Carbohydrate Polymers **16** (1991) 297–320

Effects of Potassium, Sodium and Calcium on the Microstructure and Rheological Behaviour of Kappa-Carrageenan Gels

A.-M. Hermansson, E. Eriksson & E. Jordansson

SIK, The Swedish Institute for Food Research, PO Box 5401, S-402 29 Göteborg, Sweden

(Received 19 November 1990; revised version received 15 January 1991; accepted 18 January 1991)

ABSTRACT

The effects of cations and mixtures of cations on kappa-carrageenan gelation was studied by electron microscopy and viscoelastic measurements. On cooling only potassium-kappa-carrageenan formed a transient fine structure at 54–52°C. The structure became unstable at 51°C, whereafter long stiff superstrands with two to three strands in parallel formed a coarse network. The balance between the fine and the coarse gel strands was dependent on the potassium concentration. In contrast to the potassium form, sodium-kappa-carrageenan formed relatively weak gels and showed almost no dependence on the sodium ion concentration. The sodium form had a network structure with flexible superstrands of uniform thickness. The calcium-kappa-carrageenan formed relatively weak gels in a limited calcium concentration range, whereafter salting-out effects were observed. The calcium-induced gels had a very fine network of an entanglement type without any of the coarse superstrands seen in sodium or potassium forms. A transient gel structure was not found for sodium- or calcium-kappa-carrageenan.

Strong synergistic effects were found between calcium and potassium. Depending on the ratio of potassium to calcium, it was possible to make 1% gels in the temperature range 20–40°C with storage moduli varying from 70 to 43 000 Pa. Apart from adding KCl to the pure calcium form and $CaCl_2$ to the pure potassium form of kappa-carrageenan, intermediate ion forms were produced by ion exchange where the potassium to calcium ratio was varied between 0·2 and 0·8.

Synergistic effects were also observed when sodium was added to potassium-kappa-carrageenan, but not when potassium was added to sodium-

Carbohydrate Polymers 0144-8617/91/$03.50 —

kappa-carrageenan. No synergistic effects were observed between calcium and sodium in any form.

INTRODUCTION

Kappa-carrageenan is a commonly used sulphated galactan. It has the ability to form a gel on cooling from a hot solution and can be used to control texture, stability, diffusion and waterholding properties of a wide range of products. The gelation process is complex and involves a coil–helix transition followed by aggregation and network formation.

The most common counter-ions in kappa-carrageenan preparations are potassium, sodium and calcium. The type of cations used has an impact on gel characteristics, and more knowledge is needed to take full advantage of the potential of kappa-carrageenan.

Studies on the association of monovalent ions with kappa-carrageenan have revealed specific site binding to kappa-carrageenan of K^+, Rb^+ and Cs^+ and non-specific binding effects of Li^+, Na^+ and Me_4N^+ (Gasdalen & Smidsrød, 1981; Norton *et al.*, 1983; Belton *et al.*, 1985; Nilsson *et al.*, 1989). This seems to have an impact both on the coil–helix transition and on the final gel structure. The effect of cations on the coil–helix transition has been investigated by Rochas and Rinaudo (1980). The cation concentration necessary for a coil–helix transition was found to be in the order $Rb^+ \leqslant Cs^+ = K^+ \ll Na^+ < Li^+$. As an example, a coil–helix transition at 20°C would require about 7 mM K^+ but as much as 200–250 mM Na^+. The effect of cations on gel properties has shown that monovalent cations give gels with decreasing shear and elastic moduli in the order $Cs^+ > K^+ \gg Na^+ > Li^+$ (Watanase & Nishinari, 1981; Morris & Chilvers, 1983).

Only a limited number of studies have been made on the effect of divalent cations on the gelation mechanisms of kappa-carrageenan. Rochas and Rinaudo (1980) found that there was only a slight dependence on the nature of divalent counter-ions with regard to the transition midpoint temperature for the melting of the kappa-carrageenan helices. According to their results, 20–30 mM Ca^{2+} would be required for a coil–helix transition at 20°C. The information about the effect of calcium on the rheological properties of kappa-carrageenan gels is confusing and contradictory. Morris and Chilvers (1983) found that calcium gave rise to stronger gels than potassium, whereas the opposite was found by Watanase and Nishinari (1981, 1986). However, the results were obtained under different experimental conditions, and the importance of the experimental conditions for the properties of calcium-containing kappa-carrageenan will be discussed in detail in this paper.

It is commonly accepted that a coil–helix transition is a necessary requirement for carrageenan gels to form, but the mechanisms involved in gel formation are not well understood. Morris *et al.* (1980) found that helices of potassium-kappa-carrageenan only formed under conditions that also promoted aggregation. This means that there is a strong link between helix formation and gel formation. Different results were observed for iota-carrageenan systems, where helices were able to exist as stable entities and helix formation did not necessarily lead to gel formation depending on the type of cation. So far, the prevailing theories of kappa-carrageenan gelation are based on results obtained from studies of potassium-induced gelation, and there is little known about other cationic systems (Morris *et al.*, 1980; Rochas & Rinaudo, 1984; Hermansson, 1989).

In a previous study, the microstructure and rheological behaviour revealed a transient state on gelation of the potassium form of kappa-carrageenan (Hermansson, 1989). In the presence of potassium, the storage modulus showed a sharp initial maximum during cooling and gel formation. The structure of the transient state was found to consist of a fine network, where the junction zones were believed to be double helices. When the temperature was further lowered, the structure became unstable and the helices associated into rigid rods consistent with dimers of double helices. The rods were the building blocks in the formation of a coarse supramolecular network structure, where strands aligned in parallel to form the long stiff superstrands typical of the potassium-induced gels of kappa-carrageenan. The balance between the fine network and the coarse supramolecular structure was found to be dependent on the potassium concentration. The finding of a transient state is in agreement with the model for aggregation of pairwise-interacting polymers recently suggested by Higgs and Ball (1989).

The work reported in this paper will provide additional information about the transient states of potassium-induced kappa-carrageenan gels. New information will be given about the microstructure and the rheological behaviour of the sodium and calcium forms of kappa-carrageenan. The possibilities of obtaining strong synergistic effects by combinations of cations will also be demonstrated.

MATERIALS AND METHODS

Materials

Samples of kappa-carrageenan from *Euchema cottonii* type III were purchased from Sigma Chemicals (St Louis, MO, USA). The pure potassium, sodium and calcium forms were prepared by ion exchange of a hot 1% carrageenan solution in a column thermostatted to 85°C with a commercial ion exchange resin (AG 50W-X8, BioRad) and freeze dried. A surplus of resin was used, and the batch size was 300 ml wet resin to 1·0 litres of 1% carrageenan solution. The pH was carefully checked after each step in the ion exchange procedure in order to obtain high efficiencies and eliminate the risk of hydrolysis during preparation.

Intermediate ion forms of kappa-carrageenan were produced by mixing resin with a hot carrageenan solution according to the procedure described by Morris and Chilvers (1983). Intermediate or mixed ion forms were prepared with different ratios of potassium to calcium.

The cationic concentration of the various ion forms was determined in triplicate by atomic absorption spectroscopy, of dilute solutions as well as acid hydrolysates. The results are shown in Table 1. The values are slightly below those corresponding to an idealized repeat unit for kappa-carrageenan providing there is one calcium ion per two sulphate groups. Similar results have been reported by Morris and Chilvers (1983).

Rheological measurements

Viscoelastic measurements were made in a Bohlin VOR rheometer with the C14 measuring system (DIN 53019) and a modified cupholder. The original holder made of stainless steel was replaced by a holder made of an alloy (Nilo Alloy 36), which has a thermal expansion constant that is only one-tenth of the original. The replacement was made because changes in the original holder during cooling induced strains of the kappa-carrageenan gels outside the linear region. The alloy holder was also used in the previous study of potassium-kappa-carrageenan gels (Hermansson, 1989).

