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Rheology and microstructure of Ca- and Na-κ-carrageenan and locust bean gum gels

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The viscoelastic and microstructural influences of 0.1–0.6% locust bean gum on 0.5 or 1.0% κ-carrageenan gels, in different ionic environments, have been studied using small deformation oscillatory measurements and transmission electron microscopy (TEM). The results from the rheological measurements showed synergistic effects in the storage modulus, G′, as locust bean gum, of two different mannose to galactose ratios (3 and 5), was mixed with ion-exchanged Na- and Ca-κ-carrageenan, in 0.25 M NaCl and 0.030 M $CaCl_2$, respectively. The increase in G′ was dependent on the mannose to galactose ratio, polymer concentrations, and ionic environment.

At the supermolecular level, the microstructure of dilute samples has been visualised using low angle rotary metal shadowing for TEM. In the presence of sodium and calcium ions, the self-association of κ-carrageenan helices is moderate to low. Locust bean gum did not influence the supermolecular structure of κ-carrageenan to any large extent. The microstructure of the gels at the network level was studied using plastic embedding and thin sectioning for TEM. In both sodium and calcium ionic environments, the mixed gels showed a more homogeneous and connective network structure. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Mixtures of κ-carrageenan and galactomannan are widely used additives in the food industry because of their textural and structural enhancing properties (Damásio *et al.*, 1994; Stanley, 1990). κ-Carrageenan is a polyanionic galactan extracted from red marine algae. Preceding gelation the polysaccharide goes through a thermally induced disorder–order conformational change. The disorder–order transition and subsequent aggregation and network formation are influenced by the ionic composition of the aqueous environment (Rochas *et al.*, 1980; Rochas & Rinaudo, 1980, 1984; Morris *et al.*, 1980a, b; Morris & Norton, 1983; Grasdalen & Smidsrød, 1981a, b; Norton *et al.*, 1984). In the presence of sodium or calcium ions, aggregation of κ-carrageenan helices is limited compared with aggregation in a potassium environment (Morris & Norton, 1983; Hermansson, 1989; Hermansson *et al.*, 1991; Turquois *et al.*, 1995). The κ-carrageenan gels formed in the presence of sodium and calcium ions are much weaker than the gels formed in potassium chloride (Morris *et al.*, 1980b; Watase & Nishinari, 1981, 1982; Hermansson *et al.*, 1991). Hermansson and co-workers have visualised the microstructure of self-associating κ-carrageenan, under different ionic environments, using high-resolution electron microscopy. They observed an increased κ-carrageenan self-association as the ion content was varied in the order $Ca^{2+} < Na^{+} \ll K^{+}$ (Hermansson *et al.*, 1991).

The non-gelling polysaccharide locust bean gum is a galactomannan harvested from the seeds of the tree *Ceratonia siliqua*. Locust bean gum consists of a linear chain of mannose residues that is non-regularly substituted by galactose residues (McCleary *et al.*, 1985). For locust bean gum, the ratio mannose to galactose varies between 3 and 5 depending on the source and preparation method. In solution, locust bean gum adopts a conformationally disordered random coil structure. Structural ordering by freeze–thaw treatment or long standing times can occur (Dea & Morrison, 1975; Dea *et al.*, 1972, 1977).

Mixtures of κ-carrageenan and locust bean gum show unexpected, non-additive rheological behaviour,

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i.e. synergism (Ainsworth & Blanshard, 1978; Tako & Nakamura, 1986; Carins *et al.*, 1986; Arnaud *et al.*, 1989; Fernandes *et al.*, 1991; Turquois *et al.*, 1992, 1994; Stading & Hermansson, 1993; Goycoolea *et al.*, 1995) and a reduced tendency toward syneresis compared with pure κ-carrageenan gels (Stanley, 1990). The origin of these effects has been ascribed to an interaction between κ-carrageenan helices and the random coil polymer, locust bean gum (Dea *et al.*, 1972, 1977; Williams *et al.*, 1992, 1993; Pilculeli *et al.*, 1994; Goycoolea *et al.*, 1995). Most studies on mixtures of κ-carrageenan and galactomannan have been performed in pure water or in potassium chloride (Tako & Nakamura, 1986; Fernandes *et al.*, 1991; Turquois *et al.*, 1992, 1994; Goycoolea *et al.*, 1995). These studies have shown that addition of locust bean gum significantly increases the gel strength, and that the effect is strongest when potassium ions are present. Tako and Nakamura (1986) studied the viscoelasticity of ion-exchanged Na- and Ca-κ-carrageenan mixed with locust bean gum in water. In the absence of salt, they did not find any synergistic effects of locust bean gum addition. The synergistic effects observed when κ-carrageenan is mixed with galactomannans have also been found to be dependent on the mannose to galactose ratio. Higher mannose to galactose ratio is favourable (Dea *et al.*, 1972; Dea & Morrison, 1975; Rees, 1972a, b; Fernandes *et al.*, 1991; Lundin & Hermansson, 1995b).

In an earlier study, we have elucidated the microstructural and rheological influences of locust bean gum on K-κ-carrageenan in potassium chloride (Lundin & Hermansson, 1995b). We observed non-additive effects in the viscoelastic properties of the mixtures, and that the self-association of K-κ-carrageenan was hindered by the presence of locust bean gum. To further elucidate the effects of locust bean gum on the properties of helical κ-carrageenan in different ionic environments, we have performed microscopy and viscoelastic measurements on ion-exchanged Na- and Ca-κ-carrageenan mixed with locust bean gum of two different M:G ratios. The experiments have been performed in the presence of 0.25 M NaCl or 0.030 M $CaCl_2$ (i.e., ionic environments which induce helical κ-carrageenan and moderate to low degrees of κ-carrageenan self-association at 20°C). The results demonstrate that addition of locust bean gum to κ-carrageenan in a sodium or calcium ionic environment leads to synergistic viscoelastic properties.

