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Rheology of kappa-carrageenan in mixtures of sodium and cesium iodide: two types of gels

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Recent studies on dilute solutions (Borgström *et al.* (1996), *Int. J. Biol. Macromol.* **18**, 223) have shown that kappa-carrageenan helices associate into superhelical rigid rods in mixed 0.1 M aqueous solutions of NaI and CsI above a critical mole fraction ($x_{Cs}=0.4$) of Cs. This work concerns the temperature-dependent rheology of more concentrated systems in mixed and pure solutions of the same salts. Gels with low moduli were even found in NaI alone, although this salt is known to impede the gelation of kappa-carrageenan, but only above 0.9% (w/w) of carrageenan. These gels were reminiscent of iota-carrageenan gels in two respects: the (low) magnitude of the shear storage modulus (G'), and the absence of hysteresis in the sol–gel transition. On the other hand, both the threshold concentration for gelation and the ratio between the loss and storage moduli were substantially higher for the kappa-carrageenan gels in NaI. In mixed solutions of CsI and NaI, two types of kappa-carrageenan gels could be distinguished, depending on the cesium content. The transition occurred at $x_{Cs}=0.4$, as in the previous studies on dilute solutions. Below $x_{Cs}=0.4$, the gels were similar to those in NaI alone. Above $x_{Cs}=0.4$, the gels were similar to 'conventional' kappa-carrageenan gels, formed in salts such as KCl: a pronounced thermal hysteresis appeared in the sol–gel transition, the gels showed tendencies for syneresis, and G' increased dramatically with increasing cesium content. © 1997 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The carrageenans are a family of polymeric sulfated galactans extracted from various species of red seaweed (i.e. *Eucheuma cottoni, Eucheuma spinosum, Gigartina acicularis*) (Rees *et al.*, 1982; Painter, 1983; Piculell, 1995). The gel-forming members have a linear primary structure based on a repeating disaccharide of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-D-galactopyranose residues. Two main types, with one or two half-ester sulfate groups per disaccharide units, can be distinguished and are referred to as kappa- and iota-carrageenan, respectively. Both types are widely employed in the food industry as gelling agents, since they form thermoreversible gels under certain conditions, strongly influenced by the type and quantity of ions present.

The essential step in the carrageenan gelation is a coil-to-helix transition, induced by cooling a hot solution under the appropriate ionic conditions (Anderson *et al.*, 1969; Rees *et al.*, 1969; see also the reviews by Rees *et al.*, 1982 and Piculell, 1995). Accumulated evidence from a variety of experimental techniques (Rees *et al.*, 1982; Piculell, 1995) indicates that the ordered structure adopted, in solution as well as in the solid state, is a double helix. The bimolecular solution structure was very recently confirmed by multi-angle laser light-scattering following gel permeation chromatography of a number of iota- and kappa-carrageenan samples of varying molecular weight (Viebke *et al.*, 1995). However, despite considerable research efforts, the molecular mechanisms by which the helical carrageenans may form infinite three-dimensional networks, as required for gelation, have not been satisfactorily established. There are also notable differences in the gelation of kappa- and iota-carrageenan. Kappa-carrageenan gels are typically strong and brittle, and a characteristic thermal hysteresis is seen in the setting–melting behavior, the gel setting temperature being lower than the melting temperature. In contrast, iota-carrageenan forms much weaker gels with no thermal hysteresis in the solution–gel transition.

Earlier, it was proposed that an essential part of carrageenan gelation was a network formation on the

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helical level, such that each active carrageenan chain joined in double-helical junctions with two or more other chains (Anderson *et al.*, 1969; Rees & Welsh, 1977). This attractive model would also seem to be supported by the general observation that gelation, in the normal cold-setting procedure, occurs simultaneously (or very nearly so) with the helix formation. However, it was recently shown (Viebke *et al.*, 1994) that the two processes, helix formation and gelation, could be separated for kappa-carrageenan. Thus a non-gelling solution of helical molecules of kappa-carrageenan could be transformed, isothermally and reversibly, into a gel by dialysis against an appropriate salt solution, under such conditions that the carrageenan never passed the coil state. In the same work, analyzes of the coil–helix transition of kappa-carrageenan using coil–helix transition theory indicated that the occurrence of two independent helical sections on the same carrageenan molecule (as would be necessary for cross-linking, on the helical level) was very unlikely, owing to the high cooperativity of the helix formation. These results suggested that the network formation and gelation of kappa-carrageenan occurred essentially on the super-helical level, through the association of helices.

It thus seems essential to obtain more detailed knowledge about helix–helix interactions and helix association processes for the carrageenans, in order to understand more about their gelation. Since gels are formed by many other helical polymers (and not only biopolymers; see Miller *et al.*, 1985; Berghmans *et al.*, 1987), such knowledge is also of more general interest. Kappa-carrageenan remains a particularly interesting model system, since the helix formation and association processes may be controlled by utilizing the specific ion-binding properties of the kappa-carrageenan helix. Thus, 'specific' cations such as K^+, Cs^+ and Rb^+ bind to the kappa-carrageenan double helix, thereby increasing the stability of the helix, promoting a subsequent helix aggregation, and increasing the gelling ability (Rochas & Rinaudo, 1980; Grasdalen & Smidsrød, 1981a; Belton *et al.*, 1985; Piculell *et al.*, 1989; Nilsson & Piculell, 1991). Moreover, certain anions also specifically affect the conformational transition and helix aggregation of kappa-carrageenan. Grasdalen and Smidsrød (1981b) first noticed that iodide ions stabilize the kappa-carrageenan helix, but impede aggregation and gelation. Subsequent studies (Smidsrød & Grasdalen, 1984; Norton *et al.*, 1984) have shown that anions stabilize the ordered conformation in the order $F < Cl < NO_3 < Br < I$, with particularly strong effects seen for the latter two ions. NMR studies have shown that both SCN and I bind to the helix (Grasdalen and Smidsrød, 1981b; Zhang *et al.*, 1992; Zhang & Furó, 1993) and both a stabilization of the helix and a destabilization of super-helical aggregates are expected consequences of such binding. It is notable that neither a site-binding of ions nor a pronounced ion specificity has been observed for iota-carrageenan free of kappa-carrageenan impurities (Piculell *et al.*, 1987; 1992; Piculell & Rochas, 1990; Parker, 1995).