TABLE 1
Composition of Cations

Ion form of kappa-carrageenan	*Amount of cations % (w/w)*		
	Potassium	*Calcium*	*Sodium*
Potassium form	8·5	> 0·02	> 0·02
Sodium form	> 0·02	> 0·02	5·5
Calcium form	> 0·02	3·5	> 0·02
Intermediate form I	2·1	2·7	0·52
Intermediate form II	1·4	3·2	0·50

A 1% solution of kappa-carrageenan was heated at 98°C for 15 min, poured into the rheometer and conditioned at 90°C for 5 min. The sample was covered with a thin layer of paraffin oil to avoid evaporation during the measurement. The sample was cooled at a cooling rate of 1·5°C/min to 20°C, unless otherwise stated, and kept at 20°C for 90 min. The rheometer was used in the oscillatory mode but oscillated only when G* was recorded. The frequency was 1 Hz except when the frequency dependence was measured in the range 0·01–10 Hz. The strain was well within the linear region and was kept as low as 0·001 in order not to disturb gelation. The strain dependence was measured at the end of every sequence.

Electron microscopy

Samples were prepared for electron microscopy by the mica sandwich technique, as previously described, without any additives and with a minimum of shear (Hermansson, 1989). The technique is well suited for the stable structures of the sodium and potassium ion forms, which showed good reproducibility over a wide concentration range (0·01–10 mg/ml). The calcium ion form was less stable. Phase separation easily occurred on dilution, and there was a tendency for surface-induced changes as well as interactions with other structural components. The following precautions were therefore taken. The temperature was kept constant at −95°C throughout the drying process, and the sample was prepared without any dilution steps prior to or after the onset of gelation at a concentration of 1% (10 mg/ml). The intermediate calcium-containing forms of kappa-carrageenan were more stable than the pure calcium form. Replicas were examined in a JEOL 100 CX-II at an accelerating voltage of 80 kV. Several preparations were made for every carrageenan sample.

RESULTS AND DISCUSSION

Pure forms of kappa-carrageenan

Potassium-kappa-carrageenan

In the previous paper a sharp maximum was found in the initial stage of gelation in the presence of 100–200 mM KCl. This paper will provide additional information about the time–temperature dependence of the transient state of potassium-induced gelation of kappa-carrageenan.

The temperature dependence was studied in the vicinity of the gelation temperature. A 1% carrageenan solution in 100 mM KCl was cooled at 1·5°C/min to a given temperature between 50 and 54°C and kept at that temperature for 90 min. Figure 1 shows that there was a sharp initial maximum when the samples were cooled to 50–51°C, whereafter the storage modulus dropped to a constant value. However, at 52°C the storage modulus increased slowly to a value of 3·5 kPa, and there was no maximum within the time allowed for the experiment. A weak gel developed slowly at 54°C and reached a storage modulus of 250 Pa after 90 min. Similarly to the transient gel structure of the peak maximum, the structure formed at the higher temperatures, 52 and 54°C, had lower phase angles ($\leqslant 2°$) than those of the aggregated structures ($\leqslant 4°$) regardless of the magnitude of the storage modulus. A very low strain was required to follow the initial reactions of gel formation at elevated temperatures. The linear region varied between 0·002 and 0·004, and a strain of 0·001 was used throughout this study. The gels cooled to 20°C had a linear region well above 0·003, but the results imply that even such a low strain as 0·003 may influence a system during gelation and thereby affect the final gel characteristics. Similar effects have been observed for β-lactoglobulin gels (Stading & Hermansson, 1990). This is worth pointing out since strains as high as 0·05 are commonly used in viscoelastic measurements of biopolymer gels.

The results shown in Fig. 1 are in agreement with the previously discussed changes in the network structure from a fine network where the junction zones are double helices to a destabilization on cooling and the formation of dimers of double helices (Hermansson, 1989; Higgs & Ball, 1989). The transient fine network structure and the subsequent rodlike structures formed on cooling can be seen in Fig. 2(a and b). On further cooling to 20°C, assemblies of double helices aggregate into the coarse superstrands typical of potassium-induced gels of kappa-carrageenan. As shown in Fig. 2(c), the superstrands are often composed of two to three strands in parallel with a small interspace between. At a lower potassium concentration of 10 mM no maximum in the storage modulus was observed, and the microstructure of this gel was different from that at 100 mM KCl, as shown in Fig. 3. In this case, a fine network structure makes up the continuous phase, and some superstrands are dispersed in the fine network structure. The latter seemed to be somewhat more aggregated and irregular than that of the transient structure. In contrast to the superstrands formed at higher potassium concentrations, the dispersed strands were flexible and uniform in thickness. There was no tendency towards alignment of strands into the coarse rigid superstrands observed at higher potassium concentrations.

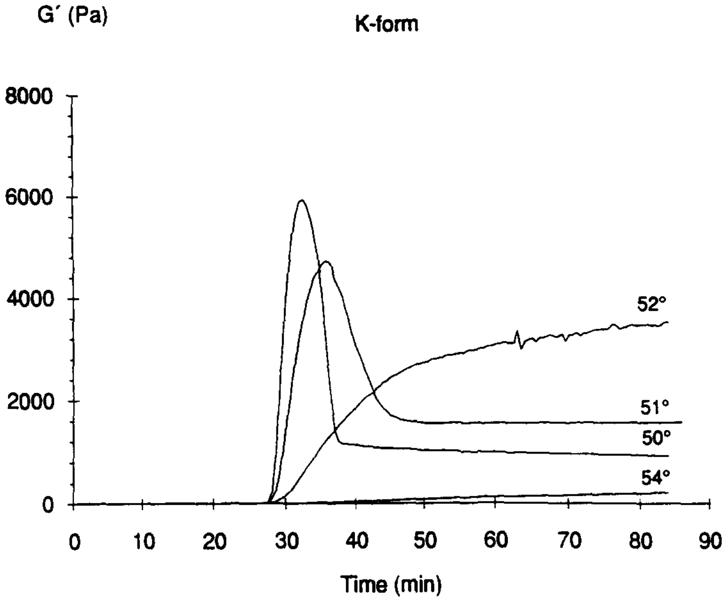

Fig. 1. The storage modulus G′ of 1% potassium-kappa-carrageenan in 100 mM KCl at 50, 51, 52 and 54°C.

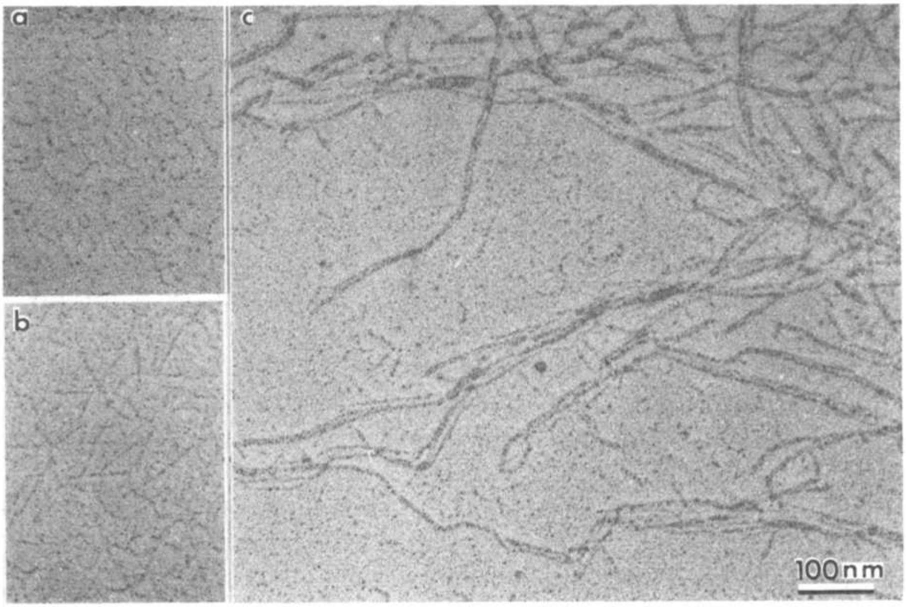

Fig. 2. Structures during gel formation of potassium-kappa-carrageenan in 100 mM KCl. (a) The transient fine structure, (b) helices associated into rigid rods, (c) completed aggregation to the stiff supramolecular network structure typical of potassium-induced gels at higher salt concentrations.