MATERIALS AND METHODS

Materials

κ-Carrageenan from *Euchema cottonii* type III (lot 54H0504) and locust bean gum (lot 40H0160) were purchased from Sigma Chemicals (St. Louis, MO, USA). The pure sodium and calcium forms of κ-carrageenan were prepared by a method described by Hermansson *et al.* (1991). 1.0% κ-carrageenan solution was ion-exchanged in a column, maintained at 90°C, with a commercial resin AG 50W-8X (Bio Rad) and freeze-dried. The locust bean gum was fractionated into two fractions depending on solubility at different temperatures, according to the procedure described in an earlier paper (Lundin & Hermansson, 1995a). The samples used are the fractions soluble at 35°C and below and at 65–80°C. The fraction will be called lbg35 and lbg80, respectively. All polysaccharide mixtures were dissolved during constant stirring, in a 90°C waterbath. Depending on the ion form of the κ-carrageenan, the polysaccharides were dissolved in 0.25 M NaCl or 0.030 M $CaCl_2$. As reported by Rochas and Rinaudo (1980), the coil–helix transition temperature for κ-carrageenan, under these conditions is in the region of 20°C. In order to be below the transition temperature, the polysaccharide mixtures have been studied at temperatures below 15°C.

Viscoelastic measurements

The small deformation oscillatory measurements were performed in a Bohlin VOR Rheometer (Bohlin Rheology, Lund, Sweden). The measuring system was a serrated coutte type cup and bob measuring system (DIN 53019). The bob was suspended in an interchangeable torsion bar with a maximum deflection of between 4.10^{-4} Nm and 9.10^{-3} Nm. The strain was kept as low as 4.10^{-4} in order not to disturb the network. This is well within the linear region. The frequency was 1 Hz when the temperature was the independent variable and 0.001–10 Hz during the dynamic spectra. Samples at 90°C were transferred to the rheometer, which was preheated to 40°C. The sample was equilibrated for 5 min at 40°C. Viscoelastic properties were recorded as the temperature was varied linearly at 1.5°C/min from 40 to 15°C and maintained at that temperature for 1 h. The frequency dependence was recorded at 15°C followed by a heating gradient. The sample was heated until the gel melted. The onset of gelation is taken as the temperature where G' is over the noise level. The onset of melting is taken as the temperature where G' starts to decrease, during heating. As a rough measure for the temperature where the gel is fully melted, the temperature where G' and G'' cross at 1 Hz is taken.

The κ-carrageenan concentration was 0.5 or 1.0%, and the locust bean gum concentration varied between 0.1 and 0.6%. All concentrations given are in weight percent (w/w). Depending on the ion form of κ-carrageenan, either 0.25 M NaCl or 0.030 M $CaCl_2$ was used as solvent.

Microscopy

Low-angle rotary metal shadowing

Samples for the mica sandwich technique were cooled to a temperature below 15°C before they were applied to the mica surface. A small volume of polysaccharide mixture was spread on a freshly cleaved mica surface and the corresponding mica surface put on top. The sandwich was rapidly frozen in liquid nitrogen, cleaved below the surface and transferred to a precooled Balzer VOR 400 freeze-etching system. The sample was sublimated at −90°C for 2 h, after which followed low angle rotary shadowing. Metal replicates of the polymer monolayer were studied in a transmission electron microscope (Jeol 100 CX-II, Jeol Ltd, Tokyo, Japan), at an accelerating voltage of 80 kV. This procedure has been described in greater detail earlier (Lundin & Hermansson, 1995a). Samples containing 0.01% pure κ-carrageenan and 0.01% κ-carrageenan mixed with 0.002% or 0.01% locust bean gum were studied.

Plastic embedding and thin sectioning

Gels containing 1.0% pure κ-carrageenan and 1.0% κ-carrageenan mixed with 0.1 or 0.5% locust bean gum were plastic-embedded and thin-sectioned for transmission electron microscopy. Depending on the ionic form of the κ-carrageenan, Na or Ca, the mixtures were dissolved in 0.25 M NaCl or 0.030 M $CaCl_2$. After 1 h storage at 15°C, small cubes of the gel, 1×1×1 mm, were cut out. The gel cubes were fixed in 2% glutaraldehyde followed by 1% OsO_4. The samples were then rinsed several times before dehydration in a graded ethanol series. To prevent osmotic effects, the fixative solutions and washing solutions contained the suitable salt, NaCl or $CaCl_2$. The gels were then plastic-embedded in LR White (Laboratories Equipment Ltd, Reading, UK) medium grade resin at 60°C. The samples were thin-sectioned (~80 nm) and stained with uranylacetate. Sections were studied in a transmission electron microscope (Jeol 100 CX-II, Jeol Ltd, Tokyo, Japan), at an accelerating voltage of 80 kV.

RESULTS

Microstructure

Two different preparation techniques for transmission electron microscopy have been used to visualise the network structure of κ-carrageenan and locust bean gum gels. The "mica sandwich" technique generates a possibility of visualising monolayers of polymer samples at the supermolecular level. Using this technique, it is possible to study how the polymers influence one another and aggregate in dilute systems. Preparation by plastic embedding and thin sectioning, on the other hand, enables examination of the network at higher concentrations. This method gives us information about the homogeneity and connectivity of the gel network.

Supermolecular structures

The effects of different amounts of locust bean gum on the supermolecular structure of κ-carrageenan have been studied using the mica sandwich technique. The random coil structure of locust bean gum is not at the moment possible to visualise by the low angle rotary metal shadowing technique. Consequently, it is possible only to observe differences in the κ-carrageenan microstructure.

Figure 1a shows Na-κ-carrageenan in 0.25 M NaCl at

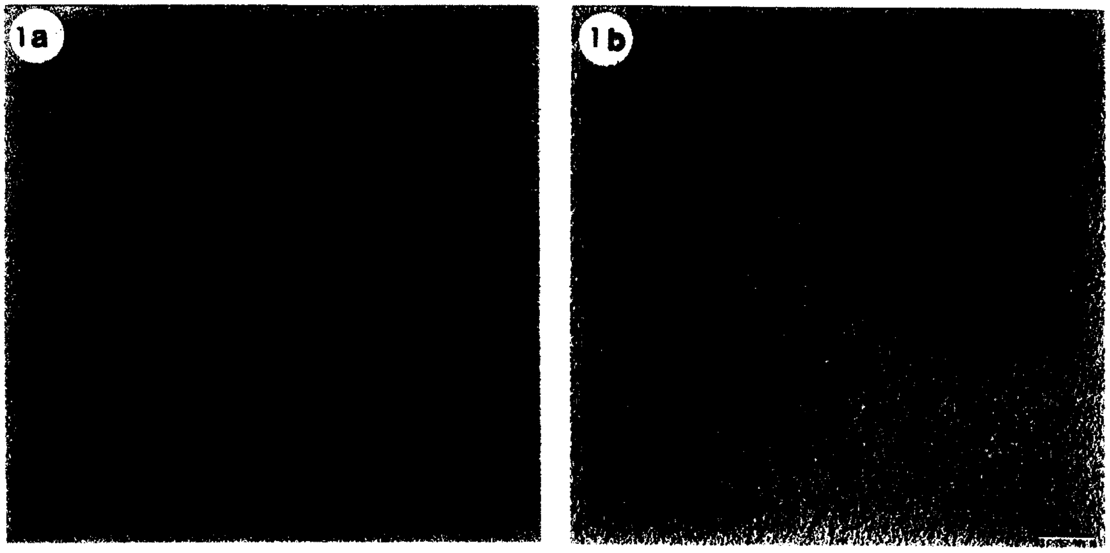

Fig. 1. Micrographs showing the supermolecular structure of (a) 0.01% Na-κ-carrageenan; and (b) 0.01% Na-κ-carrageenan mixed with 0.01% locust bean gum (M:G~5) in 0.25 M NaCl. Scale bar = 100 nm.