In our laboratory, we have recently used mixtures of gel-promoting cations and gel-impeding anions to fine-tune the helix–helix interactions of kappa-carrageenan, and to further resolve the stages of helix association (Borgström *et al.*, 1996a). We studied dilute solutions (0.1–0.5% w/w) of helical kappa-carrageenan in 0.1 M mixtures of NaI and CsI by cryo-transmission electron microscopy, optical rotation and capillary viscometry. The mole fraction of cesium salt, $x_{Cs} = [CsI]/([CsI] + [NaI])$, was varied between zero and unity. All data gave consistent evidence of the appearance of superhelical aggregates above $x_{Cs} = 0.4$, whereas, below this critical cesium content, there was no evidence of aggregates larger than the individual double helices. The superhelical aggregates were visible as very rigid rods in the electron micrographs and their appearance coincided with the onset of a thermal hysteresis in the conformational transition, as monitored by optical rotation. As the cesium content was increased beyond $x_{Cs} = 0.4$, the super-helical rods started to aggregate into bundles, according to the electron micrographs. This resulted in a continuous increase in the viscosity, whereas the extent of the thermal hysteresis remained roughly constant, suggesting that the thermal hysteresis was mainly due to the rigid rods, whereas the continuous increase in viscosity was largely due to rod–rod aggregation.

The purpose of the present study is to investigate whether the various levels of association of kappa-carrageenan helices also manifest themselves in the rheology of more concentrated systems. As will emerge below, this is indeed the case. In the course of our studies, we also found that kappa-carrageenan may even form gels in pure NaI, albeit at higher concentrations than those required with 'conventional' salts. As we know of no previous study of kappa-carrageenan gels in NaI, we have investigated their rheology at varying concentrations of kappa-carrageenan and salt here.

EXPERIMENTAL

Materials

Kappa- and iota-carrageenans were kindly donated by SBI (France). Sodium salt forms were prepared by cation exchange on a Dowex-50W strong acid cation exchange resin (Sigma). The resin was first converted to the H^+ form by elution with HCl and then to the sodium form by using NaCl. Ion exchange of 1.59% (w/w) polysaccharide solutions was carried out at 95°C to maintain the polymer in the coil form. The resultant samples were then freeze-dried and stored. To check for possible degradation, the storage moduli of the samples were checked before and after the ion exchange, and no substantial difference was observed. The carrageenans were dissolved in the desired aqueous salt solutions (NaI and/or CsI, AnalaR grade from Sigma) by heating at 95°C for 20 min while stirring. Millipore® water was used throughout.

Methods

Rheological measurements under low amplitude oscillatory shear were performed on a controlled stress Carri-Med CSL 100 rheometer (TA Instruments, UK) using a parallel plate geometry (40 mm diameter; 1 mm separation). To avoid slippage (see below), a cross-hatched acrylic upper plate (40 mm diameter. 0.5 mm separation) was used above $x_{\mathrm{Cs}}=0.45$. All measurements were performed with 0.5% strain, since strain sweeps on a few selected gels demonstrated that the working deformation was well within the linear viscoelastic region. The hot solutions were loaded on the platen of the rheometer pre-set at 80°C. The periphery of the samples was coated with silicone oil to minimize loss of solvent or absorption of atmospheric moisture. The storage modulus (G'), the loss modulus (G''), G''/G' ($\tan\delta$) and the complex viscosity (η^*) were monitored as functions of temperature, time and frequency using the following procedure. A cooling scan (1 deg/min) to 20°C was followed by a 30 min isothermal run and a frequency sweep between 0.01 and 10 Hz. The measurement routine was then completed by a heating run from 20 to 80°C (1 deg/min).

RESULTS AND DISCUSSIONS

Gels in NaI alone

As mentioned in the Introduction, we found that kappa-carrageenan forms gels with low moduli even in the presence of pure NaI—conditions that are normally considered to prevent gelation, if the carrageenan concentration is sufficiently high. (Note that gels were only found for non-degraded kappa-carrageenan. With ultrasonically degraded kappa-carrageenan, we have consistently obtained liquid samples that, at sufficiently high concentrations, phase-separate macroscopically into one isotropic and one chiral nematic liquid crystalline phase (Borgström *et al.*, 1996b). We have not obtained the latter type of phase separation for non-degraded kappa-carrageenan.)

Figure 1a shows the thermal variations in the small-deformation moduli observed on cooling and heating 1.5% w/w kappa-carrageenan (sodium form) in 0.1 M NaI. On cooling, both $G'$and G'' increased rapidly around 40°C after which a slower, further increase or a levelling off, respectively, was seen. An identical spectrum was obtained on heating with no thermal hysteresis. The latter result is consistent with the absence of hysteresis in the conformational transition which has previously been noted for kappa-carrageenan under similar salt conditions (Grasdalen and Smidsrød, 1981b; Norton *et al.*, 1984; Goycoolea *et al.*, 1995). A subsequent time sweep at 20°C (Fig. 1b) shows a relatively weak modulus with no time dependence. Figure 1c illustrates the response of the same sample to increasing the small amplitude angular frequency (ω) at 20°C. A gel-like response (G' substantially higher than G'') is obtained, with only a slight frequency dependence of the moduli. The value of $\tan\delta$ reveals a substantial viscous component ($\tan\delta=0.22$ at 1.6 Hz) compared to other biopolymer gels (gelatin and agarose, where $\tan\delta$ varies from 0.03 to 0.07). The logarithmic power law dependence of the complex dynamic viscosity, η^*, vs frequency is essentially linear, with no indication of a levelling off to a horizontal 'Newtonian plateau' at low frequencies. The slope is −0.88, a value in between the limiting value of −1 expected for purely elastic gels and the maximum value of −0.76 observed for polysaccharides coils interacting by topological entanglements (Morris, 1990a). Finally, there is also a significant linear region of the amplitude of oscillation (at 1.6 Hz, 20°C) up to a strain of ~80% (data not shown). This response is similar to that of other biopolymer gels.

The minimum amount of polymer required for the formation of an infinite network, with experimentally detectable gel-like properties, was about 0.9% w/w in 0.1 M NaI. This value is much higher than the 'typical' critical gelling concentration of kappa-carrageenan (~0.1% w/w; Rochas & Landry, 1988) in the presence of gel-promoting cations. On the other hand, it is comparable to values obtained for certain other biopolymers, such as gelatin, where the critical gelling concentration is around 1% w/w (Clark *et al.*, 1983). The concentration dependence (above the critical gelling concentration) of G' (Fig. 2a) follows the simple empirical formula $G'=Kc^n$ (c is the concentration and K and n are constants) frequently found for elastic gels. In the investigated concentration range (1–5% w/w) we find $n=2.6$ for kappa-carrageenan gels in 0.1 M NaI. For similar temperatures and salt concentrations, but in KCl salt Rochas & Landry (1988), using large deformation measurements and Braudo *et al.* (1984) found $n\approx 2$ and 2.4, respectively. Watase & Nishinari (1982) found $n\approx 2$ at concentrations above ~4% for kappa-carrageenan in various salts, whereas a larger exponent was found at lower concentrations.