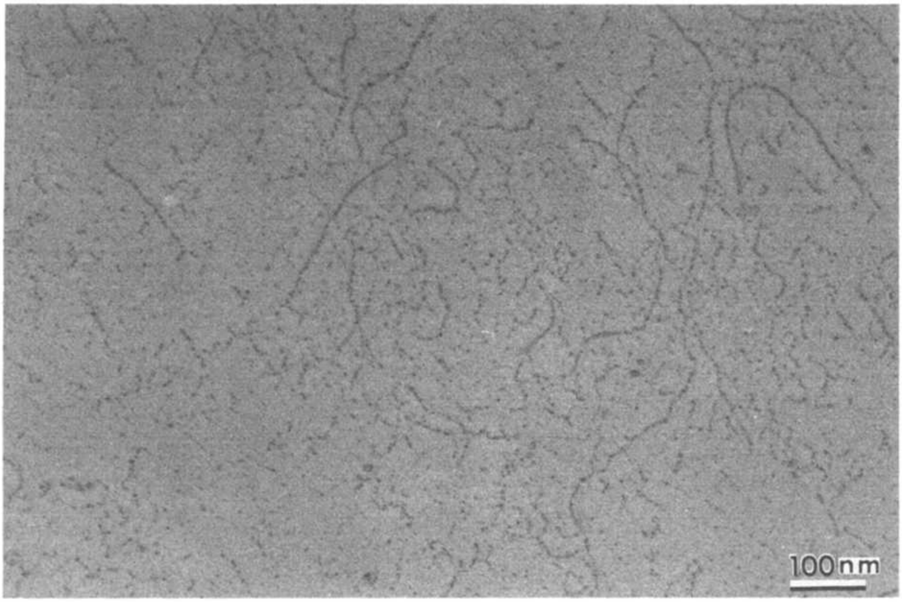

Fig. 3. The structure of potassium-kappa-carrageenan in 10 mM KCl showing flexible superstrands dispersed in a fine network structure.

The results have shown that the gelation of kappa-carrageenan in 100 mM KCl is complex and involves several aggregation steps. It is then plausible to believe that the kinetics during cooling have an influence on the final gel properties. Figure 4 shows the changes in the storage modulus during cooling to 20°C at the cooling rates 1·5, 1·0 and 0·5°C/min. The final storage modulus increased with decreasing cooling rate. A transition peak occurred at all cooling rates but became smaller when the cooling rate was decreased. The results imply that the fine structure is more stable at lower cooling rates. Preliminary results also indicate that part of the rods strengthen the fine structure, whereas they to a large extent merge into the coarser supramolecular network at higher cooling rates. In a previously reported study of the dependence on potassium concentration, it was shown that the strongest gel formed at 200 mM KCl was composed of a mixed structure, where the fine network was intertwined with the coarser supramolecular network structure (Hermansson, 1989). A mixed structure might also be the reason for the stronger gels formed at lower cooling rates.

Sodium-kappa-carrageenan

Figure 5 shows the changes in the storage modulus for the sodium-kappa-carrageenan in 250 mM and 500 mM NaCl. Sodium induced

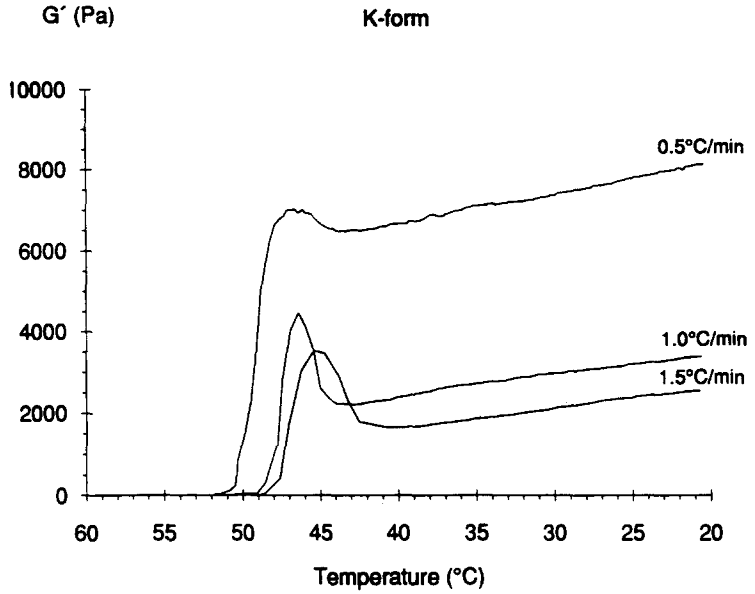

Fig. 4. The storage modulus G′ as a function of temperature for 1% potassium-kappa-carrageenan in 100 mM KCl at cooling rates of 0·5, 1·0 and 1·5°C/min.

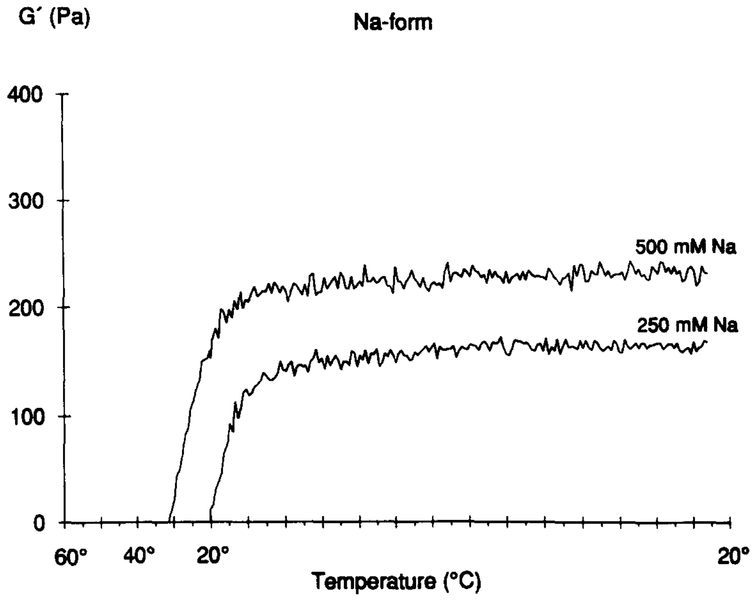

Fig. 5. The storage modulus G′ of 1% sodium-kappa-carrageenan during gelation in 250 and 500 mM NaCl.

weaker gels than potassium, with a final value of the storage modulus in the range 160–240 Pa and low phase angles (⩽ 2°) regardless of the ion concentration. Gels did not form in 200 mM NaCl. This is in agreement with the requirement of 250 mM sodium for coil–helix transition at 20°C reported by Rochas and Rinaudo (1980). The gelation temperatures at different sodium concentrations also correspond with those reported for the coil–helix transition. The results thus imply that, similarly to potassium-induced gelation of carrageenan, gelation in the presence of sodium is directly correlated with the coil–helix transition.

In contrast to the potassium-induced gels, the gels formed in the presence of sodium showed almost no dependence on the ion concentration. The sodium gels had storage moduli of the same magnitude as those of the weakest potassium-kappa-carrageenan gels in 10 mM KCl, whereas gels formed in 100 and 200 mM KCl showed 10–100 times higher storage moduli (Hermansson, 1989). This implies that there is a difference between these two cations with regard to the type of structure formed and how the structure changes with salt concentration.