15°C. At this temperature the polysaccharide is below its configurational transition temperature and in the helical form. Na-κ-carrageenan forms supermolecular strands with a low degree of helical self-association. In the micrograph, it is not possible to discern the number of helices building each strand. However, no coarse supemolecular assemblies, similar to those formed by K-κ-carrageenan in 0.10 M KCl (Hermansson, 1989Hermansson *et al.*, 1991), can be observed. Independent of the amount of locust bean gum and M:G ratio, addition of locust bean gum did not lead to any observable difference in the Na-κ-carrageenan microstructure. To illustrate the similarity between pure Na-κ-carrageenan and mixtures of Na-κ-carrageenan and locust bean gum, the mixture containing locust bean gum lbg80 is presented in Fig. 1b. In the micrograph showing the microstructure of the mixture, the relatively unperturbed Na-κ-carrageenan structure with supermolecular strands can be observed.

The microstructure of Ca-κ-carrageenan in 0.030 M $CaCl_2$ at 15°C is presented in Fig. 2a. At this temperature the polymer is below its transition temperature and in its helical conformation. The pure Ca-κ-carrageenan forms flexible supermolecular strands of a uniform dimension, which are mixed with strands of a finer dimension. The fine strands are at the limit of resolution obtained with this technique. Figure 2a shows the two structures observed for pure Ca-κ-carrageenan. A decreasing amount of flexible supermolecular strands is seen as locust bean gum is added to the Ca-κ-carrageenan solution. This is most predominant for mixtures containing lbg80. Figure 2b shows the fine structure of Ca-κ-carrageenan mixed with lbg80. Mixtures of Ca-κ-carrageenan and lbg35 and lbg80 showed a decreased tendency to form flexible supermolecular strands as the locust bean gum concentration as well as the M:G ratio increased.

Homogeneity and connectivity of the gel network

Plastic embedding and thin sectioning for the transmission electron microscope enable examination of the network structure of the mixture in a different concentration range. Thin sectioning gives overall information on the homogeneity and connectivity of the gel structure but generally less detail than the mica sandwich technique. Plastic embedding leads to large strains on the gel network, which can perturb the network structure. To limit these effects, a low viscosity resin and stepwise dehydration have been applied to the samples. Mixtures containing 1.0% Na- or Ca-κ-carrageenan have been mixed with 0.1% and 0.5% lbg35 or lbg80 and plastic-embedded.

The pure Na-κ-carrageenan network is shown in Fig. 3a. The pure ionic form of Na-κ-carrageenan forms an open network structure. It is not possible to distinguish any details of the individual strands building the network. Figure 3b and Fig. 3c show micrographs of the network structure of 1.0% Na-κ-carrageenan mixed with 0.50% lbg35 or 0.50% lbg80, in 0.25 M NaCl. The mixed network formed has a decreased porosity and it is more homogeneously spread in space. The same behaviour is observed in the mixed Ca-κ-carrageenan gels, see Fig. 4b and Fig. 4c. The structural effects of locust bean gum on the κ-carrageenan networks are enhanced with increased locust bean gum concentration and as the M:G ratio is varied from 3 to 5. Phase separation, into domains of different structure enriched in one of the biopolymers, was not observed for any of the mixtures by any microscopy methods used.

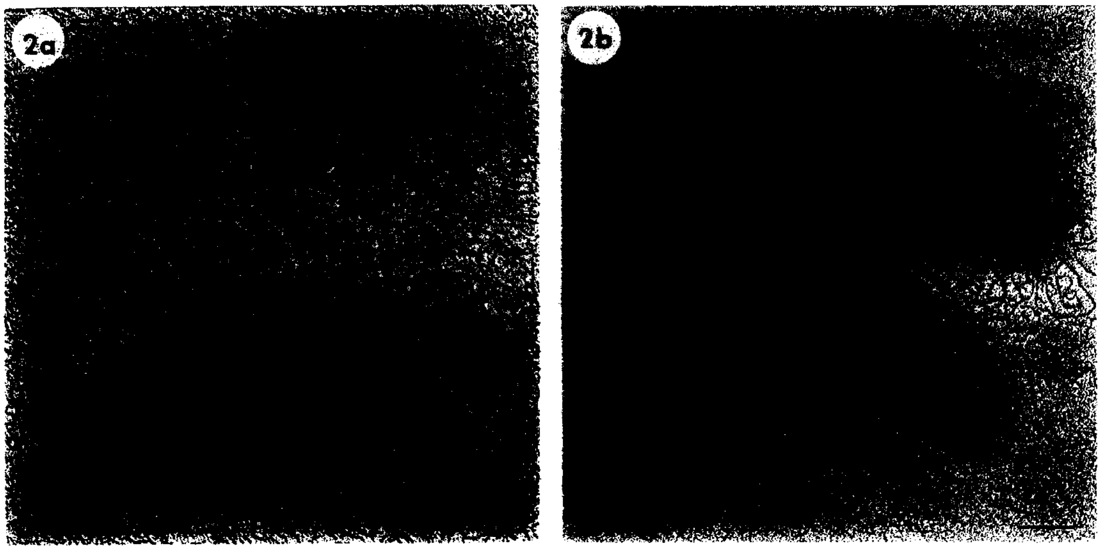

Fig. 2. Micrographs showing the supermolecular structure of (a) 0.01% Ca-κ-carrageenan; and (b) 0.008% Ca-κ-carrageenan mixed with 0.002% locust bean gum (M:G~5) in 0.030 M $CaCl_2$. Scale bar = 100 nm.