Two features of the rheology of kappa-carrageenan gels in NaI, i.e. the low value of the storage modulus and the absence of thermal hysteresis, are reminiscent of those found for iota-carrageenan free of kappa-carra-

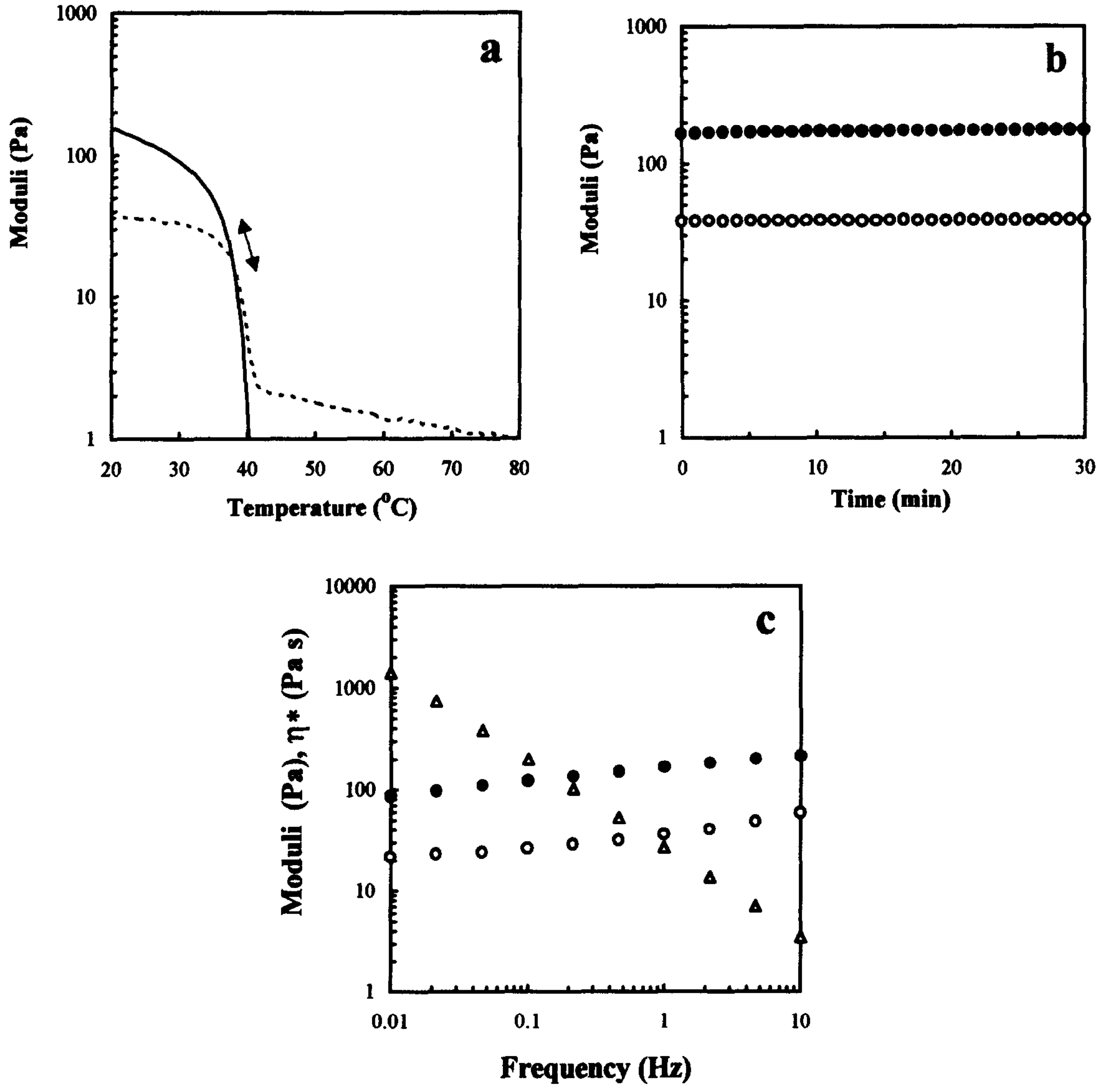

Fig. 1. Rheology of 1.5% sodium kappa-carrageenan in 0.1 M NaI. (a) Temperature dependence of G'(———) and G''(- - -) on cooling and heating (1 deg/min, 1.6 Hz, 0.5% strain); (b) time sweep of G' (●) and G''" (○) at 20°C (1.6 Hz, 0.5% strain); (c) frequency dependence of G'(●), G'' (○) and η^* (△) at 20°C.

geenan impurities. To closer examine this point, we made some comparative measurements on iota-carrageenan under the same gelling and salt conditions. (Note that in the absence of kappa-carrageenan impurities, the rheology of iota-carrageenan is largely insensitive to the identity of the monovalent salt: Piculell *et al.*, 1992; Parker, 1995.) Typical sharp transitions, like that shown for kappa-carrageenan in Fig. 1a, were found, but at higher temperatures (> 50°C) as expected for iota-carrageenan in 0.1 M salt (Piculell *et al.*, 1987; 1992). The melting scans followed an identical route, with no hysteresis. At low concentrations, slightly stronger networks were formed for iota-carrageenan as compared to kappa-carrageenan (Fig. 2a), while the reverse was observed at higher concentrations (above ~1.5%). This means that the exponent in the formula $G' = KC^n$ had a significantly lower value of $n = 1.81$ for iota-carrageenan. This value is comparable to, but slightly lower than, the value $n = 1.97$ reported by Rochas *et al.*, 1989 for iota-carrageenan in KCl.

Although kappa-carrageenan gels in NaI display some striking similarities with iota-carrageenan gels, our comparisons also revealed some notable differences. The minimum gelling concentration was much lower (about 0.1% w/w) for iota-carrageenan. This is not due to differences in the molecular weight, since these were very similar (~300 000 for both samples; Viebke *et al.*, 1995). Moreover, the viscous component was, at all concentrations, significantly larger for the kappa-carrageenan gels than for iota-carrageenan, as is shown by the tanδ values in Fig. 2b. At this stage, we will not speculate on the molecular origin of these differences.

