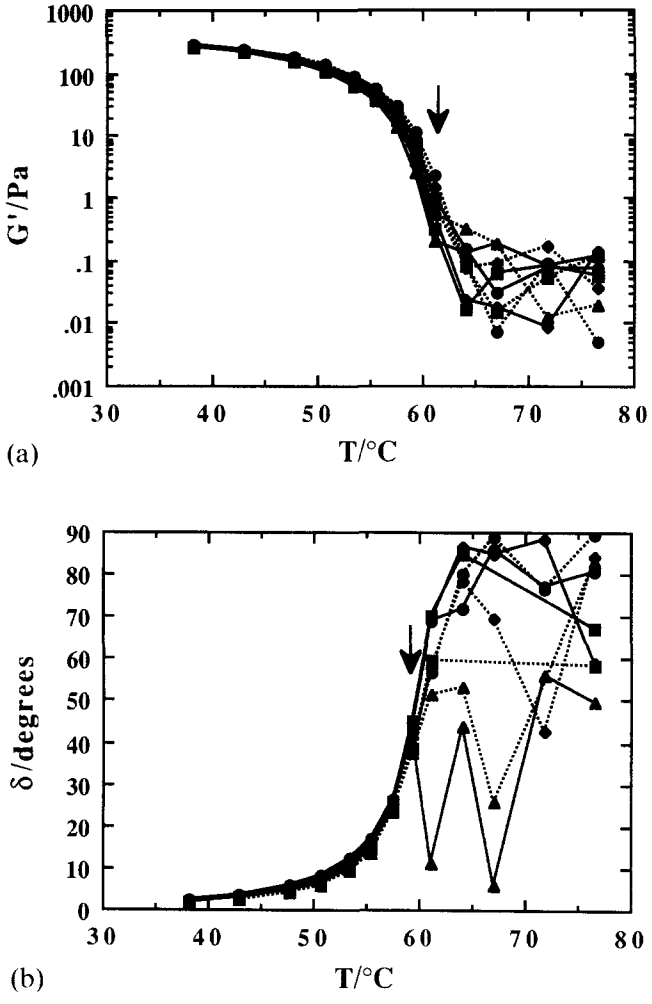

Fig. 2. Temperature dependence of shear storage modulus (a) and phase angle (b) of 2·5% purified sodium iota-carrageenan in 0·25M NaCl obtained on cooling (solid lines) and heating (dotted lines) at an oscillating frequency of 0·2 (▲), 0·5 (■), 1 (♦) or 2 (●) Hz. Arrows indicate T_e (see text).

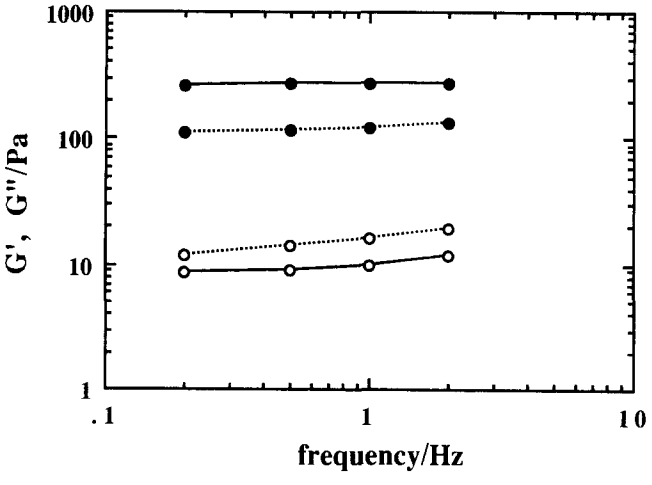

Fig. 3. Frequency dependences of shear storage (●) and loss (O) moduli at 38 °C (solid line) and 51 °C (dotted line) for the same system as in Fig. 2.

