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Influence of locust bean gum on the rheological behaviour and microstructure of K–κ-carrageenan

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Mixtures of κ-carrageenan–locust bean gum (LBG) were analysed by transmission electron microscopy and dynamic viscoelastic measurements. The effect of two different mannose:galactose (M:G) ratios of LBG on the rheology and microstructure of κ-carrageenan in KCl was studied. The rheological differences observed in the mixed gel as LBGs were added were found to be dependent on M:G ratio, salt concentration and LBG content.

Monolayers of dilute samples of κ-carrageenan mixed with LBGs in varying ratios in 0.10 M KCl were visualised by low-angle rotary metal shadowing for transmission electron microscopy. The results obtained showed that LBG hindered the self-association of κ-carrageenan, and that the effect was enhanced at a higher M:G ratio and as the LBG contents were increased.

Small deformation viscoelastic measurements were performed on κ-carrageenan–LBG mixtures in 0.050 and 0.10 M KCl at constant temperatures of 38 and 48°C, respectively. Increased rheological stability was observed for mixtures containing a relatively high LBG content. Mixtures with a relatively low LBG content showed a maximum in storage modulus and an increase in phase angle. The stabilising effect of LBG on the rheology of the blends was more effective for the LBG with high M:G and as the LBG content was increased.

INTRODUCTION

Mixtures of carrageenan and galactomannan have long been known to have special features that were not able to be predicted from the performance of either of the polysaccharides. The synergistic effects on gel strength and elasticity obtained when κ-carrageenan and the galactomannan, locust bean gum (LBG), are mixed have led to the widespread application of this system in the food industry, e.g. in ice cream, yoghurt and other dairy products.

κ-Carrageenan is a polyanionic polysaccharide extracted from red marine algae. The gelation of κ-carrageenan is preceded by a coil–helix transition followed by aggregation and network formation (Morris *et al.*, 1980*b*). The coil–helix transition is influenced by anions and cations that are present, and by polymer length and salt concentration (Grasdalen & Smidsrød, 1981*a,b*; Morris *et al.*, 1980*a, b*; Norton *et al.*, 1983*a,b*, 1984; Rochas & Rinaudo, 1980*a,b*, 1984; Rinaudo & Rochas, 1986; Rochas & Landry, 1987; Rochas *et al.*, 1990*a*). The helical state of κ-carrageenan is favoured by high salt concentration and low temperature. The microstructure of ion-exchanged κ-carrageenan in different ionic environments has been studied by Hermansson and co-workers using transmission electron microscopy (Hermansson, 1989; Hermansson *et al.*, 1991).

The non-gelling polysaccharide, LBG, is a galactomannan extracted from seeds of carob trees. The polysaccharide consists of a linear chain of α-D-mannopyranosyl residues, which is substituted by single α-D-galactopyranosyl groups along the chain. The D-galactose distribution along the mannan backbone has been shown to be non-regular, with a high proportion of substituted couplets, a smaller amount of triplets and an absence of long regions of substitution (McCleary *et al.*, 1985). The mannose to galactose ratio has been found to range from 3 to almost 5, depending on the source and preparation method. Galactomannans are known to self-associate under certain conditions (Dea & Morrison, 1975; Dea *et al.*, 1977). Self-association of galactomannans is enhanced by a low degree of substitution. In a previous paper, we showed that visualisation of the unperturbed random coil structure of LBG by electron microscopy techniques is at the limit of

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resolution obtained by the high resolution rotary shadowing technique (Lundin & Hermansson, 1995).