For both carrageenan types, the gel–sol transition temperatures increased with increasing polymer concentration (Fig. 3). In Fig. 3, as in the following, we have chosen to characterize the transition by the

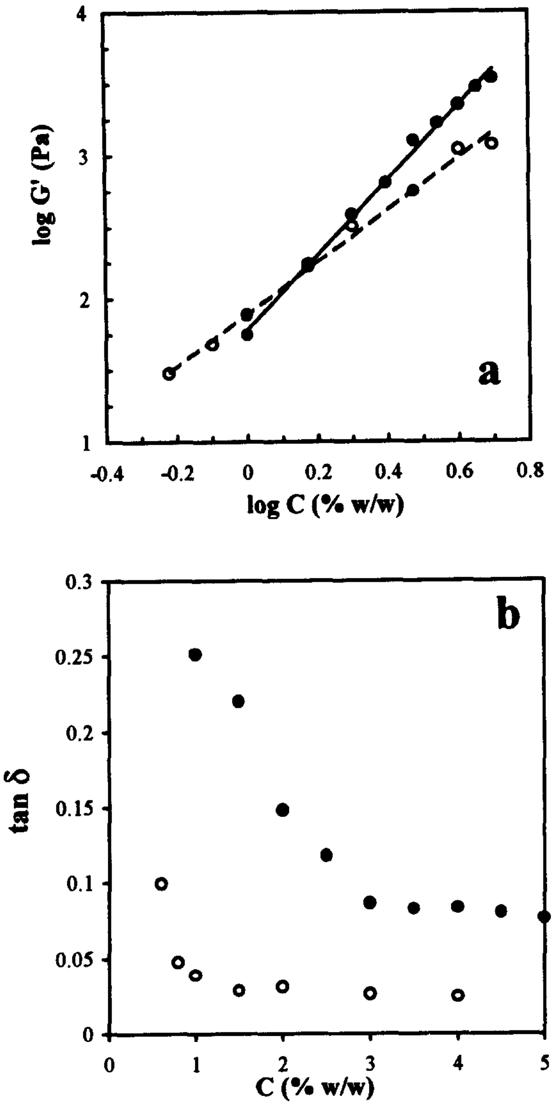

Fig. 2. Concentration dependence of: (a) G' (at 20°C, 1.6 Hz, 0.5% strain at the end of 30 min isothermal run); and (b) tanδ (from frequency sweeps after the isothermal run) for sodium kappa-(●) and iota-(○) carrageenan in 0.1 M NaI.

temperature where G' just exceeds the noise in our measurements. In general, the transition onset temperature on cooling, T_o, may be lower than the corresponding temperature of total loss of a measurable storage modulus on heating, T_c; however, $T_o = T_c$, for the perfectly thermoreversible transitions in pure NaI. To a large extent, the variation seen in Fig. 3 should be due to the increased stability of the helix conformation at increasing carrageenan concentration, due to the decreasing cost in electrostatic free energy for making helices (Nilsson *et al.*, 1989; Piculell & Nilsson, 1990). This also explains why the variation is stronger for the more highly charged iota-carrageenan.

Note that the transition temperatures defined here should not be regarded as 'gel points', signifying the appearance/disappearance of an infinite carrageenan network (Winter, 1987; te Nijenhuis & Winter, 1989; Cuvelier *et al.*, 1989; Michon *et al.*, 1993). Our concern, here, is not to determine such gel points, but rather to monitor those thermal shifts of the sol–gel transition that follow changes in the content of carrageenan or salt. The transition temperatures, T_o and T_c, are well suited for such purposes, since they may be obtained with good accuracy, experimentally. The variation of G', with temperature, is quite steep at these temperatures (Figs 1a, 6, 10, 11 and 12).

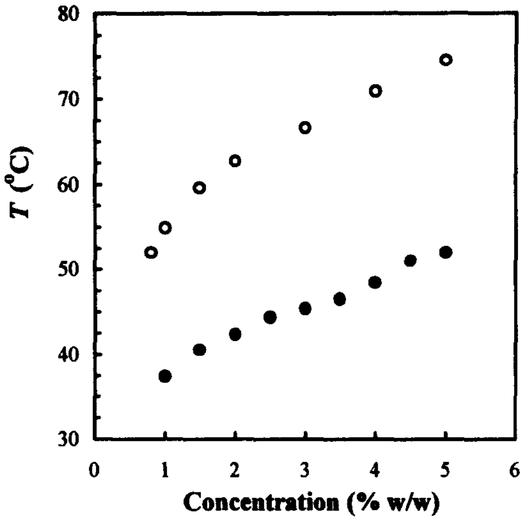

Fig. 3. Concentration dependence of the transition onset temperatures (see text) for sodium kappa-carrageenan (●) and iota-carrageenan (○) in 0.1 M NaI (1 deg/min, 1.6 Hz, 0.5% strain).

We also studied the variation of the storage modulus vith increasing concentration of NaI for gels with a fixed concentration (1.5%) of kappa-carrageenan (Fig. 4). The network rigidity increased with increasing salt concentration up to a maximum of 0.2 M, then it decreased gradually to level off above ~0.5 M NaI. Similar patterns, with maxima around 0.2–0.4 M of salt,

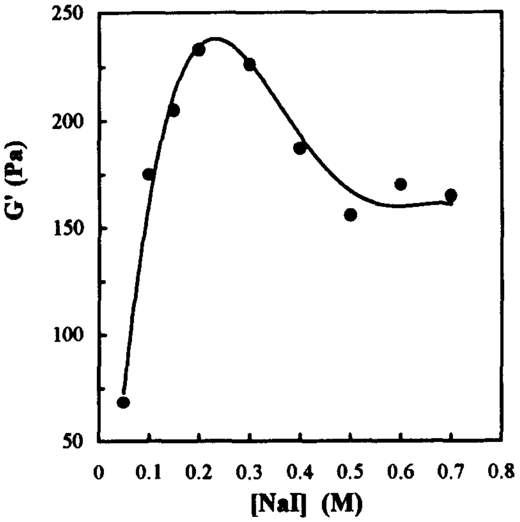

Fig. 4. Modulus of 1.5% (w/w) sodium kappa-carrageenan after 30 min at 20°C vs NaI concentration (conditions as in Fig. 3).

have been reported in previous studies dealing with other ions that promote the coil-to-helix transition (Watase & Nishinari, 1981; Watase & Nishinari, 1982; Watase *et al.*, 1990a, b). However, the origin of these effects does not seem to be well understood. In principle, the effect could be caused either by an increase or a decrease in the helix–helix association, since both mechanisms may eventually lead to a destruction of the infinite cohesive gel network. Too much association will cause the gel to collapse into a suspension of disconnected microgel particles, while too little association will give a true solution of disconnected polymer molecules. With NaI, in particular, it is difficult to decide which mechanism is the dominating one. The screening effect of the added salt is expected to facilitate association of a polyelectrolyte like carrageenan. On the other hand, both the binding of iodide to the carrageenan helix and the more general Hofmeister effect of the iodide ion (von Hippel & Schleich, 1969; Piculell & Nilsson, 1990) should give the opposite effect.