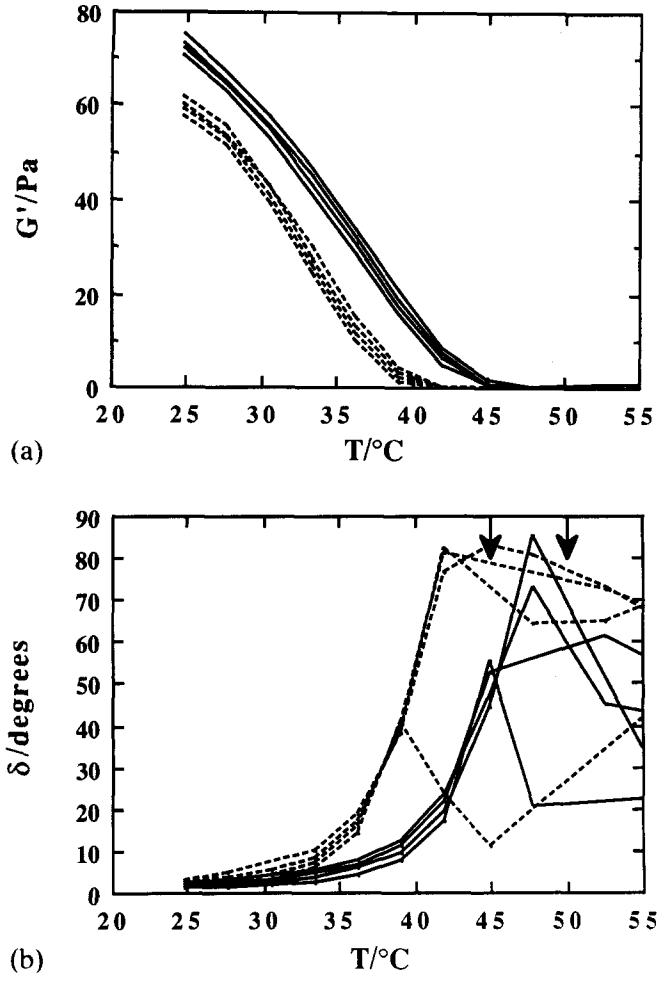

Fig. 4. Temperature dependence of shear storage modulus (a) and phase angle (b) at four frequencies for native 1% iota-carrageenan in 0·1M NaCl (dashed line) and KCl (solid line) solutions. Arrows indicate T_o (see text) for the sodium (left) and potassium (right) forms.

conformational order, T_o, differs by the same amount for the potassium and the sodium forms of iota-carrageenan. The values of T_o, as determined by optical rotation, for iota-carrageenan in 0·1M KCl or NaCl are displayed in Fig. 4(b), and it is clear that T_e is correlated with T_o. (As both T_e and T_o are operationally defined, their exact values depend on the methods of measurement. Therefore, it should not be expected that T_e and T_o are identical even in instances when both temperatures reflect the same molecular events.) Thus, the onset of elastic behavior may be attributed to an onset of conformational ordering of iota-carrageenan. This result agrees with the prevailing notion that helix formation is a prerequisite for gel formation in carrageenans. Below T_o, the fraction of the polymer in the ordered conformation increases progressively with decreasing temperature, and it seems likely that the increase in G' on lowering the temperature (Fig. 4(a)) may, at least in part, be attributed to this increase in the helical fraction.

1% systems with added kappa-carrageenan

To investigate the effects of small amounts of kappa-carrageenan on the rheology of the two ion forms of iota-carrageenan, the authors also made measurements on 1% samples of iota-carrageenan to which 0·05% or 0·10% of kappa-carrageenan had been added. The added kappa-carrageenan had little effect on the sodium form, as is evident from Fig. 5. T_e is unaffected, and, if anything, G' decreases slightly with the addition of kappa-carrageenan. The size of the latter effect is so small, however, that it is uncertain whether it is significant. In 0·1M NaCl, kappa-carrageenan is in the disordered conformation in the entire temperature interval investigated (Rochas & Rinaudo, 1980). Thus, a small fraction of kappa-carrageenan in the coil conformation has little or no effect on the rheology of the iota-carrageenan gel.

The situation is quite different for the potassium form of iota-carrageenan (Fig. 6). Most conspicuously,