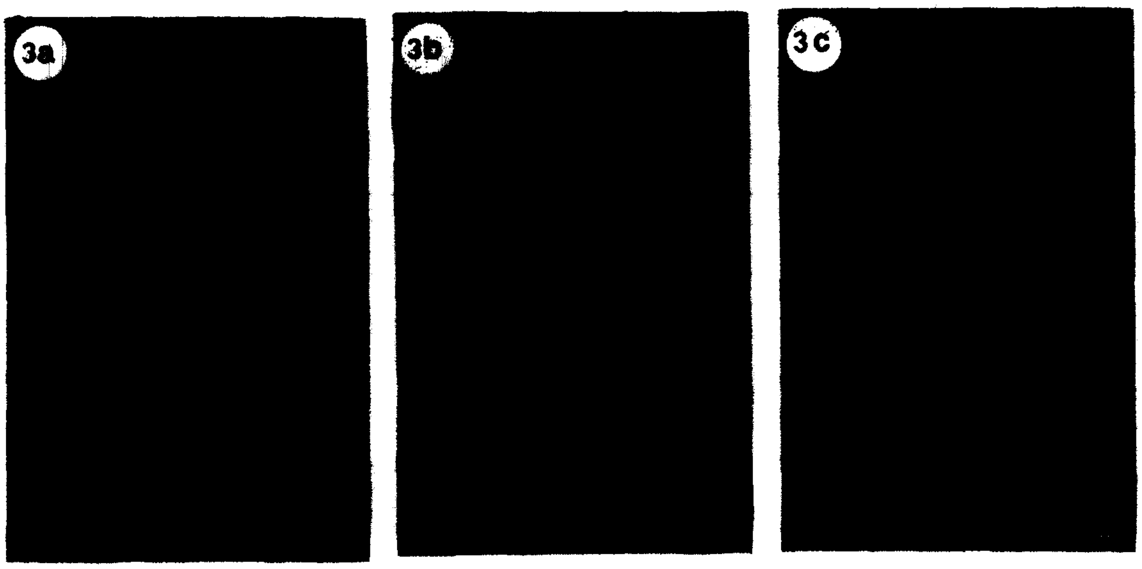

Fig. 3. Micrograph showing the network structure (a) 1.0% Na-κ-carrageenan and 1.0% Na-κ-carrageenan mixed with (b) 0.5% locust bean gum (M:G~3); (c) 0.5% locust bean gum (M:G~5), in 0.25 M NaCl. Scale bar = 500 nm.

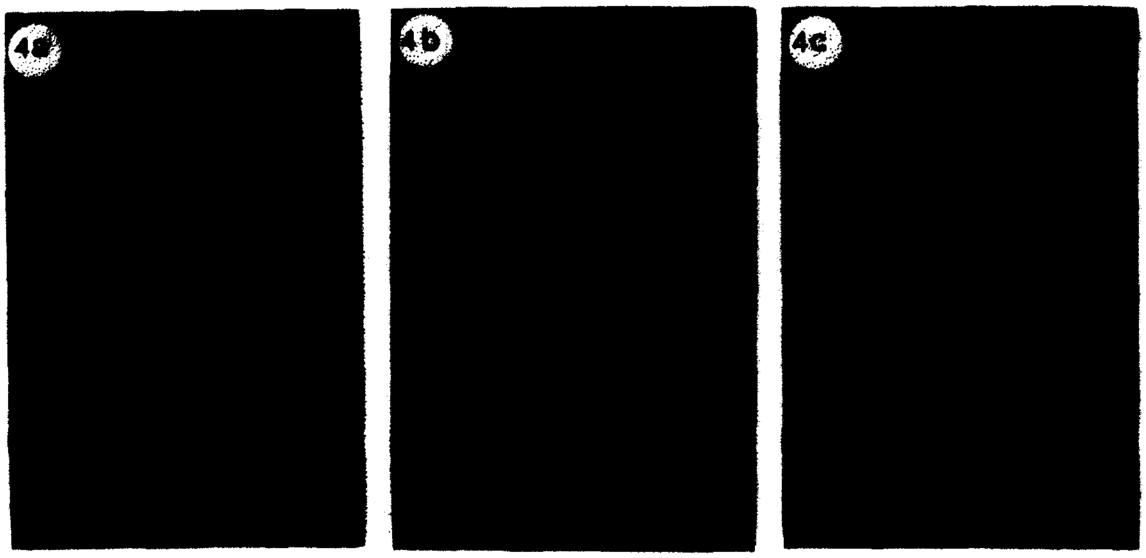

Fig. 4. Micrograph showing the network structure (a) 1.0% Ca-κ-carrageenan and 1.0% Ca-κ-carrageenan mixed with (b) 0.5% locust bean gum (M:G~3); (c) 0.5% locust bean gum (M:G~5), in 0.030 M $CaCl_2$. Scale bar = 500 nm.

Viscoelastic behaviour of mixed networks

To study the effect of the differences in microstructure on the viscoelastic behaviour of κ-carrageenan–locust bean gum mixtures, we have performed small deformation oscillatory measurements on the pure ionic forms of κ-carrageenan and mixtures containing lbg35 or lbg80. The rheological influences of locust bean gum on Na- and Ca-κ-carrageenan gels have been evaluated in 0.25 M NaCl and 0.030 M $CaCl_2$, respectively. The viscoelastic properties were recorded during cooling from 40°C, at 15°C for 1 h, and during heating from 15°C (gradient 1.5°C/min).

Figure 5 shows the storage modulus, G′, and phase angle for 1% Na-κ-carrageenan in 0.25 M NaCl during cooling and heating. At 15°C, pure Na-κ-carrageenan forms an elastic gel at 1.0% concentration. After 1 h at 15°C, the storage modulus (G′) was 150 Pa and the phase angle was 3°. At 1 Hz, the onset of the gelation occurred at 19°C. The onset of melting occurred at 20°C and the gel was fully melted at 42°C. At the lower polysaccharide concentration, 0.5% Na-κ-carrageenan, no measurable gel was detected.

When we studied the mixtures, the Na-κ-carrageenan concentration was kept constant at either 0.50 or 1.0% and an increasing amount of lbg35 or lbg80 was added. Figure 6 describes the storage modulus as a function of added locust bean gum concentration, at 15°C.