Samples with NaI concentrations larger than 0.7 M could not be investigated, because of their very high transition temperatures. Figure 5 shows the increase in T_o, with increasing NaI concentration. At high concentration, the expected (Rochas & Rinaudo, 1980; Nilsson *et al.*, 1989; Zhang *et al.*, 1991) linear variation of T_o, with the logarithm of the concentration of added salt is seen here according to the relation $T_o = 97 + 58 \log (c_{NaI})$. At low salt concentration the dependence is weaker, and it is evident that the contribution from the counterions of the kappa-carrageenan is not negligible.

Gels in 0.1 M mixtures of NaI and CsI

Having investigated the properties of the kappa-carrageenan gels in pure NaI, we proceeded to study gels in mixed solutions of CsI and NaI at fixed concentrations of total salt (0.1 M) and carrageenan (1.5%). Figure 6 shows the variation of G' and G'' during, cooling and heating for two representative samples with $x_{Cs} = 0.22$ and 0.9, respectively. At the lower cesium content (Fig. 6a), the storage modulus of the gel is comparable to that observed in pure NaI and there is no detectable thermal hysteresis. In contrast, a much higher G' was observed at the higher cesium content (Fig. 6b), and there was also a strong thermal hysteresis in the gel setting–melting behavior. These features are both typical for 'conventional' kappa-carrageenan gels formed in the KCl or CsCl. Moreover, the gels formed at high cesium content were brittle and showed syneresis, causing an observed 'slippage' transition when measured with smooth plates. A cross-hatched upper plate was, therefore, used for measurements of these samples. This point is discussed further below.

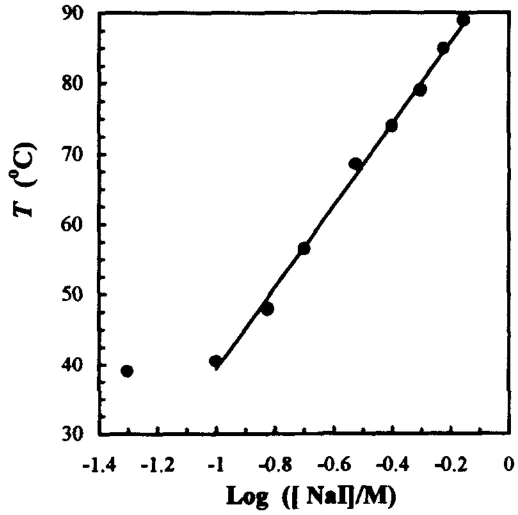

Fig. 5. Transition temperatures of 1.5% (w/w) sodium kappa-carrageenan gels vs NaI concentration (conditions as in Fig. 3).

The mechanical properties of both types of gels (low and high cesium content) are typically gel-like (Fig. 6c, d). The elastic response (G') is substantially higher than the viscous flow (G'') and both moduli show only a slight variation with frequency. The slopes of log η^* vs log ω are linear and have values of −0.88 and −0.98 for x_{Cs}, 0.2 and 0.9, respectively. The variation in tanδ with the cesium content is displayed in Fig. 7. At low cesium contents, the gels display a comparatively high tanδ. As the cesium fraction increases, tanδ progressively decreases to approach an almost constant value (similar to that of conventional carrageenan gels) at an excess of the gel-promoting ion.

Figure 8 illustrates the variation of the transition temperatures, T_o and T_c, as functions of the cesium content. The transition onset temperature on cooling, increases smoothly as a function of x_{Cs} over the whole range of salt compositions. This smooth variation supports our implicit assumption that the measured onset temperature reflects the same molecular event, i.e. a transition to the same helical conformation, at all salt compositions. In contrast, there is a change in the behavior of T_c around $x_{Cs} = 0.4$. Above this value $T_c > T_o$, whereas the two transition temperatures are the same, within experimental accuracy, at lower cesium contents. The data in Fig. 8 are in excellent agreement with the variation in the helix formation and melting behavior observed by optical rotation for dilute solutions under the same salt conditions (Borgström *et al.*, 1996a), confirming previous observations (Piculell, 1995) that the onset/disappearance of a measurable elasticity in gelling samples (well above the critical-elation concentration) practically coincides with the onset/disappearance of the helical conformation.

The salt-composition dependence of G' shown in Fig. 9 gives further clear evidence that important changes in the gels occur around $x_{Cs} = 0.4$. Above this value, G' increases dramatically with cesium content, giving at $x_{Cs} = 1$ a gel that is nearly 60 times stronger than at $x_{Cs} = 0.4$. There is also a slight variation of G' below