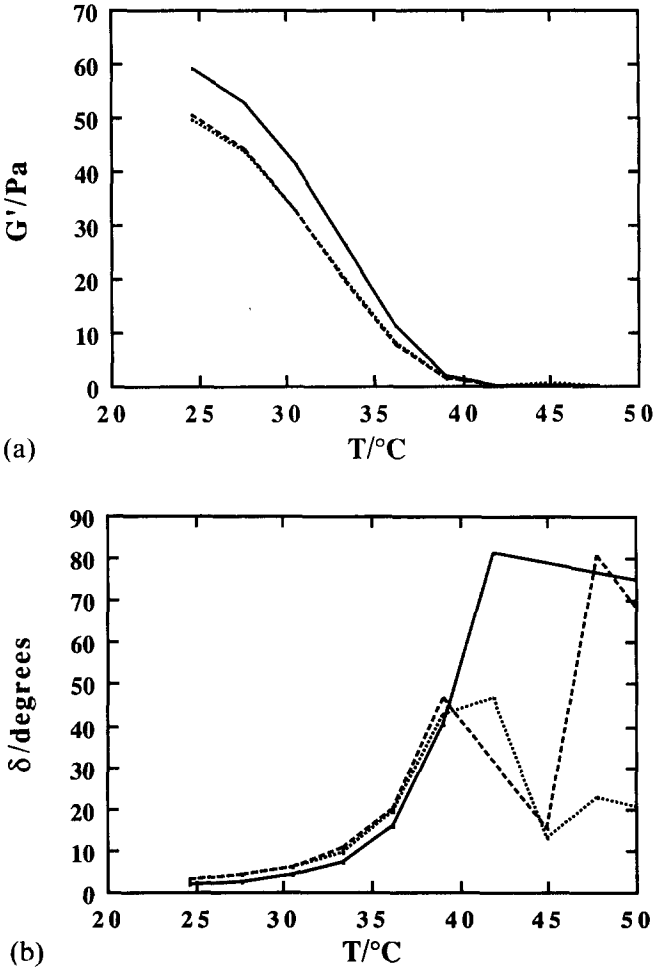

Fig. 5. Temperature dependence of shear storage modulus (a) and phase angle (b) for native 1% iota-carrageenan in 0·1M NaCl with 0 (solid lines), 0·05 (dashed lines) or 0·10% (dotted lines) added sodium kappa-carrageenan. For clarity, only results obtained at 0·5 Hz are shown.

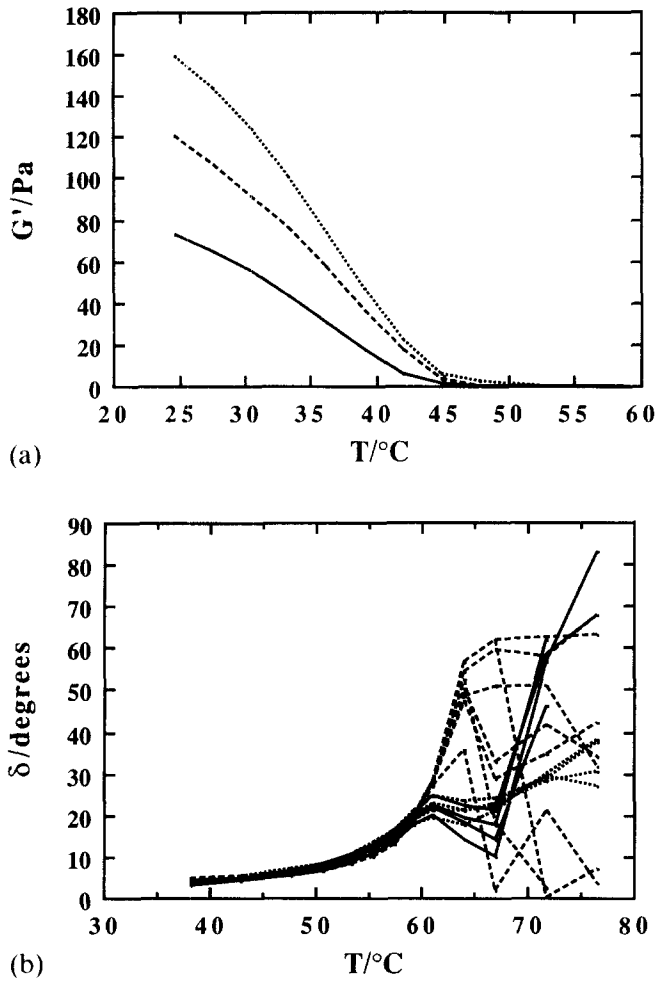

Fig. 6. Temperature dependence of shear storage modulus (a) and phase angle (b) for native 1% iota-carrageenan in 0·1M KCl with 0 (solid lines), 0·05 (dashed lines) or 0·10% (dotted lines) added sodium kappa-carrageenan. For clarity, only results obtained at 0·5 Hz are shown in (a).

the increase in G' which accompanies the progressive conformational transition of iota-carrageenan is significantly larger for samples which contain added kappa-carrageenan, and this effect increases with an increasing content of kappa-carrageenan. A further effect of added kappa-carrageenan is that T_e is shifted to slightly higher temperatures (Fig. 6(b)). The latter effect may be attributed to the onset of conformational order of the added kappa-carrageenan, since $T_{o,kappa} > T_{o,iota}$ in 0·1M KCl. $T_{o,kappa} = 53 \pm 2$ °C in 0·1M KCl (Piculell *et al.*, 1987), and T_e of the mixed system correlates well with this temperature.