The origin of the enhanced gel properties when κ-carrageenan and galactomannan or glucomannan are mixed has been a subject of debate. Several types of structure in the network have been suggested to explain the synergistic behaviour of mixed gels. The earliest model, presented by Rees and Dea and co-workers, suggested that the κ-carrageenan helix interacted with unsubstituted regions of the galactomannan backbone (Dea *et al.*, 1972; Dea & Morrison, 1975; Rees, 1972*a, b*). X-ray diffraction studies performed by Cairns *et al.* showed that no specific interaction occurs between κ-carrageenan and LBG or konjac mannan. On the basis of these findings, a model was presented which suggested that interpenetrating LBG polymers stabilise the κ-carrageenan network structure (Cairns *et al.*, 1986, 1987, 1988, 1991). Contradictory results from ^{13}C-NMR studies show a loss of mobility of the galactomannan, which indicates that specific interactions occur between κ-carrageenan and LBG (Rochas *et al.*, 1990b). Rochas' group suggested that the mixed gel consists of a coupled network with specific junction zones of κ-carrageenan and LBG. Further support for this model was presented in rheological measurements by Turquois *et al.* (1992, 1994). Both Rochas' and Turqouis' groups did not rule out the possibility that the loss of galactomannan mobility could be the result of self-aggregation of LBG induced by the presence of κ-carrageenan. Williams and co-workers studied κ-carrageenan and konjac mannan or LBG mixtures using differential scanning calorimetry, DSC, and electron spin resonance spectroscopy, ESR (Williams *et al.*, 1992, 1993). They found that addition of glucomannan had effects on the self-association of κ-carrageenan. The restrictions in κ-carrageenan aggregation were suggested to be due to an association of konjac mannan to the κ-carrageenan helices (Williams *et al.*, 1993). A ^{113}Cs-NMR study by Piculell and co-workers on mixtures of κ-carrageenan and galactomannan gave further support for an association between κ-carrageenan helices and galactomannan (Piculell *et al.*, 1994).

The effects of mixing ratio, galactose substitution of the galactomannan and ionic environment on κ-carrageenan–LBG mixtures have been studied by several different techniques. Light scattering experiments on blends of κ-carrageenan and galactomannans in different ionic environments have shown that the association between κ-carrageenan and galactomannan occurred in conditions promoting κ-carrageenan self-association, i.e. with the addition of the salt potassium chloride, as compared with sodium iodide (Viebke, 1995). Viscoelastic measurements have shown that the synergism between κ-carrageenan and LBG could be observed only for mixtures of potassium–κ-carrageenan and LBG in a potassium ion environment. Mixed gels with sodium or calcium ions present did not show any synergism in gel strength (Tako & Nakamura, 1986; Stading & Hermansson, 1993). Tako and Nakamura noted that self-association of κ-carrageenan occurred as the κ-carrageenan content was increased above the optimal mixing ratio for synergy, i.e. κ-carrageenan was added in excess (Tako & Nakamura, 1986). Stading and Hermansson did not observe any increase in gel strength when the KCl concentration was greater than 0.10 M. They showed that the composition favouring maximum synergy shifted from 90% κ-carrageenan in distilled water to 35% κ-carrageenan in 0.10 M KCl (Stading & Hermansson, 1993). Similar results have been observed in large deformation tests (Turquois *et al.*, 1992). It was found that the composition for maximal synergy was shifted from 60 to 50% κ-carrageenan as the salt concentration was varied from 0.050 to 0.10 M KCl. Fernandes *et al.* studied mixtures of κ-carrageenan and galactomannans of different origin and M:G ratio and showed that the rheological and thermal properties of the mixtures are dependent on molecular size and galactose substitution of the galactomannan (Fernandes *et al.*, 1991, 1992).

This study presents the microstructural and rheological effects of the random-coil polysaccharide, LBG, on the coarse supermolecular network of potassium–κ-carrageenan. The effects of LBG with two different M:G ratios on the supermolecular network of potassium–κ-carrageenan were visualised for different mixing ratios by rapid freezing and sublimation combined with the low-angle rotary metal shadowing technique for transmission electron microscopy. The viscoelastic properties of κ-carrageenan–LBG gels were elucidated for different mixing and M:G ratios and KCl concentrations. The results demonstrate that the addition of LBG has an effect on the self-aggregation of potassium–κ-carrageenan leading to differences in viscoelastic behaviour after the onset of gelation.