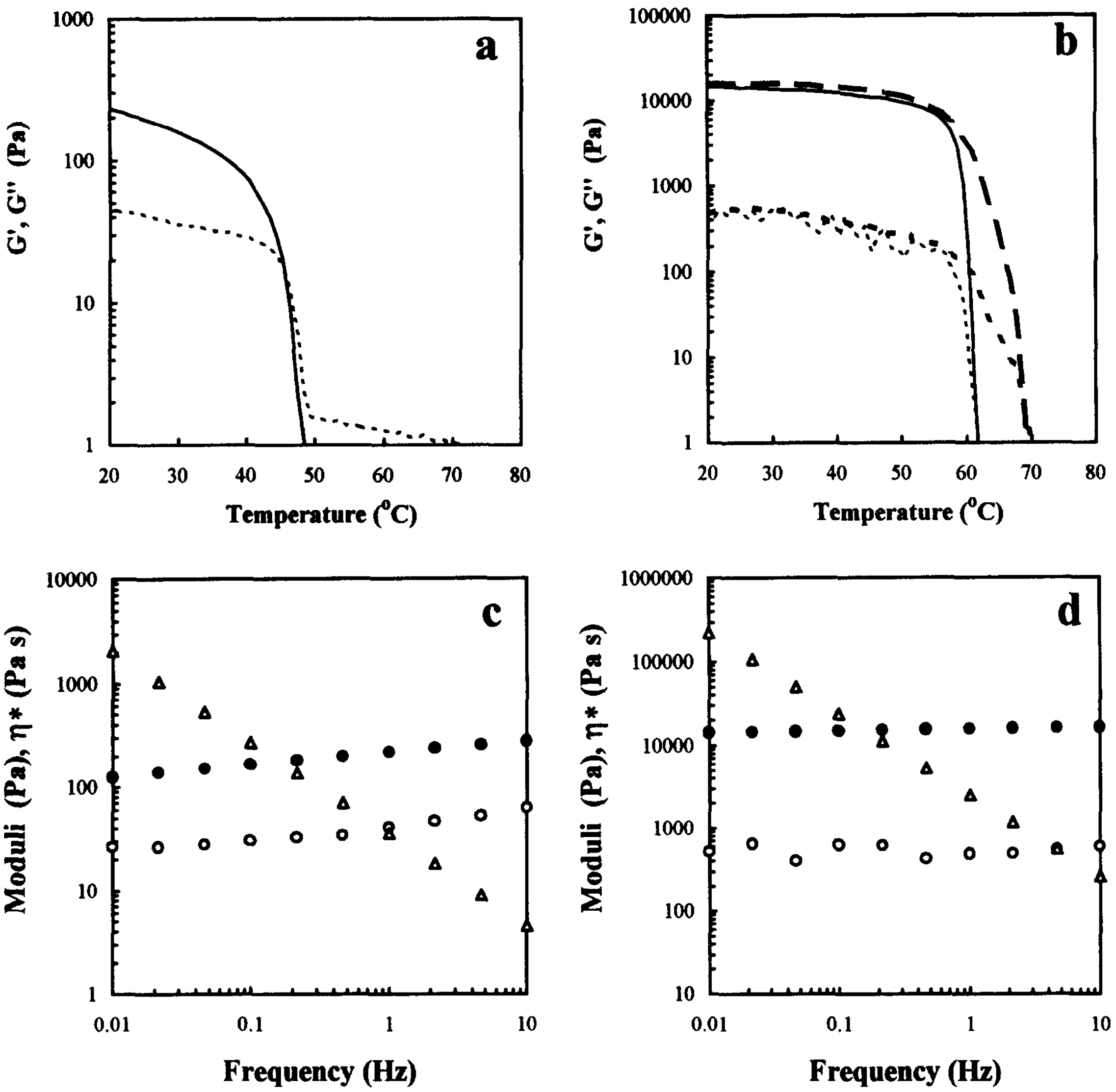

Fig. 6. Rheology of 1.5% sodium kappa-carrageenan in 0.1 M mixtures of NaI and CsI. (a) Variation of G' (———) and G'' (– – –) on cooling or heating for $x_{\mathrm{Cs}}=0.2$; (b) variation of G'(———), G'' (·····) on cooling and G' (— — —), G'' (– – –) on heating for $x_{\mathrm{Cs}}=0.9$. Frequency dependence of G'(●), G'' (○) and η^* (△) at 20°C for: (c) $x_{\mathrm{Cs}}=0.2$; and (d) $x_{\mathrm{Cs}}=0.9$ (conditions as in Fig. 3).

$x_{\mathrm{Cs}}=0.4$, but we believe that most, if not all, of this variation may be attributed to the increase in the transition temperatures with increasing cesium content (Fig. 8). In fact, when the data for these samples were plotted against the relative temperature, $T-T_0$, rather than the absolute temperature. The curves obtained for the various samples below $x_{\mathrm{Cs}}=0.4$ almost superimposed. The trends in Fig. 9 are very similar to those observed by viscometry for dilute solutions (Borgström *et al.*, 1996a), which also showed that important changes in the mode of association of kappa-carrageenan helices occur above $x_{\mathrm{Cs}}=0.4$ in 0.1 M mixtures of NaI and CsI. There can be little doubt that the transition around $x_{\mathrm{Cs}}=0.4$ reflects the formation of super-helical rods as evidenced by electron microscopy (Borgström *et al.*, 1996a). It is tempting to attribute at least part of the continuing increase in G', with cesium content above $x_{\mathrm{Cs}}=0.4$, to the increasing extent of aggregation of the super-helical rods into larger aggregates, as was also seen in the electron micrographs. However, this effect could also, at least in part, be due to a gradual increase of the fraction of super-helical rods at the expense of non-aggregated helices. At this stage, we do not know if the helix-to-superhelix transition is very sharp, or if there is a coexistence of significant fractions of both types of aggregates over a large range of cesium contents above $x_{\mathrm{Cs}}=0.4$.

The rheological response of a few selected samples above $x_{\mathrm{Cs}}=0.45$ was investigated using ranges and rates of the cooling–heating cycle differing from those given in the Experimental section. Figure 10 shows results for a sample with x_{Cs} 0.5 that was cooled from 80 to 45°C (rather than 20°C) and then back again to 80°C. The extent of the hysteresis is virtually the same as for the

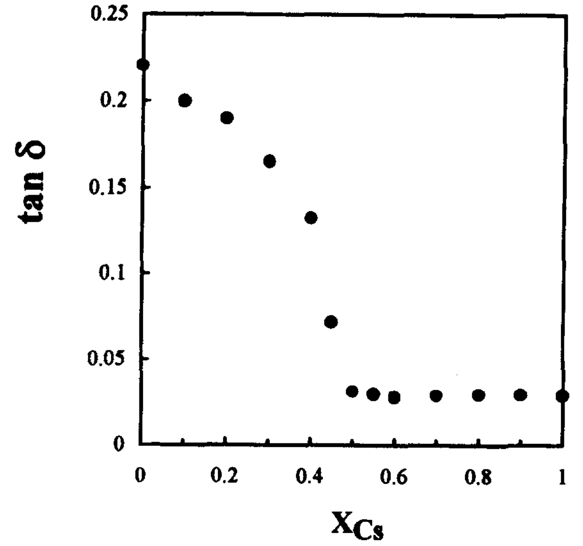

Fig. 7. tanδ vs x_{Cs}, for 1.5% (w/w) sodium kappa-carrageenan at 20°C.