Figure 6 shows the network structure of the sodium form in 250 mM NaCl. The structure is homogeneous with flexible superstrands of a constant thickness. Details at a higher magnification can be seen from Fig. 7. Similarly to the potassium-induced gel the network is formed on

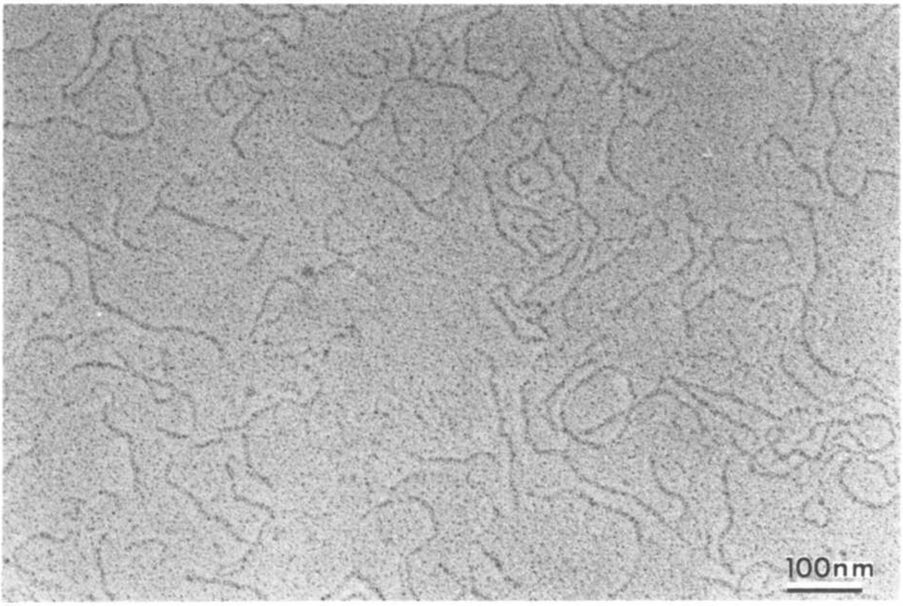

Fig. 6. The structure of sodium-kappa-carrageenan in 250 mM NaCl showing a network of flexible superstrands.

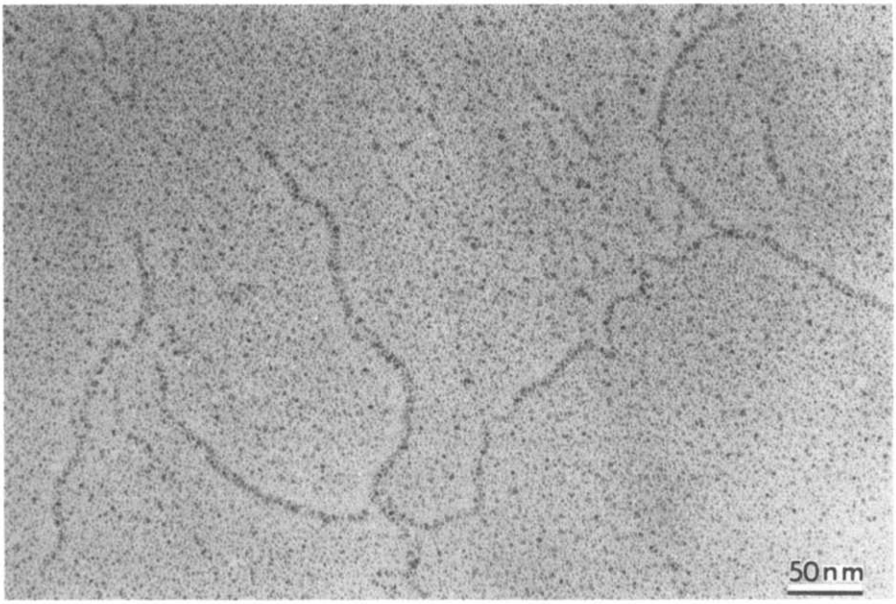

Fig. 7. Details of junction zones of sodium-kappa-carrageenan in 250 mM NaCl.

the supramolecular level by alignment of at least two double helices which deviate and align with other double helices or assemblies thereof. The type of superstrands formed in the presence of sodium is similar in appearance to those found at the lower concentration limit for the coil–helix transition of potassium shown in Fig. 3. However, the rigid coarse supramolecular strands of two to three strands aligned in parallel formed at higher potassium concentration do not form in the presence of sodium. These rigid superstrands are specific for the potassium form, and their formation is one contributing factor for the dependence on the potassium ion concentration with regard to the rheological behaviour of potassium-kappa-carrageenan. The other contributing factor is probably the decrease in solubility of the double helices and the partial stabilization of the fine network structure in the presence of potassium. In the presence of sodium, it is likely that domains of the double helical structure are formed, but that this structure cannot span the whole volume and form a gel structure. In this respect, the sodium form is more similar to the domain model proposed by Morris *et al.* (1980).

The results presented in this study show that gel formation is closely related to the coil–helix transition both for the sodium and the potassium forms of kappa-carrageenan. The flexible superstrands of the sodium form are similar in appearance to those found at low concentrations of potassium, whereas specific potassium effects can be seen on the supramolecular level at higher potassium concentrations.

Calcium-kappa-carrageenan

The pure calcium-kappa-carrageenan gave rise to weak gels in 30 and 100 mM calcium, as shown by Fig. 8. Similarly to the other ion forms, the gels were almost frequency-independent in the range 0·01–10 Hz, and the phase angles were low. Gels did not form at 20 mM calcium or below, which is in agreement with the requirements of 20–30 mM calcium for a coil–helix transition at 20°C (Rochas & Rinaudo, 1980). There was no dependence on the calcium concentration in the range 30–100 mM, and salting out effects were observed at higher concentrations. The gelation temperature was 20°C and 25°C at 10 mM and 30 mM calcium, respectively. The temperatures reported for the coil–helix transitions at these salt concentrations were approximately 20°C and 30°C (Rochas & Rinaudo, 1980).

The concentration dependence of G′ for pure calcium-kappa-carrageenan in distilled water was investigated, and the results are shown in Fig. 9. Gels formed at a concentration of 4% and developed slowly to form strong gels without reaching equilibrium after 90 min at 20°C. The

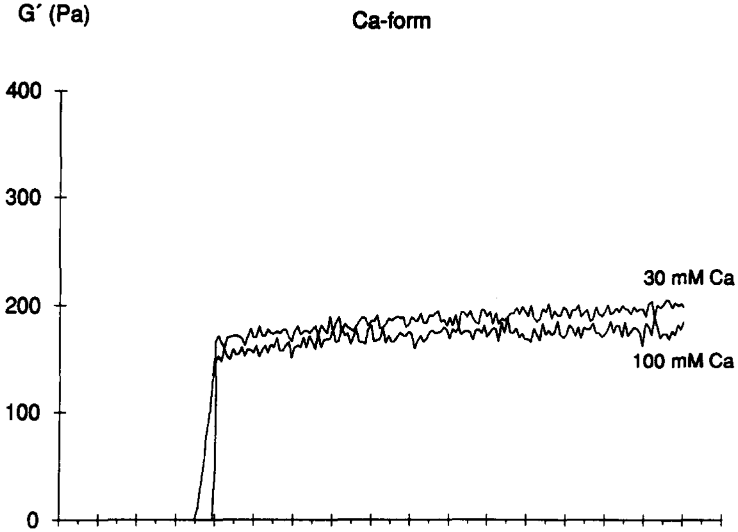

Fig. 8. The storage modulus G′ of 1% calcium-kappa-carrageenan during gelation in 30 and 100 mM $CaCl_2$.