It is of interest to investigate whether the enhanced G' of the mixed gels is dependent on the presence of kappa-carrageenan impurities in the native iota-carrageenan sample. Evidence suggests that at least some of the kappa-carrageenan residues are situated within the iota-carrageenan molecules (Bellion *et al.*, 1981; Piculell & Rochas, 1990). A mechanism could be envisaged whereby added kappa-carrageenan molecules

Table 1. Mechanical properties of carrageenan samples in 0·1M KCl at 25°C

Sample	G' (Pa)
1% iota (native) + 0·1% kappa	160
1% iota (purified) + 0·1% kappa	150
1% iota (native)	73
1% iota (purified)	35
1% kappa[a]	7 300

[a]Data from Nilsson and Muhrbeck (1992).

associate with kappa-carrageenan blocks in the iota-carrageenan molecules, thereby providing efficient cross-links. Such cross-links would presumably enhance the elastic modulus of the resulting gel. To check for the latter possibility, the authors also made measurements on enzymically purified iota-carrageenan with added kappa-carrageenan. The results (Table 1) were identical to those obtained with native iota-carrageenan. Thus, the enhancement of G' observed in Fig. 6(b) is not dependent on the (possible) presence of kappa/iota-carrageenan copolymers in the native sample.

2·5% systems

In view of the previous observations of cation-specificity in the rheology of native iota-carrageenan, the similarity of the results for the 1% potassium and sodium ion forms (Fig. 4) is somewhat surprising. Evidently, the kappa-carrageenan fraction in the native iota-carrageenan used here is too small to contribute measurably to the elastic modulus at a total concentration of 1%. The authors therefore made measurements on more concentrated samples, where the salt content was also increased, in order to increase the contribution to G' from the kappa-carrageenan impurities (Rochas & Landry, 1988). In these measurements, the authors compared the two ion forms (sodium and potassium), and also compared the enzymically purified iota-carrageenan with the native sample. Figure 7 summarizes the results. For the two ion forms of purified iota-carrageenan and for the sodium form of native iota-carrageenan the values of G' are quite similar at all temperatures, while they are distinctly higher for the potassium form of the native sample. The latter effect must, therefore, be due to the kappa-carrageenan fraction present in native iota-carrageenan.

A closer comparison of the results for the two potassium samples reveal some interesting details (Fig. 8). Plots of $\log(G')$ and of δ versus temperature show that for the native sample, T_e falls well above T_0 of 2·5% iota-carrageenan in 0·25M KCl ($T_{o,iota} = 66$ °C, as obtained by optical rotation). Furthermore, a distinct hysteresis in the rheological parameters is seen at $T > T_{o,iota}$. None of these features is evident in the results for the purified sample, where the solution

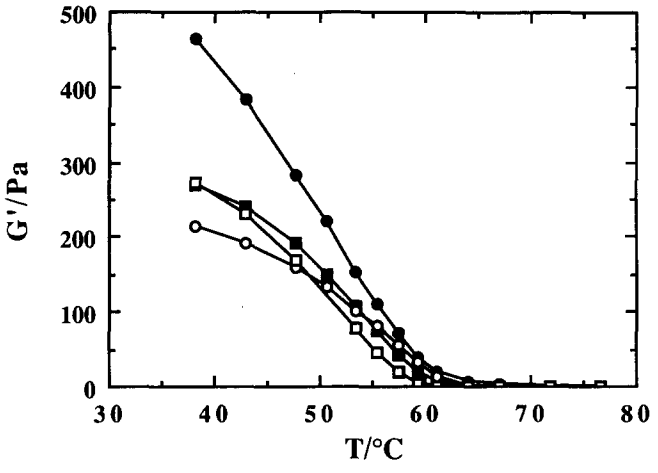

Fig. 7. Storage moduli for 2·5% native (closed symbols) or purified (open symbols) iota-carrageenan in 0·25M KCl (circles) or NaCl (squares).

behavior extends down to $T_{o,iota}$. These results imply that the hysteresis in the native sample is due to kappa-carrageenan impurities (optical rotation measurements showed that $T_{o,kappa}$ = 76°C for the kappa-carrageenan fraction), and that the enzymic purification successfully removes these impurities.

DISCUSSION

Rheology of iota-carrageenan

The above results demonstrate that for sufficiently pure systems there is no marked difference in the rheology of the sodium and potassium forms of iota-carrageenan. The small temperature shift observed is readily understood as a consequence of the small shift in the transition temperature. (The latter effect may be caused by small differences in the distance of closest approach between the cation and the sulfate group due to differences in the radii of the hydrated cations.) The onset of elastic behavior of aqueous iota-carrageenan closely follows the onset of helix formation, and the modulus of elasticity increases monotonically with an increasing helical content.