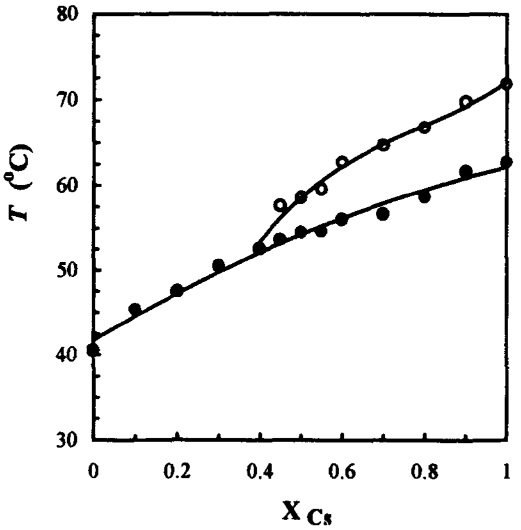

Fig. 8. Transition temperatures T_o (●) and T_c (○) vs cesium content for 1.5% (w/w) sodium kappa-carrageenan in 0.1 M mixtures of NaI and CsI.

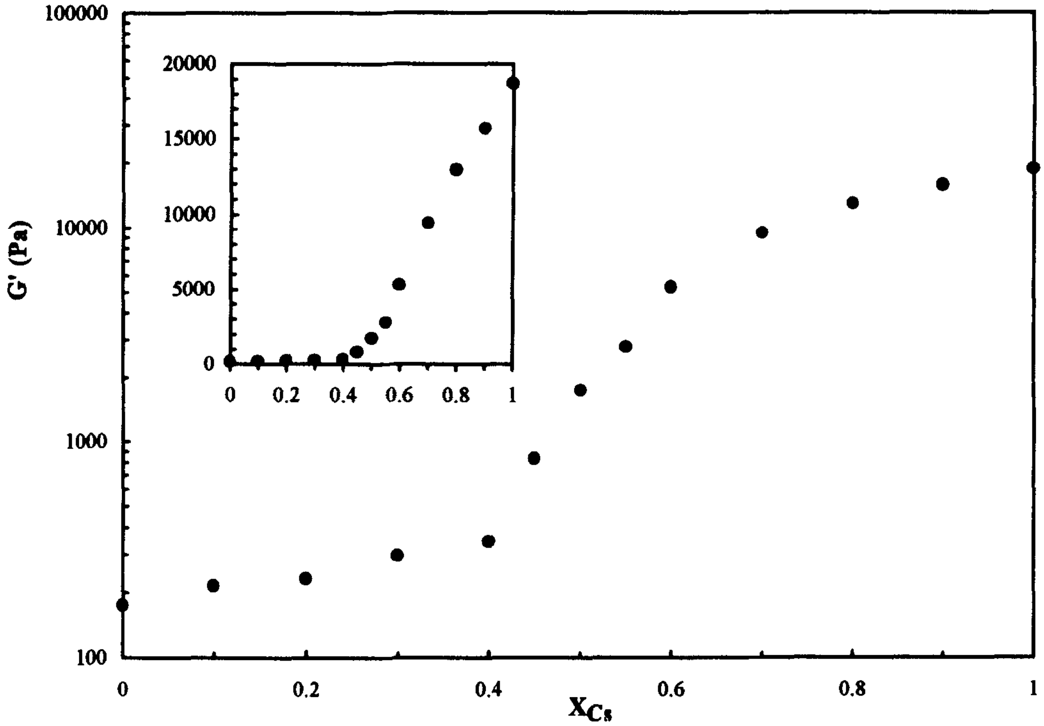

Fig. 9. Storage modulus vs cesium content for 1.5% (w/w) sodium kappa-carrageenan in 0.1 M mixtures of NaI and CsI after 30 min isothermal run at 20°C (conditions as in Fig. 3). The inset shows the same data on a linear vertical scale.

gel that was cooled to 20°C, indicating that the formation of super-helical rods starts early, maybe simultaneously with the helix formation. When the cooling rate was decreased for the same sample, the modulus increased, as is shown in Fig. 11. For this particular sample, cooling at 1 deg/min produced a curve that might be taken to suggest that the gelation occurred in two discrete steps, one above and one below ~40°C. On lowering the cooling rate, however, the increase in the gel modulus occurred in one single, smooth step.

As mentioned above, problems with syneresis and contraction of the gels were noted at high contents of cesium ions ($x_{Cs} \geq 0.5$–0.55). This is illustrated in Fig. 12, where we compare results for $x_{Cs} = 1$ using smooth and crosshatched upper plates, respectively. With the smooth plate we repeatedly obtained, soon after the initial increase, a sharp drop in the moduli on cooling, followed by a levelling off at a much lower value. Similar effects have been noted for kappa-carrageenan gels by a number of other workers (Hermans-

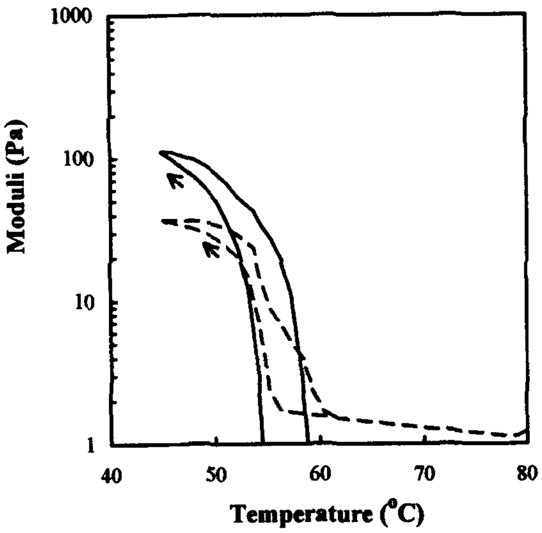

Fig. 10. Temperature dependence of G' (———) and G'' (– – –) on cooling and heating of 1.5% (w/w) sodium kappa-carrageenan in a 0.1 M mixture of NaI and CsI at $x_{Cs}=0.5$. Arrows indicate cooling runs (1 deg/min, 0.5%; strain, 1.6 Hz).