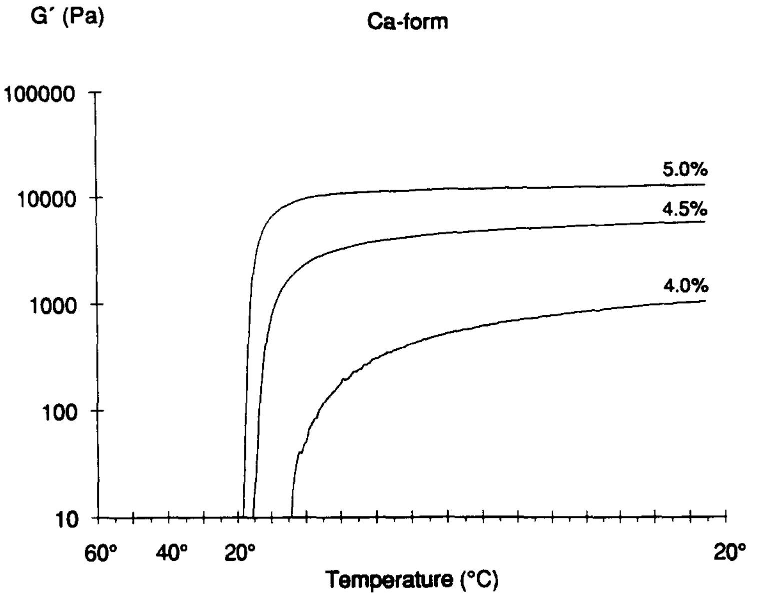

Fig. 9. The storage modulus G′ of 4%, 4·5% and 5% calcium-kappa-carrageenan during gelation in distilled water.

gels formed faster at 5%, and the storage modulus reached an equilibrium as high as 12 kPa. Gels did not form under the experimental conditions at polysaccharide concentrations of 3·8% or below. The calcium concentration of the 4% calcium-kappa-carrageenan solution was 35 mM. This is somewhat above the concentration range of 20–30 mM required for the coil–helix transition (Rochas & Rinaudo, 1980). However, the results are contradictory to results reported by Morris and Chilvers (1983) on the concentration dependence of calcium-kappa-carrageenan in distilled water. They found that gels formed at 1·5% (w/w). They cooled their solutions to room temperature and the measurements were made after 48 h of storage. No information was given about the storage temperature. We have found that gels formed when 1% calcium-kappa-carrageenan solutions were stored in a refrigerator at + 4°C for 48 h. In our study, a 1% calcium-kappa-carrageenan solution contained 9 mM calcium according to the data given in Table 1. This corresponds to the concentration of divalent cations required for a coil–helix transition at 4°C according to Rochas and Rinaudo (1980). At present, there is no information available about aggregation reactions on long-term storage at low temperatures of any cation form of kappa-carrageenan.

Unlike the sodium and potassium forms, there was a small delay between the coil–helix transition temperature and the gelation temperature of the calcium form. This implies that there might be a different gelation mechanism for the calcium than for the other cation forms. It has already been pointed out that the calcium form was more difficult to prepare for electron microscopy than the other ion forms. Phase separation problems made it impossible to dilute the calcium system to obtain the best possible information about structural components. Special care also had to be taken during sublimation of water during preparation in order to avoid rearrangements on the mica surface. Figure 10 shows the structure of a 1% calcium gel in 30 mM $CaCl_2$. The low contrast is due to the restraints on the preparation conditions as discussed above. However, the resolution is quite good and, providing the quality of the final print is good enough, it can be seen that calcium gives rise to a very fine network structure without any of the superstrands typical of the sodium or potassium forms of kappa-carrageenan. No frequency dependence was observed in the range 0·01–10 Hz, which implies a low mobility of the network chains. From the differences observed between gel formation of different cation forms, one may speculate whether there is a fundamental difference between the calcium-induced helices and the

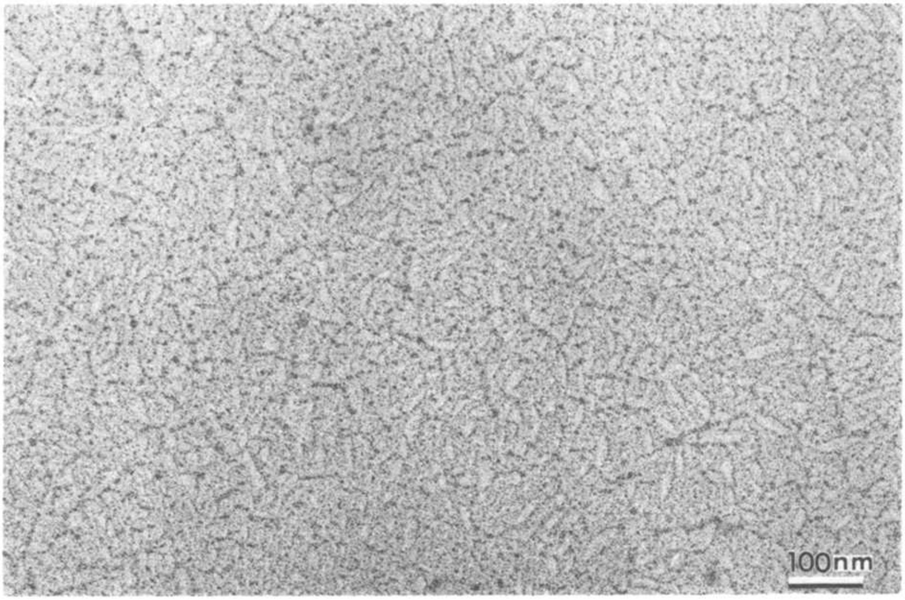

Fig. 10. The structure of calcium-kappa-carrageenan in 30 mM $CaCl_2$ showing a very fine network structure without superstrands.

double helices induced by sodium and potassium. In addition the aggregation of the dimers in the presence of divalent cations may be limited either by accumulation of net positive charge, if all binding sites are occupied or by interrupted co-operativity, if electrical neutrality is preserved. Further studies of the coil–helix transition as well as the aggregation steps of the pure calcium form of kappa-carrageenan would be of interest.

Interactions between cations

So far, there are no systematic studies available on the effect of mixtures of cations on gel formation of kappa-carrageenan. Commercially available carrageenan preparations vary in their cation composition, and this may result in differences in the gel characteristics. Thus, the characteristic potassium-induced maximum in the storage modulus can be seen for some but not for all commercial samples of kappa-carrageenan. It is, furthermore, quite common that specific ion forms are produced by ion exchange without information about the final ion composition. As will be shown in this section, even a small amount of a specific cation might have a dramatic effect on the gel characteristics of kappa-carrageenan. In addition to studies of the three pure ion forms of kappa-carrageenan, cations have been added to intermediate forms, where the ratio of potassium to calcium has been varied.

Sodium-kappa-carrageenan

When potassium was added to sodium-kappa-carrageenan, gels formed in 20 mM but not in 10 mM KCl. A critical potassium concentration of 27 mM was found for the pure potassium form of kappa-carrageenan. The potassium contribution from 1% carrageenan is 20 mM according to Table 1, and an addition of 7 mM KCl was necessary for gelation of the pure potassium form. In total, more than twice as much potassium was needed for gel formation than for the coil–helix transition at 20°C. This may be due to the requirement of potassium for the formation of the coarse supramolecular network structures typical of potassium-induced kappa-carrageenan gels.

The storage moduli of sodium-kappa-carrageenan gels in 20 mM and in 100 mM potassium are shown in Fig. 11. As expected, addition of 20 mM potassium resulted in a relatively weak gel. Addition of 100 mM potassium to the sodium form resulted in a strong gel with the typical transient behaviour of potassium-induced gels. The peak maximum was as high as 6 kPa, and the storage modulus dropped to 2·4 kPa after 90 min at 20°C. The peak was not as narrow as those of the pure potassium systems. The gelation temperature was 44°C, which is about 6°C lower than the temperature of the coil–helix transition reported by Rochas and Rinaudo (1980) at 100 mM K^+ and 20°C, whereas the transition and gelation temperature coincided for the pure potassium systems.