It is appropriate here to emphasize that the gelation of iota-carrageenan is not well understood. As far as the authors are aware, no attempt at a molecular explanation has been advanced since the so-called domain model (Morris *et al.*, 1980; Robinson *et al.*, 1980) was suggested more than a decade ago. The domain model, which was based on experimental observations of the conformational transition and gelation behavior of native iota-carrageenanan, explains the gelation of carrageenans in terms of a coil-to-helix transition followed by an aggregation of domains of carrageenan double helices, where the latter step was found to require the presence of gel-inducing ions. In

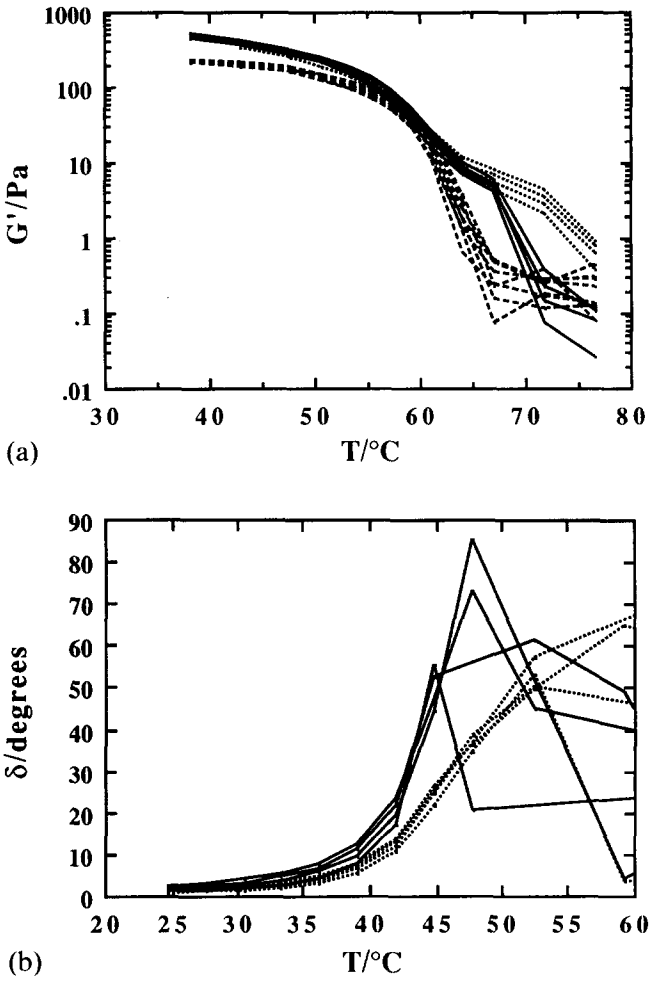

Fig. 8. Shear storage moduli (a) and phase angles (b) for 2·5% native (solid lines: cooling; dotted lines: heating) and purified (dashed lines: cooling and heating) iota-carrageenan in 0·25M KCl.

the light of the experimental evidence gathered since then, the domain model must be rejected for pure iota-carrageenan. Aqueous systems of pure iota-carrageenan display a reversible gelation process, where the absence of thermal hysteresis implies that the system is at *equilibrium* at each temperature. The latter feature, which is uncommon among biopolymer gels, should make the iota-carrageenan gel network attractive for theoretical modeling.

A model which would seem to be relevant for pure iota-carrageenan is that of Higgs and Ball (1989). They consider the equilibrium network formation in a system of pairwise interacting polymer chains, such as double-helix forming polymers. If the average length of a helical region is short compared to the average chain length, a chain may simultaneously participate in more than one helical region, whereby cross-linking is possible. However, as the helical content is made to increase (by, for example, lowering the temperature), the helical regions become longer and eventually, in the limit of infinite chains, a pairing transition is

reached, after which the helical fraction becomes unity. A consequence of the pairing transition is that the network is destroyed. Thus, the model predicts that the gel should only exist in a certain temperature interval: at high temperatures, the network is destroyed owing to coil formation, and, at sufficiently low temperatures, it is again destroyed because of the pairing transition. The latter effect is not seen in the authors' rheological data on iota-carrageenan. The authors can think of various possible reasons why this may be so. One possibility is that the network formation is really due to an association of helices, rather than to the helix formation itself. This explanation has been invoked to explain the formation of weak gels of xanthan, a system which has properties closely similar to those of iota-carrageenan in that a weak gel is formed with an absence of thermal hysteresis (Frangou *et al.*, 1982; Ross-Murphy *et al.*, 1983). Another possibility is the original proposition by Rees *et al.* (1969) that helix formation is interrupted by hetero-units ('kinks') present (at a small fraction) in the primary structure of iota-carrageenan. Further detailed studies of enzymically purified samples are needed to obtain a clearer picture of the gelation of iota-carrageenan.