MATERIALS AND METHODS

Materials

κ-Carrageenan from *Euchema cottonii* type III (lot 120H0502) and LBG (lot 40H0160) were purchased from Sigma Chemicals (St. Louis, MO, USA).

The pure potassium form of κ-carrageenan was prepared by the method described by Hermansson *et al.* (1991). 1.0% (w/v) κ-carrageenan solution was ion-exchanged in a column thermostatted to 90°C with a commercial ion-exchange resin AG 50W-X8 (Bio Rad) and freeze-dried. In this study, two batches of ion-exchanged potassium-κ-carrageenan were used. The cation content was determined by atomic absorption spectroscopy to 6.8% K ions, 0.1% Na ions and <0.02% Ca ions per dry weight κ-carrageenan, for both of the batches.

LBG was fractionated depending on the solubility of the polysaccharide at different temperatures, according to the procedure described in an earlier paper (Lundin & Hermansson, 1995). The samples used were the fractions soluble at 35°C and below and at 65–80°C. The fractions will be called LBG35 and LBG80, respectively. The M:G ratio was determined by gas chromatography of the alditol acetate derivatives to be 3 and 5, respectively.

Sample preparation

Polysaccharide blends were dispersed in 0.050 or 0.10 M KCl at room temperature and stirred continuously until dissolved in a 90°C water bath. The samples for electron microscopy were prepared at a concentration of 0.01% in 0.10 M KCl and allowed to cool before microscopy preparation. The mixtures for rheological measurements were prepared in different concentrations and proportions. The polymers were dissolved in either 0.050 or 0.10 M KCl. The samples were transferred at 90°C to the preheated rheometer. All concentrations given are in weight per cent (w/w).

Electron microscopy

Dilute samples of κ-carrageenan and κ-carrageenan mixed in different ratios with LBG were prepared, at a total polymer concentration of 0.01%, by the mica sandwich technique for transmission electron microscopy. A suitable amount of sample was transferred with a pipette to a freshly cleaved mica surface. With a minimum of shear, the corresponding mica surface was put on top and the 'sandwich' was plunge-freezed in liquid nitrogen. The sandwich was cleaved below the surface of liquid nitrogen before it was rapidly transferred to a pre-cooled Balzer VOR 400 freeze-etching system. The sample was sublimated at −90°C for 2 h, after which followed low-angle rotary metal shadowing. The procedure has been described in detail earlier (Lundin & Hermansson, 1995). The replicas of monolayer of polymers were examined in a TEM, 100CXII (Jeol Ltd, Tokyo, Japan) at an acceleration voltage of 80 kV.

The mixtures containing κ-carrageenan and LBG35 were mixed at a ratio of 1:1 and 1:4, while the mixtures of κ-carrageenan and LBG80 were mixed at proportions of 4:1 and 1:1.

Dynamic viscoelastic measurements

The rheological 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). For dynamic measurements, the bob was suspended in an interchangeable torsion bar with a torque at maximum deflection of between 4×10^{-4} and 9×10^{-3} Nm. The frequency was 1 Hz. The strain was kept as low as 10^{-3} so as not to disturb the network. This is well within the linear region. The viscoelastic properties were recorded as the temperature was varied linearly at a gradient of 1.5°C/min from 90 to 48 or 38°C and kept at that temperature for 12 h.

The viscoelastic properties were measured on pure κ-carrageenan samples at a concentration of 0.4% and on mixtures containing κ-carrageenan at a concentration of 0.4%. In the mixture containing 0.10 M KCl, the following concentrations of galactomannan were used: LBG35 at concentrations of 0.4 and 1.2% and LBG80 at concentrations of 0.1 and 0.2%. In the mixture containing 0.050 M KCl, the following concentrations of galactomannan were used: LBG35 at concentrations of 0.4 and 1.2% and LBG80 at concentrations of 0.05 and 0.1%.