Similar effects were observed when calcium was added to the sodium form. No gel formed in 30 mM calcium, and a very weak structure with an unstable phase angle and a storage modulus of approximately 70 Pa formed in 40 mM calcium. A total salt concentration of 39 mM calcium was required for gelation of the pure calcium systems, and there were no signs of synergistic effects at low salt concentration for the sodium form. The addition of 60 mM calcium resulted in a gel with a storage modulus of 280 Pa, as shown in Fig. 12, and a low phase angle (~ 2°). This gel was somewhat stronger than those obtained for the pure calcium form in 30–100 mM $CaCl_2$.

Potassium-kappa-carrageenan

When sodium was added to potassium-kappa-carrageenan, only 25 mM sodium was required for gel formation and very strong gels formed in the presence of 50 and 100 mM sodium, as depicted in Fig. 13. The gels did not show the sharp initial peaks typical of potassium-induced gels, but

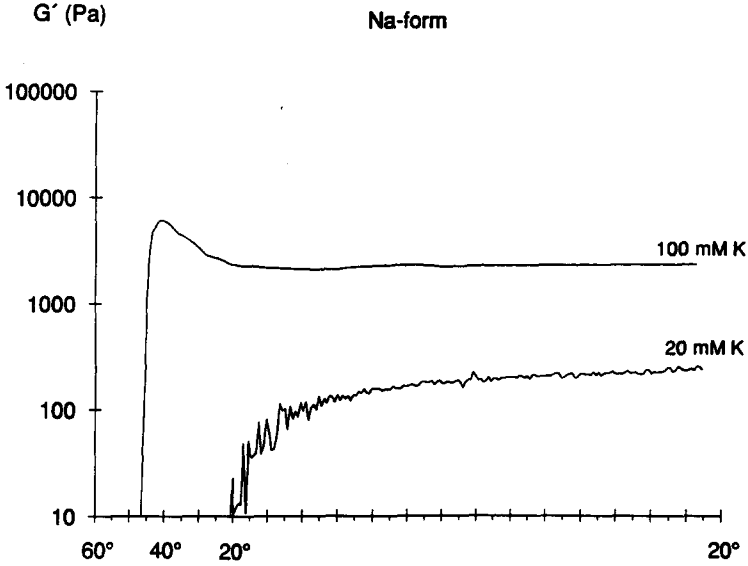

Fig. 11. The storage modulus G′ of 1% sodium-kappa-carrageenan during gelation in 20 and 100 mM KCl.

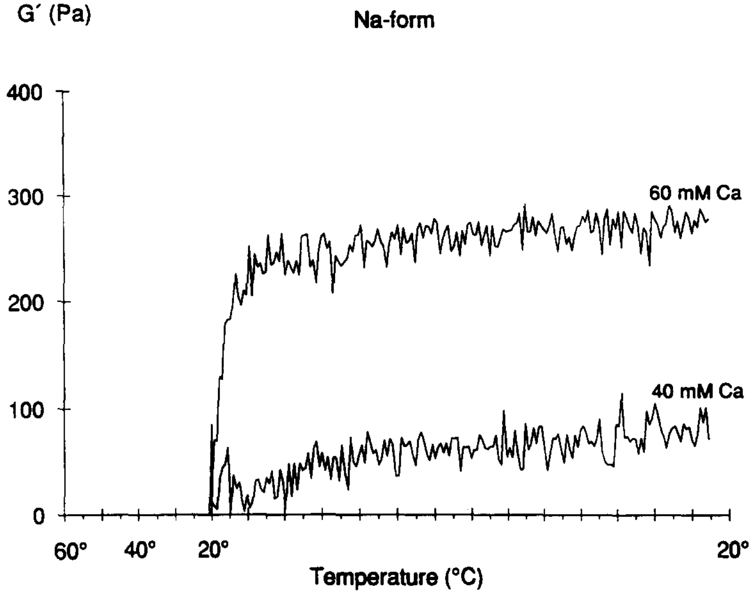

Fig. 12. The storage modulus G′ of 1% sodium-kappa-carrageenan during gelation in 40 and 60 mM $CaCl_2$.

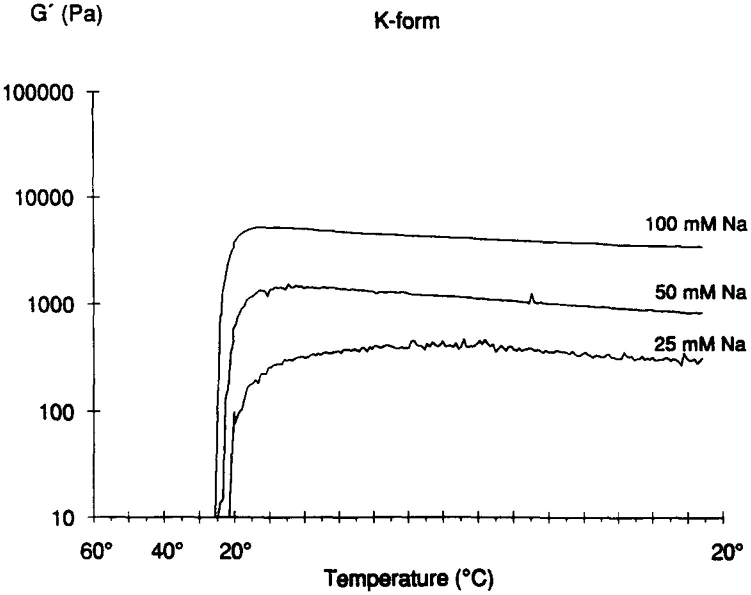

Fig. 13. The storage modulus G′ of 1% potassium-kappa-carrageenan during gelation in 25, 50 and 100 mM NaCl.

the storage modulus decreased slowly with time at 20°C. The results imply that synergistic effects were obtained when small amounts of sodium were added to potassium-kappa-carrageenan. As discussed above, 1% potassium-kappa-carrageenan contains 20 mM potassium, which is above the concentration necessary for a coil–helix transition but below that necessary for gel formation at 20°C. For pure sodium-kappa-carrageenan, on the other hand, as much as 250 mM sodium had to be added for the formation of a relatively weak gel at 20°C.

The presence of synergistic effects between potassium-kappa-carrageenan and other cations became even more evident when calcium was added to the potassium form. A gel with a storage modulus of 360 Pa already formed after an addition of 2 mM calcium whereas 30 mM calcium was required for a gel with a storage modulus of 160 Pa for the pure calcium form. Figure 14 shows that the storage modulus increased drastically with increasing calcium concentrations and a gel with a storage modulus as high as 35 kPa formed when 20–30 mM calcium was added to the potassium form at 20°C. The sharp initial peak typical of potassium-induced gels could not be seen when calcium was added to the potassium form. The curves increased to a plateau value except for the addition of 10 mM, where there was a broad maximum. At the lowest level of calcium addition the gel developed slowly, and equilibrium was not reached within the experimental time. The synergistic effects between calcium and potassium resulted in considerably stronger gels than any gels of pure ion forms of kappa-carrageenan.

Calcium-kappa-carrageenan

The addition of sodium to the calcium form resulted in a weak gel when 250 mM sodium was added, as shown in Fig. 15. No gel formed at sodium concentrations of 200 mM and below. Neither did a gel form at a higher sodium concentration of 500 mM, where salting out took place. The calcium form also showed salting out effects at moderate calcium concentrations (100 mM Ca^{2+}). No synergistic effects were observed between sodium and calcium, and addition of sodium to the calcium form resulted in poor gel-forming stability.