Effects of kappa-carrageenan

From the results presented here, it may be concluded that small fractions of kappa-carrageenan give rise to a considerable enhancement of the gel strength of iota-carrageenan gels in the presence of cations (such as potassium) which specifically induce helix formation and aggregation of kappa-carrageenan. (These results agree with those of Rochas *et al.* (1989), who measured the dependence of Young's modulus on the composition of mixed kappa/iota-carrageenan gels in 0·25M KCl.) The effect is absent under conditions when kappa-carrageenan is in the coil conformation (NaCl solutions). At sufficiently high concentrations of carrageenan and KCl, the effect of kappa-carrageenan impurities is also seen for conventionally prepared iota-carrageenan without added kappa-carrageenan. Added kappa-carrageenan affects the rheology of enzymically purified and of native iota-carrageenan alike, so the effect is not caused by iota/kappa block-copolymers, which may be present in native iota-carrageenan.

Recently, two of the authors have performed rheological studies of kappa- and iota-carrageenan systems where increasing fractions of the polyelectrolyte poly(styrene sulfonate) (PSS) had been added (Nilsson & Muhrbeck, 1992). PSS was chosen as it should act as an 'inert' polyelectrolyte (the formation of mixed PSS–carrageenan complexes was considered unlikely). With the addition of a sufficient amount of PSS, a demixing was observed, confirming the absence of PSS–carrageenan aggregation. Before the phase separation occurred, however, the added PSS had little effect on the rheology of the gels formed. This resembles the authors' present finding that kappa-carrageenan in the coil conformation (NaCl systems) has no effect on the gel properties of iota-carrageenan. Most likely, the NaCl systems studied here are true one-phase systems, where the effect of the added kappa-carrageenan is merely that of a slight increase in the concentration of chains which are not incorporated into the network, together with an increase (here negligible) in the activity of the counterion.

For the gels of enhanced strength formed in the presence of ordered kappa-carrageenan (KCl systems), we have to consider three possible structures; *viz.* two interpenetrating independent networks in a one-phase mixture, an interconnected network involving association between unlike chains, and a phase-separated structure. In the first case, we would expect a mixed gel where the resulting modulus is simply the sum of the moduli of the two contributing networks. Such an additivity has indeed been found by Zhang and Rochas (1990) for Young's modulus in mixed gels of kappa-carrageenan and agarose. (Since the modulus of a gel based on a single biopolymer usually varies with (at least) the square of the polymer concentration (Clark & Ross-Murphy, 1985), additivity is not a trivial result, but really indicates that each polymer of the mixed gel forms its network as if the other polymer were not present. This situation might be expected for a dilute, one-phase mixture of two biopolymers.) For the present system, the authors were unable to check conclusively whether there was additivity or not, for two reasons. First, the measurements of G' on dilute (0·1%) kappa-carrageenan gels produced results which varied widely (between 3 and 80 Pa), depending on the exact conditions (cooling rate, geometry) of the measurements. The results of Moldenaers *et al.* (1988) suggest that these variations were due to syneresis (causing slippage at the wall), which we could also observe independently for these dilute kappa-carrageenan gels. Secondly, to check for additivity, we should know the *total* concentration of *helix-forming* kappa-carrageenan (added kappa-carrageenan + impurities) in the authors' native iota-carrageenan samples. An accurate value for the latter quantity is not readily obtained.

Information on the association and phase behavior of mixed carrageenans can be gained from independent experiments, however. Thus, in mixtures of kappa- and iota-carrageenan the general observation is that the conformational transition of each carrageenan type is unaffected by the presence of the other (Piculell *et al.*, 1987, 1989). This would not be expected in the case of formation of mixed aggregates, since such aggregates, if formed, should stabilize the helical conformation of iota-carrageenan, giving rise to an increase in T_0, and, possibly, a thermal hysteresis of the iota-carrageenan conformational transition. Experiments also show that, under aggregating conditions, aggregates of

kappa-carrageenan phase-separate from iota/kappa mixtures (Rochas *et al.*, 1989). This also speaks against the formation of mixed kappa/iota aggregates. Indeed, the observation by Rochas *et al.* (1989), that kappa-carrageenan phase-separates from iota-carrageenan in dilute systems already at 0·03M KCl, strongly suggests that a phase-separated (composite) gel is formed under the conditions of the present study.