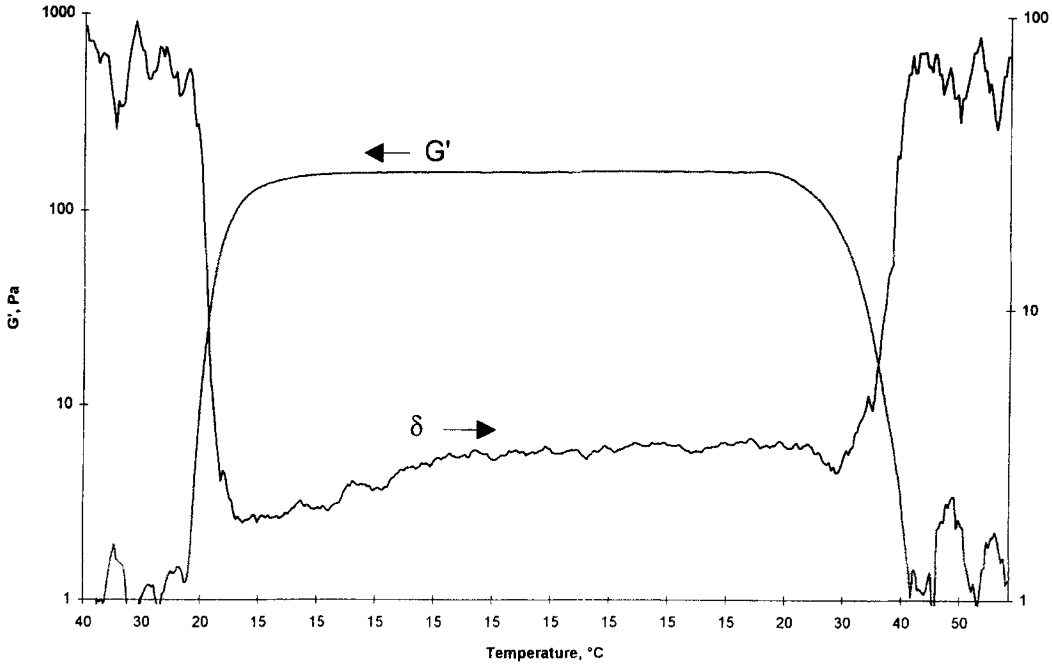

Fig. 5. Storage modulus (G′) and phase angle (δ) as a function of time during cooling and heating, 1.5°C/min, for 1.0% Na-κ-carrageenan, in 0.25 M NaCl.

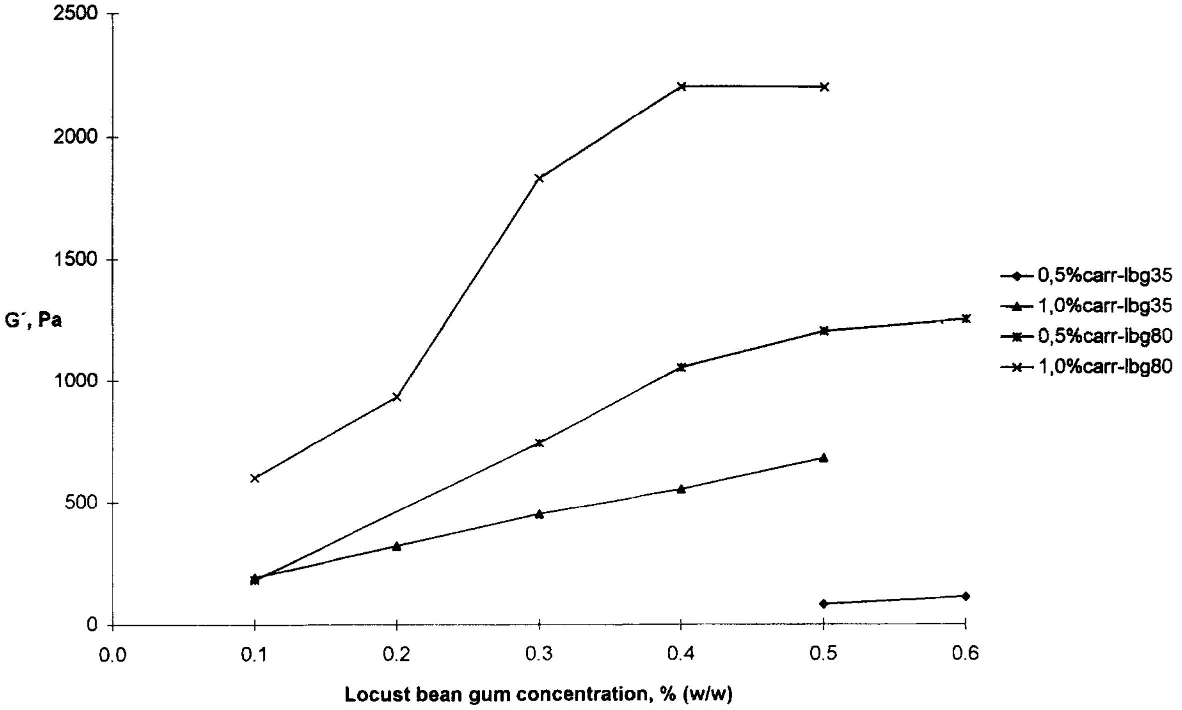

Fig. 6. Storage modulus (G′) as a function of added locust bean gum concentration for 0.50% and 1.0% Na-κ-carrageenan mixed with 0.1–0.6% locust bean gum, in 0.25 M NaCl after 5 min at 15°C.

Addition of lbg35, which has a M:G ratio of 3, to κ-carrageenan led to a moderate increase in G′. The non-gelling system of 0.50% κ-carrageenan formed a network as 0.50% lbg35 was added. A slight increase was observed when the lbg35 concentration was increased to 0.60%. The phase angles were around 6°. At an increased κ-carrageenan concentration (1.0%), the measured effects of lbg35 on G′ were larger. An increase in lbg35 concentration from 0.10% to 0.50% led to an increase in G′ from 200 to 680 Pa and phase angles between 3 and 4°.