RESULTS AND DISCUSSION

Microstructure of κ-carrageenan–LBG mixtures

The gelation of κ-carrageenan has been attributed to a two-stage process involving the coil–helix transition followed by aggregation. The aggregation process has been shown to be dependent on counterions present in the solution (Morris *et al.*, 1980*b*). Of the more commonly used salts, potassium is by far the most effective counterion in promoting κ-carrageenan aggregation when compared with sodium and calcium ions. Hermansson *et al.* visualised the structure of dilute κ-carrageenan in different ionic environments by transmission electron microscopy. They found that, in 0.10 M KCl, κ-carrageenan forms a mixed structure of coarse supermolecular strands and finer strands (Hermansson, 1989; Hermansson *et al.*, 1991). A micrograph of the structure typical for κ-carrageenan in 0.10 M KCl is presented in Fig. 1. Arrow A points at a supermolecular strand which is interpreted as consisting of a large number of aligned κ-carrageenan double helices. In the micrograph, it is also possible to observe finer strands which are believed to consist of double helical κ-carrageenan strands. Arrow B points at one of the finer strands (Fig. 1).

It is currently not possible to clearly visualise the random coil structure of LBG with the resolution obtained with Pt/C rotary shadowing used in combination with the mica sandwich technique (Lundin & Hermansson, 1995). This implies that it is only possible to observe changes in the microstructure of κ-carrageenan for mixtures containing LBG by the use of this technique.

To visualise the differences in the microstructure of κ-carrageenan as LBGs are added in different amounts, samples of κ-carrageenan–LBG were prepared by rapid

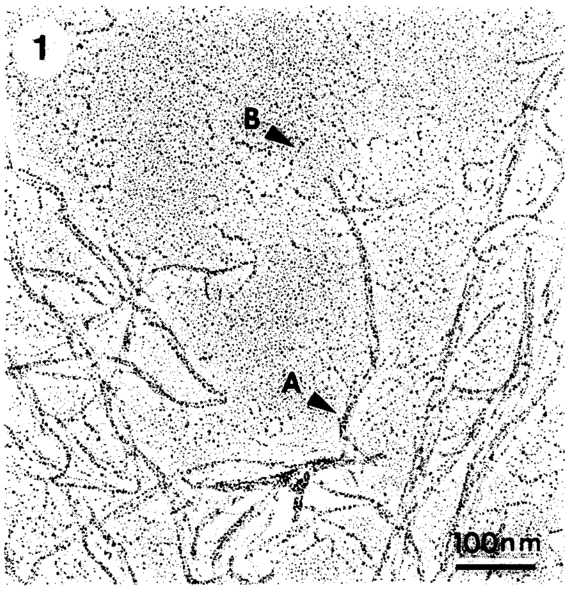

Fig. 1. Micrograph of 0.01% κ-carrageenan in 0.10 M KCl.

freezing and sublimation combined with low-angle rotary metal shadowing for transmission electron microscopy. Depending on the M:G ratio of the LBG, the mixing ratios were chosen for the purpose of showing the large structural changes in κ-carrageenan. Figures 2–5 show transmission electron micrographs of mixtures of κ-carrageenan and either LBG35 or LBG80 in 0.10 M KCl. The polysaccharides were mixed at different ratios of κ-carrageenan:LBG at a total polymer concentration of 0.01%. Mixtures containing LBG35 were mixed in a κ-carrageenan:LBG35 ratio of 1:1 (Fig. 2) and 1:4 (Fig. 3). The samples with LBG80 were mixed in a κ-carrageenan:LBG80 ratio of 4:1 (Fig. 4) and 1:1 (Fig. 5). Note the differences in mixing ratios between the LBG samples.

Figures 2 and 3 show micrographs of a monolayer of κ-carrageenan–LBG35 at mixing ratios of 1:1 and 1:4, respectively. Coarse supermolecular strands of aggregated κ-carrageenan helices, as well as the finer strands of κ-carrageenan can be seen in Fig. 2. Both the assembled supermolecular strands and the finer strands can be recognised from micrographs of pure potassium–κ-carrageenan in 0.10 M KCl (Fig. 1).