Previous studies demonstrate a lack of universality in the rheology of composite gels. Dea *et al.* (1977) have reported that the elastic modulus of a gel of locust-bean gum formed in the presence of dextran and sucrose is an order of magnitude larger than the modulus of a corresponding gel with locust-bean gum alone. In contrast, the addition of gelatin to agarose (whereby a composite gel consisting of two gel-forming polymers is formed) initially results in an unexpected decrease in the elastic modulus (Clark *et al.*, 1983). For the PSS–carrageenan mixtures mentioned above, which represent mixtures of a non-gelling and a gelling polymer, the addition of PSS at concentrations high enough to induce phase separation could result in either an increase (kappa-carrageenan) or a decrease (iota-carrageenan) in the gel strength, compared to that of the corresponding pure carrageenan system (Nilsson & Muhrbeck, 1992).

In the context of polymer blends the mechanical properties of binary composites have been thoroughly studied (Manson & Sperling, 1976), and it is found that the properties depend not only on composition, but also on the detailed morphology of the composite. Recent attempts (Clark *et al.*, 1983; Ross-Murphy, 1984; Nilsson & Muhrbeck, 1992) to apply theories for composites to the elasticity of composite gels have started from the limiting Takayanagi models. The Takayanagi models provide upper (G'_u) and lower (G'_l) bound limits to G' for a two-component composite according to the expressions:

$$G'_u = \phi_A G'_A + \phi_B G'_B \quad (1)$$

and

$$(G'_l)^{-1} = \phi_A (G'_A)^{-1} + \phi_B (G'_B)^{-1} \quad (2)$$

Here, G'_A and G'_B are the moduli for the pure components A and B, respectively. Physically, the upper and lower limits correspond to the cases of uniform strain or uniform stress across the sample, respectively. It is expected (Manson & Sperling, 1976) that for a simple phase-separated structure, the two limits should correspond to different cases of connectivity of the system. Thus, the modulus of a system which is continuous in the stronger (weaker) phase but discontinuous in the weaker (stronger) phase is expected to approach the upper (lower) limiting behavior. A third equation, applicable for the intermediate case of a bicontinuous composite material (subscript bc), has been derived by Davies (1971):

$$(G'_{bc})^{1/5} = \phi_A (G'_A)^{1/5} + \phi_B (G'_B)^{1/5} \quad (3)$$

In the case of a composite two-polymer *gel* (which contains at least three components), G'_A and G'_B should be understood as the moduli of the two separating phases, both of which, in general, contain both polymers at different concentrations. Furthermore, the two phases generally differ in their total polymer concentration. An analysis of the composition of the separating phases in a composite gel, where no macroscopic phase separation occurs, is difficult indeed. Here, we will only use eqns (1)–(3) to make rough estimates of G'_u, G'_l and G'_{bc} in composite gels of iota- and kappa-carrageenan. To this end, we will make the simplifying assumptions that each of the two separating phases contains only one of the carrageenan types, and that the polysaccharide concentration is the same (i.e. equal to the average total polysaccharide concentration) in both phases. The volume fractions of the two phases then become equal to the molar fractions of the respective polymers.

For the calculation of the moduli of the respective phases, we use the experimental values obtained for pure kappa- or iota-carrageenan gels (Table 1 gives values in 0·1M KCl), scaled to the appropriate total concentration using the relation $G' \propto c^2$ (c is the concentration of carrageenan), which, to a very good approximation, is obeyed by kappa- and iota-carrageenan gels (Rochas & Landry, 1988; Rochas *et al.*, 1989) in the concentration range considered. The moduli thus calculated for the 1% samples in 0·1M KCl are given in Table 2, and it is seen that the experimentally obtained moduli fall in between the expected upper and lower limits for composite iota/kappa-carrageenan gels. It is noteworthy that a good agreement is found with the prediction for a bicontinuous structure. However, given the uncertainties in the volumes and moduli of the respective phases, not too much emphasis should be put on the exact numbers of the calculated data of Table 2.