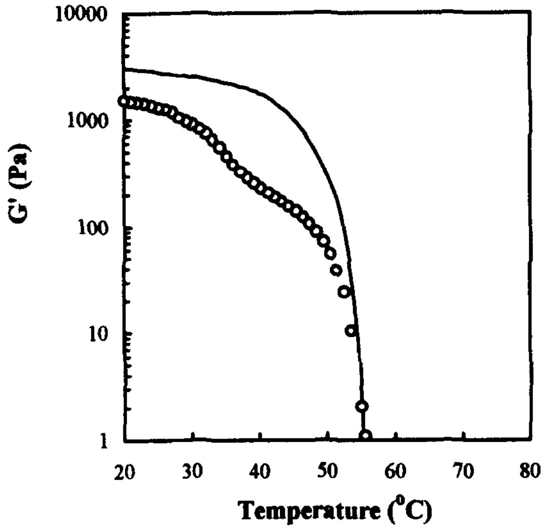

Fig. 11. Effect of cooling rate on G' of 1.5% (w/w) sodium kappa-carrageenan in a 0.1 M mixture of NaI and CsI at $x_{Cs}=0.5$. (———) 0.2 deg/min, (o) 1 deg/min (0.5% strain, 1.6 Hz).

son, 1989; Hermansson *et al.*, 1991; Roesen, 1991; Richardson and Goycoolea, 1994), but the interpretations have differed. We found that the drop disappeared when we used a cross-hatched plate (Fig. 1a). Since the two types of results were obtained on identical samples, with the same rheometer and procedures, while only varying the plates we feel confident that the effect is due to syneresis, as previously observed by Moldenaers *et al.* (1988) and Richardson and Goycoolea (1994). Indeed, the appearance of syneresis is rather an expected consequence of an increase in the degree of kappa-carrageenan aggregation (as a result of the formation and subsequent association of super-helical rods).

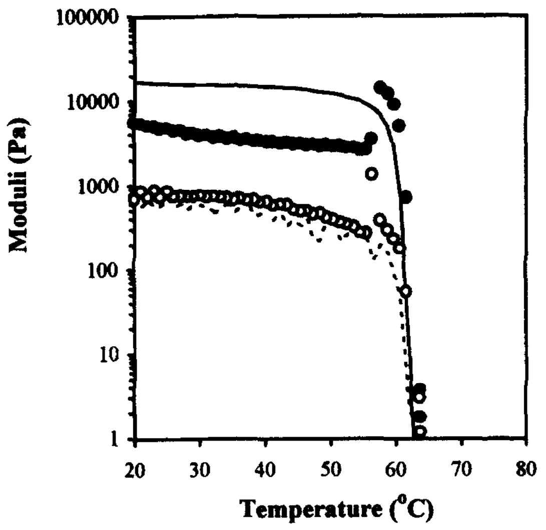

Fig. 12. Temperature dependence of G'(———) and G''(······) using cross-hatched upper plate and G'(●) and G''(○) using smooth plates for 1.5% (w/w) sodium kappa-carrageenan in pure 0.1 M CsI (40 mm plates, 0.5 mm gap, 1 deg/min, 0.5% strain, 1.6 Hz).

The notion of syneresis, or shrinking of the gel network, at high cesium contents was further confirmed by the results of the following swelling experiments. Hot 2% kappa-carrageenan solutions in mixed 0.1 M Na/CsI solutions at different x_{Cs}, were allowed to set at room temperature in 1.2 mm glass tubes. Each gel was then taken out of its tube and immersed at room temperature in a large excess of salt solution with the same salt composition as the one in which the gel was originally prepared. The changes in diameter of the cylindrical gels were then followed by measurements with a calibrated video microscope over several days. Three types of results were obtained, depending on x_{Cs}. At very low $x_{Cs}(<0.35)$, the gels simply dissolved. Gels prepared and swollen at the intermediate values of $0.35 \leq x_{Cs} < 0.45$ swelled significantly in the excess salt solution, although the degree of swelling decreased with increasing x_{Cs}. At high cesium contents ($x_{Cs} \geq 0.5$), finally, there was a significant shrinking of the gel.

CONCLUDING REMARKS

The results presented here indicate that kappa-carrageenan may form gel networks by, at least, two different routes, as indicated in the following schemes.

$$\text{coil} \rightarrow \text{helix} \rightarrow \text{gel} \quad (1)$$

$$\text{coil} \rightarrow \text{helix} \rightarrow \text{super-helical rod} \rightarrow \text{gel} \quad (2)$$

The first type of gel (formed in pure NaI or in mixtures with low levels of CsI) involves the reversible association of helices, in a fashion that is not well understood. This seems to be a new type of gel for kappa-carrageenan, but it has important similarities with iota-carrageenan gels. The second type of gel is the conventional kappa-carrageenan gel, which shows thermal hysteresis and has a much higher modulus than the first type. The results presented here, together with the previous structural findings from our laboratory (Borgström *et al.*, 1996a), strongly suggest that the 'building blocks' in the latter type of gel are super-helical rods, rather than individual double helices. The strength of this type of gel may be tuned with salts, possibly through changes in the degree of association of the super-helical rods.

Our conclusions regarding two types of kappa-carrageenan networks have notable similarities, but also important differences, with those previously drawn by Hermansson and coworkers on the basis of electron microscopy and rheology (Hermansson, 1989; Hermansson *et al.*, 1991). Their electron micrographs showed clear evidence of two types of structures, a coarse and a fine network, formed by kappa-carrageenan in the presence of potassium chloride. The coarse network was found to be built up by the parallel alignment of rodlike structures, which were larger than individual helices. Our view of the mode of aggregation (scheme 2 above) at large contents of site-binding cations (cesium or potassium) agrees well with this picture. It is, furthermore, possible that the type (1) network that we find in pure NaI or at low contents of cesium corresponds to the transient fine network seen in the images of Hermansson and coworkers, although this remains to be confirmed, since the latter images were obtained under salt conditions differing from those investigated here.

In conflict with the conclusions by Hermansson and co-workers, however, we clearly find that the network built up by the super-helical carrageenan rods gives rise to a much higher (not lower) modulus, and that the modulus further increases when the degree of association of the rods increases. We note that the conclusions of the previous authors were based on results of the type shown in Fig. 12 (with smooth plates), which we interpret in terms of syneresis.

The findings that there exist different levels and modes of helix association and gelation of kappa-carrageenan, point to many important questions to be resolved in future research. One concern is the possible generality of schemes 1 and 2 for other (bio)polymers that form gels as a result of a coil–helix transition, with or without thermal hysteresis. Do these other polymers, or do they not, form intermediate superhelical aggregates in the gelation process? More specifically, for the carrageenans, it seems important to further study the structure and association of the super-helical rods found for kappa-carrageenan. It is also important to understand the nature of the helix association that gives rise to the completely reversible type (1) gelation that occurs for iota-carrageenan and, sometimes, for kappa-carrageenan. Work along these lines is in progress in our laboratory. Lastly, the possible role of the super-helical rods for the synergistic interactions between carrageenans and galactomannans (for example, the recent reviews by Morris, 1990b; Williams & Phillips, 1995) is an interesting question that will be addressed in a forthcoming article.

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