The micrograph in Fig. 3 shows a mixture of κ-carrageenan and LBG35 at a ratio of 1:4. At this 'high' degree of added LBG35, the strands formed are fine and relatively short. Coarse supermolecular strands are sparse in this sample. The fine strands observed from this mixture are of the same dimension as the fine strands of pure κ-carrageenan in 0.10 M KCl (Fig. 1, arrow B). The coarse supermolecular structure of κ-carrageenan almost completely disappears as LBG35 is added at a ratio of 1:4 (compare Figs 2 and 3). These findings are also observed in mixtures of κ-carrageenan–LBG80, although the ratios of κ-carrageenan:LBG80 are completely different.

Micrographs of κ-carrageenan mixed with LBG80 either at the ratio of 4:1 or in equal amounts are presented in Figs 4 and 5, respectively. The micrograph in Fig. 4 shows supermolecular assemblies of κ-carrageenan helices as well as the finer strands. When larger amounts of LBG80 are added to the mixture, it is only possible to observe the fine strands of κ-carrageenan helices (Fig. 5).

The micrographs of the mixtures show an increased tendency for κ-carrageenan to form high molecular superstrands as the LBG concentration is decreased. The results show that the addition of a sufficient amount of the random coil polysaccharide, LBG, impedes κ-carrageenan helices from aggregating. The ability of LBG to inhibit κ-carrageenan aggregation depends on the degree of galactose substitution of LBG. A low substitution degree, i.e. a high M:G ratio, enhances the inhibiting effect. These results suggest that, in dilute systems, the presence of LBG hinders the self-association of κ-carrageenan. It should be noted that the ability to prevent κ-carrageenan association is observed for both of the LBGs, but the mixing ratios needed to obtain this effect are completely different. LBG35 must be added in larger amounts than LBG80 in order to obtain the same effects.

On the basis of the results of Hermansson *et al.* (1991) on pure κ-carrageenan gels, it seems reasonable to expect that the structural differences observed in the micrographs of κ-carrageenan and LBG would lead to differences in the rheological behaviour of concentrated mixtures.

Viscoelastic properties of κ-carrageenan–LBG mixtures

The exact mechanism of κ-carrageenan–LBG gel formation is not yet known. To further evaluate the behaviour, κ-carrageenan was mixed with two samples of LBG with different M:G ratios. The κ-carrageenan:LBG mixing ratios were chosen in order to illustrate extremes of the behaviour.

The time dependence of the gel formation of κ-carrageenan–LBG mixtures was studied at a constant temperature just below the transition mid-point temperature. As reported by Rochas and Rinaudo, the transition temperatures for κ-carrageenan in 0.10 and 0.050 M KCl are about 50 and 42°C, respectively (Rochas & Rinaudo, 1984).

Hermansson *et al.* (1991) performed dynamic viscoelastic measurements just above and below the coil–helix transition temperature. In agreement with Rochas and Landry (1987), they showed that the gelation process is time-dependent and that it slows down in the vicinity of the transition temperature. Therefore, in order to reduce the kinetics of aggregation and gel formation for the system, experimental temperatures of 48 and 38°C were chosen. All samples were cooled at a gradient of 1.5°C/min from 90 to 48°C or 38°C, depending on the KCl concentration, followed by a 12 h ageing period.

Fig. 2. Micrograph of κ-carrageenan–LBG35 mixture at a ratio of 1:1 in 0.10 M KCl; total polymer concentration 0.01%.