A similar calculation of the expected enhancement of G' in the 2·5% native sample in 0·25M KCl would require a knowledge of its content of kappa-carrageenan impurities which are located in sequences long enough to form helices. Lacking precise knowledge of the latter quantity, we will here, instead, reverse the calculation and ask what proportion of such kappa-carrageenan impurities would be needed in order to produce the

Table 2. Experimental and calculated (see text) shear storage moduli for 1% native iota-carrageenan with added kappa-carrageenan in 0·1M KCl at 25°C

C_{kappa} (%)	G'_{exp} (Pa)	G'_u (Pa)	G'_{bc} (Pa)	G'_l (Pa)
0·05	121	460	114	84
0·10	160	883	168	97

observed effects. With the same assumptions as before, and with G' = 130 kPa for 2·5% kappa-carrageenan in 0·25M KCl (from the data of Rochas and Landry (1988), using $E = 3G'$) we calculate that, for a bicontinuous gel (eqn (3)), a fraction of 5% of helix-forming kappa-carrageenan impurities would be needed to yield an enhancement of the gel strength from 300 to 650 Pa, which, based on a rough extrapolation of the curves in Fig. 7, should correspond to the effect at room temperature. This fraction of kappa-carrageenan is of the expected order of magnitude. The rough calculation just presented is, of course, merely a consistency test; it should not be used for a quantitative estimate of the fraction of kappa-carrageenan impurities (which, strictly, should also be included in the kappa-carrageenan fraction for the calculations in Table 2).

For the 1% samples, no effect is seen from the kappa-carrageenan impurities in native iota-carrageenan alone, although a clear effect is seen already with 0·05% added kappa-carrageenan *or* when the concentration of native iota-carrageenan is increased sufficiently. Evidently, some threshold global concentration of kappa-carrageenan (possibly dependent on salt concentration) must be exceeded for a strong effect to occur. The authors suggest that this threshold corresponds to the concentration required for the formation of a continuous kappa-carrageenan gel phase, so that the composite kappa/iota gel becomes bicontinuous. Below the threshold concentration, the kappa-carrageenan phase may only form isolated 'droplets'. In the latter case, isostress behavior is expected and, hence (eqn (2)), kappa-carrageenan should have a negligible effect on the gel strength. If we accept that the fraction of helix-forming impurities in native iota-carrageenan is close to 5%, we conclude that the threshold concentration is somewhere in the interval 0·05–0·1% under the conditions of Fig. 6. In the 2·5% system in 0·25M KCl, the same estimate indicates that the concentration of helix-forming kappa-carrageenan is of the order of 0·13%, which means that it is reasonable to assume that the threshold concentration is reached.

Within the authors' preferred interpretation of the mixed kappa/iota gels in terms of a composite gel structure, the observation that the effect on G' of added kappa-carrageenan increases with an increasing helical content of iota-carrageenan deserves some comment. First, we may note that the moduli of both the separating phases, i.e. G'_{iota} and G'_{kappa}, should increase with decreasing temperature, since the elastic modulus of a kappa-carrageenan gel increases significantly with decreasing temperature even after the conformational transition is complete (Zhang & Rochas, 1990). Secondly, if eqn (3) holds, a non-linear enhancement of the gel strength due to the kappa-carrageenan network is predicted even with G'_{kappa} independent of temperature.

A consistent rationalization of the results obtained here in terms of a composite structure of kappa/iota gels thus seems possible. A more quantitative theoretical prediction seems to be out of reach at present, however. The gels studied here are the results of a complex scheme of events: as the temperature is lowered, kappa helices and aggregates are first formed in a solution of iota-carrageenan coils. Presumably, a phase separation occurs already at this stage. At still lower temperatures, iota-carrageenan begins to form helices, which should affect the phase equilibrium. However, because of the network formation of kappa-carrageenan and iota-carrageenan, it is uncertain to what extent the compositions of the separating phases, once formed, are able to change. Therefore, it is possible that the domains which constitute the composite gel are not the true equilibrium phases, but something rather more ill-defined. Besides creating a complex morphology, this should further complicate the assignment of correct values to the parameters ϕ_a and G'_a which appear in eqns (1)–(3) as well as in more complicated expressions derived to describe the elasticity of composite materials (Manson & Sperling, 1976).

It is important to distinguish the bicontinuous gel structures discussed here, where two continuous *phases* are formed in a composite gel, from the one-phase mixture of interpenetrating networks mentioned earlier. The latter situation arises when there is no demixing of the two network-forming polymers. It is interesting to note that, in general, an aqueous mixture of an uncharged polymer and a polyelectrolyte (cf. agarose and kappa-carrageenan, which seem to form independent networks according to the study by Zhang and Rochas (1990)) has a low tendency towards demixing (Perrau *et al.*, 1989), whereas in a mixture of two similarly charged polyelectrolytes (cf. kappa- and iota-carrageenan) the latter tendency is much stronger (Piculell *et al.*, 1991).

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