The mixtures containing lbg80 showed the same behaviour, although the effect on the viscoelastic properties was more pronounced. The mixture with 0.50% κ-carrageenan and lbg80 gelled as 0.10% locust bean gum was added, and a significant increase in G′ was measured as the concentration was increased to 0.60%. The phase angles were in the region of 2–6°. At 1.0% κ-carrageenan, the G′ also increased as the lbg80 concentration was increased. The strongest gels were obtained as 0.40 and 0.50% lbg80 was added. The increase in G′ levels off at these concentrations. The phase angles for these mixtures were in the region of 8–11°. For the mixtures of κ-carrageenan–locust bean gum in 0.25 M NaCl, the onset of melting occurred around 20°C, and the gels were fully melted at between 40 and 60°C. It was observed that the gels containing lbg80 showed a tendency to be fully melted at temperatures closer to 60°C. Figure 7 shows the dependence of G′ and phase angle on temperature during cooling and heating for the mixture of 1.0% Na-κ-carrageenan and 0.5% lbg80. During the 1 h standing time at 15°C a slow increase in phase angle from 1 to about 10° is observed. The melting behaviour for the mixture shows two-step melting. During melting the phase angle goes through a maximum before the gel fully melts.

The changes in G′ and phase angle during cooling and melting for 1.0% Ca-κ-carrageenan are presented in Fig. 8. Pure Ca-κ-carrageenan does not form a gel at 0.50% at 15°C in 0.30 M $CaCl_2$, although, at 1.0%, an elastic network, with G′ around 150 Pa, is formed. The phase angle was 1.5°. The onset of gelation, taken as the temperature where G′ increases sharply, occurred at 18°C. The gel started to melt immediately upon heating from 15°C and, at the frequency used for the measurements, G′ was equal to G″ at 41°C. The G′ dependence on locust bean gum concentration, at 15°C, is presented in Fig. 9. To induce gelation in the 0.50% κ-carrageenan system, at least 0.50% lbg35 had to be added. Further addition of 0.60% lbg35, led to a slight decrease in G′. The phase angles for these mixtures were 10 and 13°, respectively. At the higher κ-carrageenan concentration (1.0%), the mixture with 0.10% lbg35 formed the strongest gel. Addition of 0.30 or 0.50% led to a decrease in G′ and a slight increase in phase angle from 1 to 4°. The mixtures with lbg80 showed synergistic effects in G′ for all concentrations of added lbg80. As we increased the lbg80 concentration from 0.30 to 0.50%, the mixtures with 0.50% κ-carrageenan showed a slight increase in G′. The phase angle for these mixtures varied between 1 and 2°. At the higher κ-carrageenan concentration (1.0%), addition of 0.10–0.50% lbg80 led to more pronounced synergistic effects. The strongest gel was formed with 0.30% lbg80. The phase angle was 1° for all these mixtures.

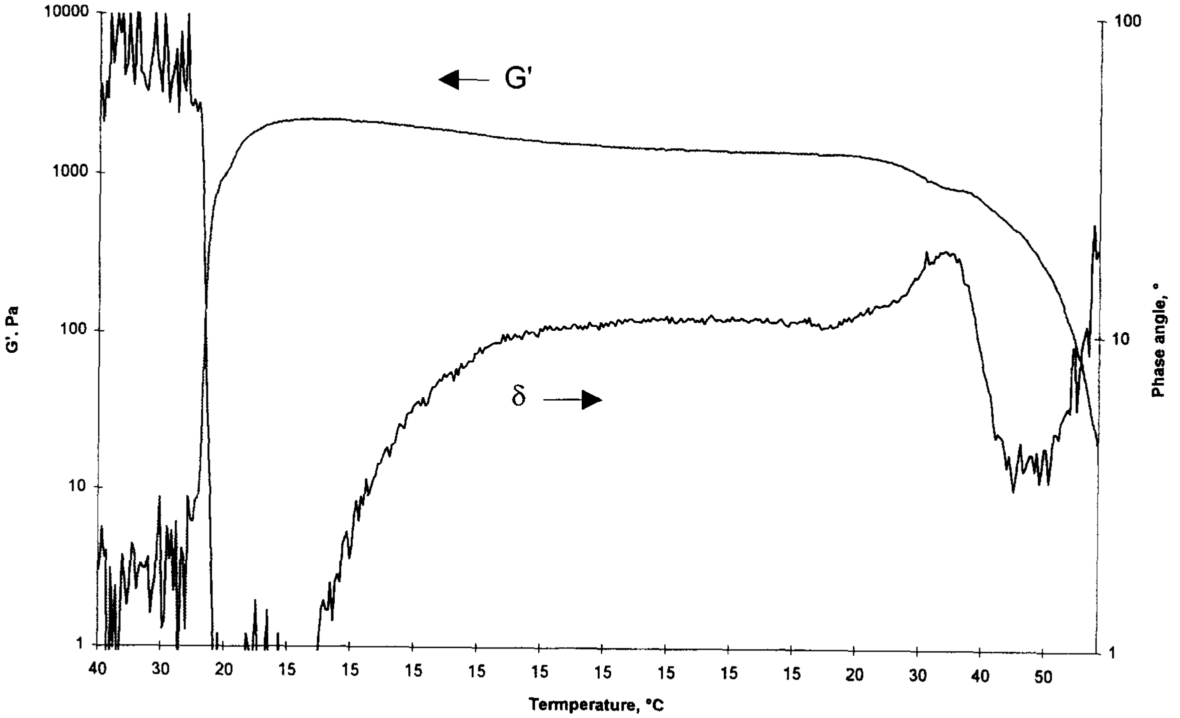

Fig. 7. Storage modulus (G′) and phase angle (δ) as a function of time during cooling and heating, 1.5°C/min, for 1.0% Na-κ-carrageenan mixed with 0.5% lbg80 in 0.25 M NaCl.