Strong synergistic effects between potassium and calcium were seen when potassium was added to the calcium form of kappa-carrageenan. Figure 16 shows that an addition of 10 mM potassium resulted in a storage modulus of 7 kPa, whereas 230 Pa was obtained for the pure potassium form in 10 mM KCl. A storage modulus of as much as 43 kPa was obtained when 100 mM potassium was added to the calcium form. The storage modulus increased rapidly to the final value, and the transient peak typical of the pure potassium systems was not observed when potassium was added to the calcium form. Interesting to note is that the gelation temperatures were 5–7°C lower than the temperatures of the coil–helix transitions in the concentration range of 30–100 mM potassium. This indicates that the aggregation steps in the potassium–calcium systems are different in character to those of the pure potassium system in this concentration range.

Intermediate forms

The effects of mixed ions on the gelation of the pure ion forms of kappa-carrageenan show that it is not adequate to make any general statements about a cation effect without specifying the ion form. It was then of interest to see whether any synergistic effects could be obtained by manipulating the composition of the counter-ions. Since the strongest synergistic effects were observed between potassium and calcium, intermediate forms were studied with varying ratios of potassium to calcium.

Figure 17 shows the effects on the storage modulus when small amounts of calcium were added to the pure calcium form, intermediate forms and the pure potassium form of kappa-carrageenan. As previously discussed, 30 mM potassium was needed for gel formation of the pure calcium form. When the potassium to calcium ratio was 0·4 gels formed

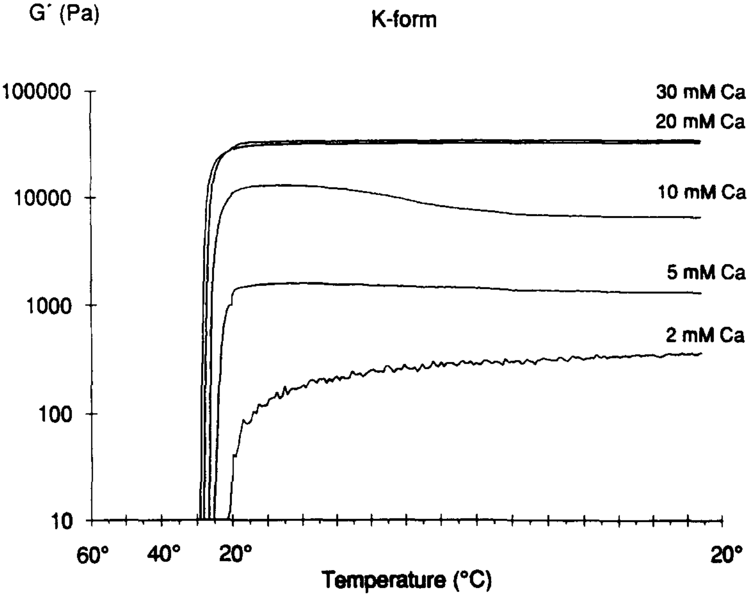

Fig. 14. The storage modulus G′ of 1% potassium-kappa-carrageenan during gelation in 2, 5, 10, 20 and 30 mM $CaCl_2$.

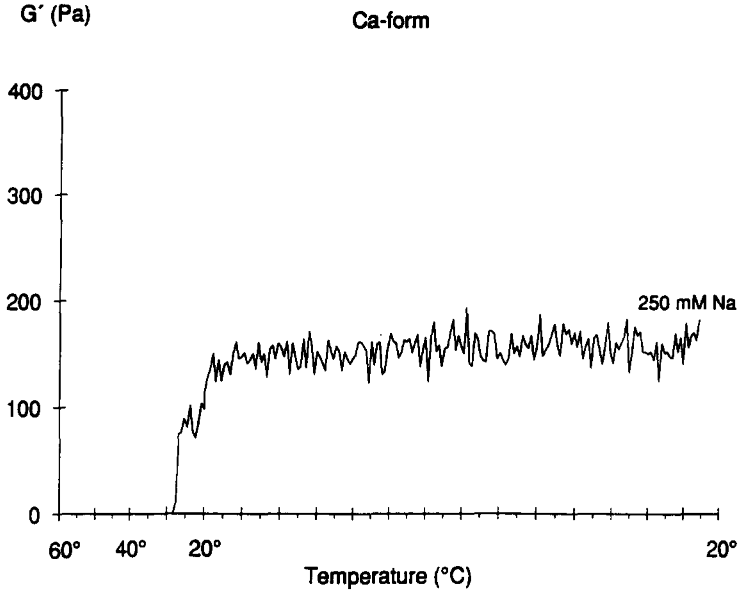

Fig. 15. The storage modulus G′ of 1% calcium-kappa-carrageenan during gelation in 250 mM NaCl.

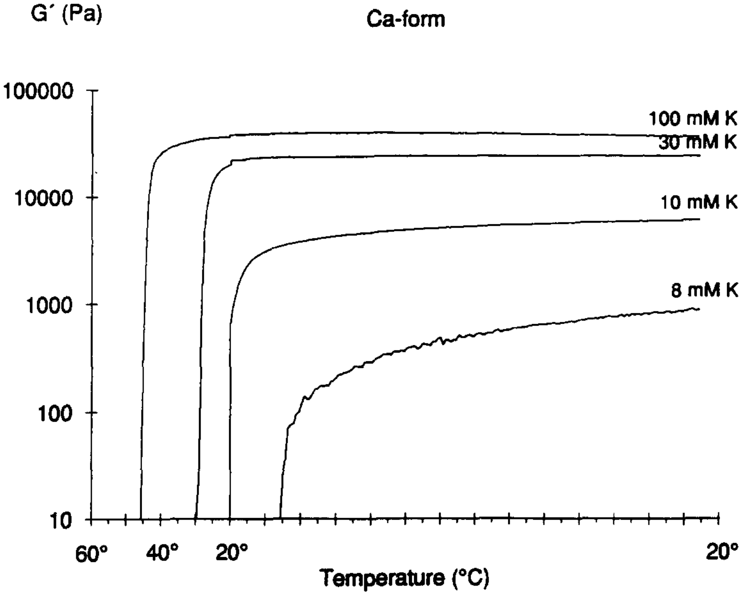

Fig. 16. The storage modulus G′ of 1% calcium-kappa-carrageenan during gelation in 8, 10, 30 and 100 mM KCl.

on the addition of 10 mM calcium, and addition of 30 mM calcium resulted in a very strong gel. At the higher potassium to calcium ratio a gel already formed in 3 mM calcium, and the addition of 10 and 30 mM resulted in strong gels. The strongest effect was obtained for the pure potassium form, where an addition of only 3 mM calcium resulted in a fairly strong gel.

Figure 18 shows the effects when small amounts of potassium were added to pure and intermediate forms of kappa-carrageenan. For the pure calcium form 10 mM potassium was needed for gelation. The intermediate form with a K/Ca ratio of 0·4 needed an addition of only 5 mM potassium for gels to form. When the ratio of K/Ca was increased to 0·8, gels already formed after an addition of 2 mM potassium. For the pure potassium form, an addition of 2 or 5 mM potassium was not enough for gels to form. This clearly illustrates the presence of strong synergistic effects between potassium and calcium.

Both Figs 17 and 18 show that very strong gels can form with mixtures of cations. In the previous paper, it was demonstrated that strong gels also formed when a high concentration of 200 mM potassium was added to the pure potassium form (Hermansson, 1989). This gel was composed of a mixed network structure, where the stabilized fine

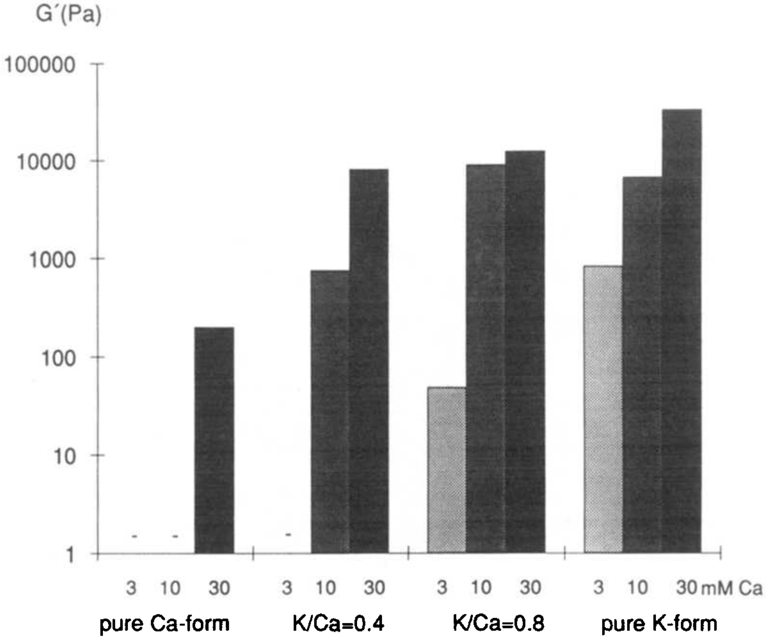

Fig. 17. The storage modulus G′ of pure and intermediate ion forms of kappa-carrageenan showing synergistic effects on addition of $CaCl_2$.

structure was intertwined with the coarse supramolecular network. It seems reasonable to assume that the strong gels discussed in this paper are composed of mixed networks. Figure 19 shows a micrograph of the intermediate form with a K/Ca ratio of 0·8 in 5 mM calcium. A fine as well as a coarse supramolecular network can be seen. The fine structure is similar in character to that of the pure calcium form shown in Fig. 10. The coarse network has long stiff superstrands. The stiffness is similar to that of the pure potassium superstrands but the composition of the superstrands seems to differ. The superstrands of the intermediate form are built up of many very fine strands, and some of these fine strands seem to interact with the strands of the fine network structure. In the pure potassium system, two to three assemblies of double helices are aligned into the superstrand, often with a small interspace between. The interactions between the fine and the coarse network probably contribute to the gel strength. So far little information is available about the formation of mixed gels from a single polysaccharide, and further studies will be made on the kinetics involved.

The results have shown that both the gel temperature and the gel strength can be varied by the choice of cations. As an example, weak gels with a storage modulus of 70 Pa formed when calcium was added to the

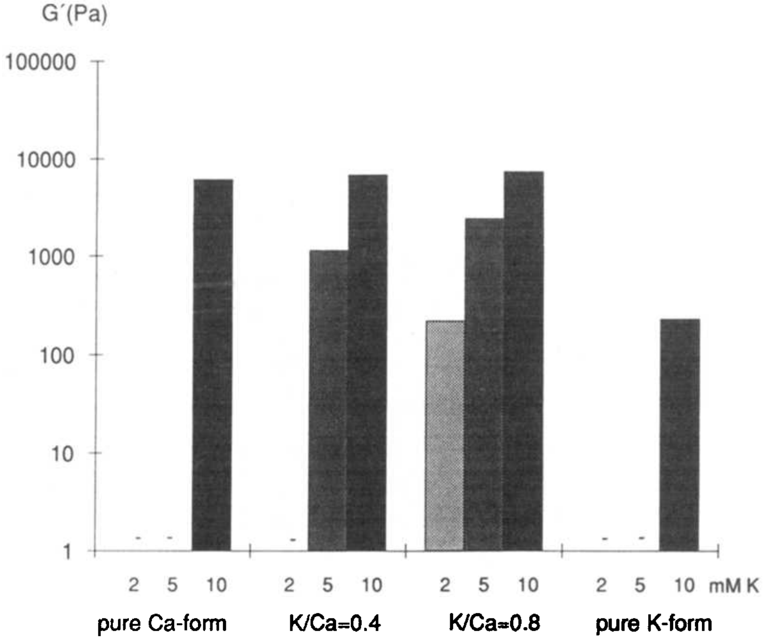

Fig. 18. The storage modulus G′ of pure and intermediate ion forms of kappa-carrageenan showing synergistic effects on addition of KCl.

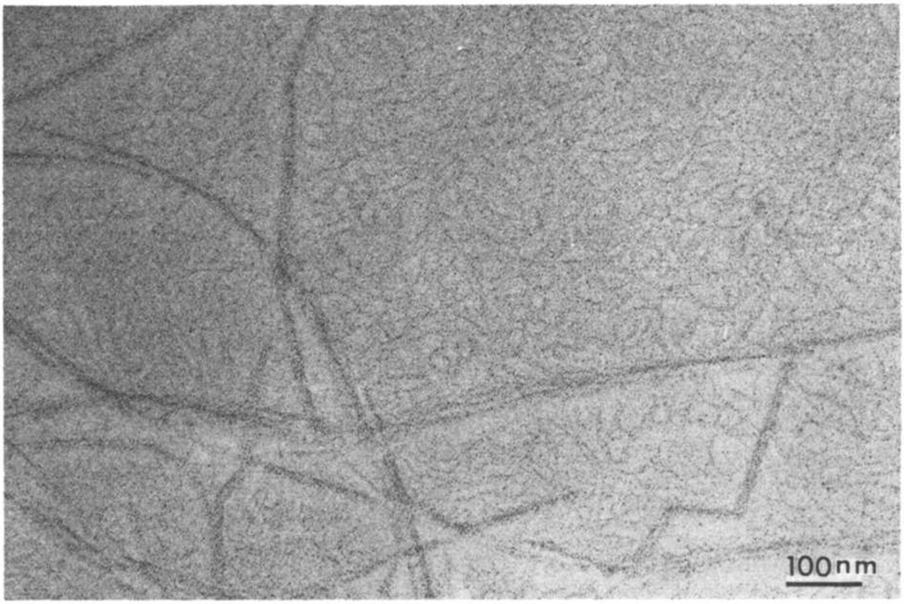

Fig. 19. The structure of an intermediate form of kappa-carrageenan with a K/Ca ratio of 0·8 in 5 mM $CaCl_2$ showing a mixed network structure.

sodium form, whereas gels with storage moduli of 43 kPa formed due to synergistic effects between calcium and potassium at a kappa-carrageenan concentration of 1%. The intermediate forms indicate the possibilities of controlling the gel strength at a given temperature by the composition of counter-ions. Structure engineering in the temperature range 20–40°C is of importance for the food as well as for the pharmaceutical industry.

ACKNOWLEDGEMENTS

The authors would like to thank Conny Haraldsson and Stig Westerlund for atomic absorption measurements. The financial support from STU — The Swedish Board of Technical Development — is gratefully acknowledged.

REFERENCES

Belton, P. S., Morris, V. J. & Tanner, S. F. (1985). *Int. J. Biol. Macromol.*, **7**, 53.
Grasdalen, H. & Smidsrød, O. (1981). *Macromolecules*, **14**, 229.

Hermansson, A. M. (1989). *Carbohydrate Polymers*, **10**, 163.
Higgs, P. G. & Ball, R. C. (1989). *J. Phys. France*, **50**, 3285.
Morris, E. R., Rees, D. A. & Robinson, G. (1980). *J. Mol. Biol.*, **138**, 349.
Morris, V. J. & Chilvers, G. R. (1983). *Carbohydrate Polymers*, **3**, 129.
Nilsson, S., Piculell, L. & Jonsson, 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.
Rochas, C. & Rinaudo, M. (1980). *Biopolymers*, **19**, 1675.
Rochas, C. & Rinaudo, M. (1984). *Biopolymers*, **23**, 735.
Stading, M. & Hermansson, A. M. (1990). *Food Hydrocolloids*, **4**, 121.
Watanase, M. & Nishinari, K. (1981). *J. Texture Studies*, **12**, 427.
Watanase, M. & Nishinari, K. (1986). In *Gums and Stabilizers for the Food Industry 3*, ed. G. O. Phillips, J. D. Wedlock & P. A. Williams. Elsevier Applied Science Publishers, London, pp. 185–94.