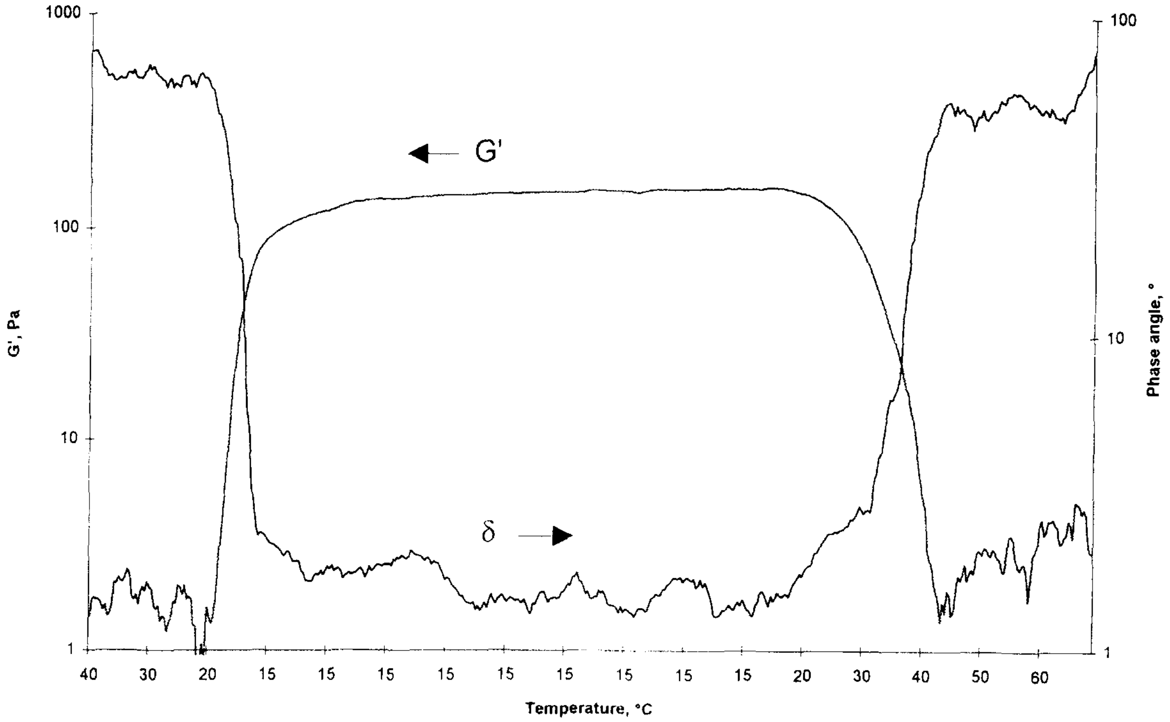

Fig. 8. Storage modulus (G′) and phase angle (δ) as a function of time during cooling and heating, 1.5°C/min, for 1.0% Ca-κ-carrageenan, in 0.030 M $CaCl_2$.

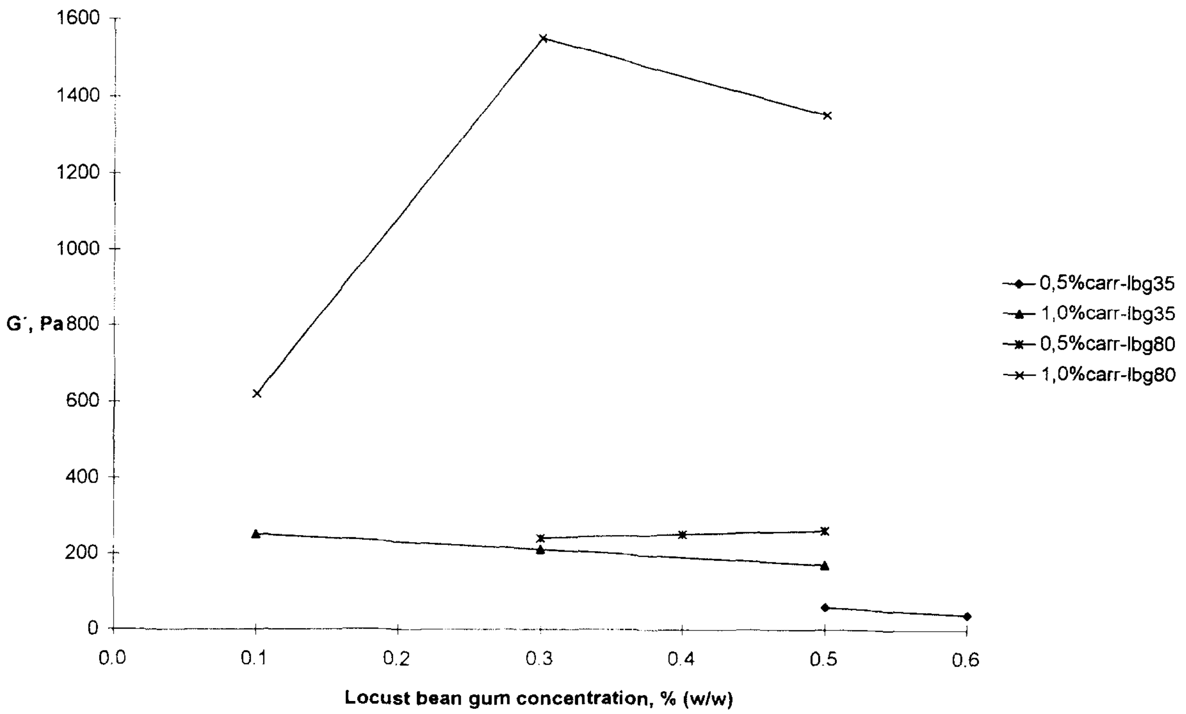

Fig. 9. Storage modulus (G′) as a function of added locust bean gum concentration for 0.50% and 1.0% Ca-κ-carrageenan mixed with 0.1–0.6% locust bean gum, in 0.030 M $CaCl_2$ after 5 min at 15°C.

DISCUSSION

Viscoelastic properties

In accordance with the results from our study of K-κ-carrageenan–locust bean gum in KCl (Lundin & Hermansson, 1995b), we have found that in the presence of sodium or calcium ions a significant increase in G′ is observed as κ-carrageenan is mixed with locust bean gum. The effect on the viscoelastic properties was more pronounced for the mixtures in NaCl compared with $CaCl_2$. The strongest effects were observed for the locust bean gum with the highest mannose to galactose ratio (lbg80 with M:G around 5).

For the mixture with 1.0% κ-carrageenan in NaCl, the increase in G′ levels off as the lbg80 concentration is above 0.40%. This could indicate that the system has reached a state where further addition of locust bean gum does not increase the strength of the network. The increase in phase angle during the 1 h at 15°C and the maximum in phase angle during melting observed for the systems with high Na-κ-carrageenan and lbg80, indicate that a structural rearrangement occurs. At 1.0% Na-κ-carrageenan, the strong synergistic effect could be explained by the formation of a mixed network consisting of supermolecular Na-κ-carrageenan strands and fine locust bean gum–κ-carrageenan strands. For the other mixtures of 0.5% Na-κ-carrageenan and locust bean gum, the Na-κ-carrageenan concentration is too low to obtain maximal viscoelastic effects of the locust bean gum.

The mixtures in $CaCl_2$ show maximum or plateau values of G′ as the galactomannan concentration is varied. The balance between the polymers seems more delicate, and the maximal viscoelastic effects are reached at lower locust bean gum concentrations. For both Na- and Ca-κ-carrageenan, the viscoelastic properties vary with the M:G ratio, polymer concentration and salt conditions.

Studying mixtures with a constant polymer concentration and varying mixing ratios, Stading and Hermansson did not observe any effect of locust bean gum on the viscoelasticity of Ca- and Na-κ-carrageenan samples in 0.030 M $CaCl_2$ and 0.25 M NaCl, respectively (Stading & Hermansson, 1993). In their study, they used a locust bean gum fraction which dissolves at 20°C, which would suggest that the mannose to galactose ratio is around 3. When this locust bean gum is used, the synergistic effects are not large enough to be detected as the κ-carrageenan concentration is decreased. Tako and Nakamura (1986) measured the viscoelastic properties of Na- and Ca-κ-carrageenan mixed with locust bean gum ($M{:}G = 4.4$) at 0°C in water. They did not observe any synergistic effects on the viscoelastic behaviour of the mixtures. The absence of synergistic viscoelastic responses is probably due to their choice of water as solvent, which suggests that the experiments were performed with κ-carrageenan in the coil state.

Influences of the ionic environment

The ionic environment plays an important part in the viscoelastic behaviour of κ-carrageenan–locust bean gum mixtures. The effects of locust bean gum vary with the ionic environment. The synergistic effect of locust bean gum on the rheology decreases in the order $K^+ > Na^+ > Ca^{2+}$. An explanation for the ionic dependence could be that the synergy is dependent on the degree of self-association of the κ-carrageenan, i.e., there is a critical degree of self-association at which the interaction between κ-carrageenan and the galactomannan is most favourable. Similar discussions have been held by Goycoolea and co-workers (Goycoolea *et al.*, 1995), who suggest that, for locust bean gum–κ-carrageenan interactions, "efficient binding requires some aggregation of the algal polysaccharide helices..." and "extensive aggregation restricts synergistic interaction by competition with heterotypic association". Piculell and co-workers have performed a model study of the adsorption of a flexible polymer onto a rigid rod. They concluded that there is a limiting strand dimension below which the curvature of the rigid rod affects the polymer adsorption (Viebke, 1996). It is possible that in the presence of sodium and calcium ions the frequency of strands with a suitable dimension is too low to obtain the strongest effects as the polymers are mixed.

Another explanation could be that the ions induce different configurations of the κ-carrageenan helices, i.e. the flexibility and elongation of single κ-carrageenan strands are influenced, and because of that the ability to interact with locust bean gum varies.

Network microstructure

Taking the limitations of the plastic embedding–thin sectioning technique into consideration, the transmission electron micrographs of thin-sectioned samples indicate that the mixed gels are more homogeneous and continuous, and that the locust bean gum concentration and M:G ratio influence the network structure. Further, the micrographs indicate that the mixtures do not separate into a κ-carrageenan-rich phase and a locust bean gum-rich phase, at the concentrations and under the conditions examined. The micrographs of dilute samples show that the self-association of κ-carrageenan in a sodium or calcium environment is low. This agrees with the low degree of thermal hysteresis, which has been thought to indicate a low degree of self-association of κ-carrageenan helices (Morris & Norton, 1983; Williams *et al.*, 1993). In the presence of sodium ions, the addition of locust bean gum did not have any observable effects on the supermolecular structure of Na-κ-carrageenan. From the micrographs of dilute samples, it is not possible to quantitatively determine the hindering effects of locust bean gum on the self-association of κ-carrageenan, since the initial degree of helical self-association in NaCl is low. Nevertheless, the capability of locust bean gum to hinder κ-carrageenan self-association may come into effect at a higher polymer concentration. The micrographs of mixtures in $CaCl_2$ show that the flexible supermolecular structure became more sparse in the mixtures as the locust bean gum concentration was increased and the M:G ratio was changed from 3 to 5. The microscopy results suggest that the ability of locust bean gum to influence the microstructure is dependent on the M:G ratio of the galactomannan, the relative concentration of the polymers and the ionic environment.

Microstructure in relation to rheology

Williams and co-workers have discussed the reason for synergy in mixtures of κ-carrageenan and konjac mannan (Williams *et al.*, 1992, 1993; Pilculell *et al.*, 1994). They suggested that there is a competitive situation between κ-carrageenan self-association and κ-carrageenan–konjac mannan interaction, and that the stoichiometry of the system significantly influences the properties of the gels. Our results support this line of reasoning. Earlier, we have shown that locust bean gum hinders the self-association of κ-carrageenan helices in KCl environment, which usually induces strong self-association, and that strong synergy is observed for these mixtures (Lundin & Hermansson, 1995b). In the sodium and calcium ionic environment studied in this paper, the self-association of helices is comparatively low, and the structural effects of locust bean gum are consequently small. However, we have observed synergistic increases in the viscoelastic properties. We believe that these effects are due to interactions between strands containing self-associated κ-carrageenan helices and locust bean gum coils, which lead to a reinforcement of the network. Depending on the relative amounts of the polymers in the binary system, mixed networks of self-associated κ-carrageenan strands and locust bean gum–κ-carrageenan strands may constitute the gel. The presence of ions seems to influence the strength of the reinforcement and, as discussed above, it is possible that the ionic effect is due to the necessity for a critical degree of κ-carrageenan self-association or to structural influences of the configuration of the κ-carrageenan helices. The effects of ions on the synergy have to be further studied in order to fully elucidate these effects.

We have elucidated the effects of locust bean gum on κ-carrageenan of different degrees of self-association. The degree of κ-carrageenan self-association has been varied by using different ionic environments, NaCl, $CaCl_2$ and, in an earlier study, KCl (Lundin & Hermansson, 1995b). It can be concluded that it is possible to observe synergy in the viscoelastic properties as locust bean gum is added to Na and Ca-κ-carrageenan in 0.25 M NaCl and 0.30 M $CaCl_2$, respectively. The synergistic effect depends on the mannose to galactose ratio, the polymer concentration as well as the ionic environment. The effects are more pronounced in the presence of sodium ions in comparison with calcium ions. Sodium and calcium induce a low degree of κ-carrageenan self-association. Addition of locust bean gum to κ-carrageenan under these conditions leads to relatively small influences on the supermolecular structure of the mixture. Nevertheless, at the network level, it was possible to observe that the mixed gels form more homogeneous networks with a higher degree of connectivity.

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