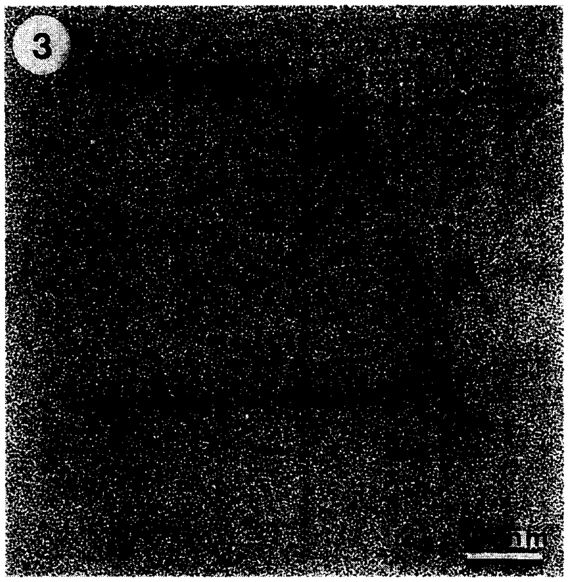

Fig. 3. Micrograph of κ-carrageenan–LBG35 mixture at a ratio of 1:4 in 0.10 M KCl; total polymer concentration 0.01%.

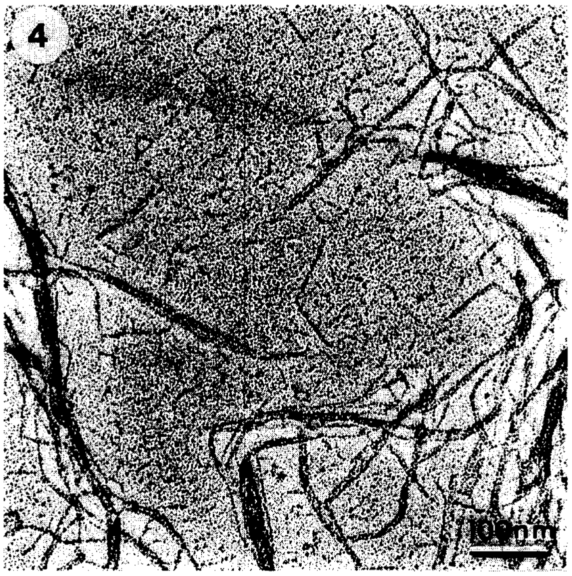

Fig. 4. Micrograph of κ-carrageenan–LBG80 mixture at a ratio of 4:1 in 0.10 M KCl; total polymer concentration 0.01%.

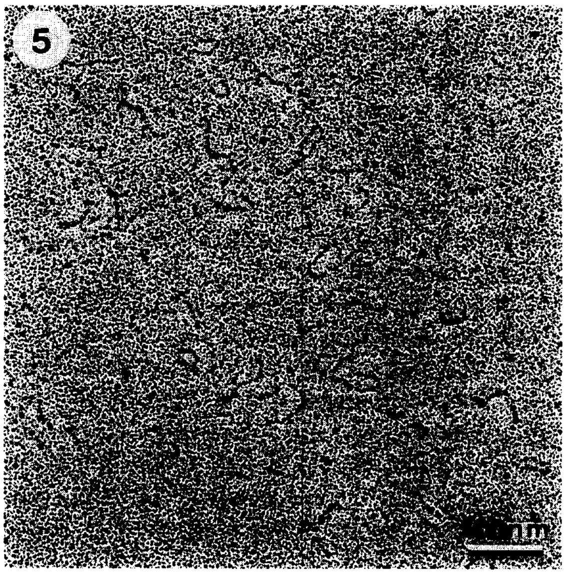

Fig. 5. Micrograph of κ-carrageenan–LBG80 mixture at a ratio of 1:1 in 0.10 M KCl; total polymer concentration 0.01%.

Figures 6–9 present the storage modulus, G', and phase angle, δ, for the mixtures of 0.4% κ-carrageenan and varying concentrations of LBG in 0.10 M KCl during 12 h of ageing at 48°C. In the mixtures containing LBG35, the galactomannan was added in concentrations of 0.4% (Fig. 6) and 1.2% (Fig. 7), while LBG80 was added in concentrations of 0.1% (Fig. 8) and 0.2% (Fig. 9). Note the differences in LBG concentrations.

Figure 6 shows G' and phase angle for the mixture containing 0.4% LBG35 during the 12 h ageing period. The initial increase in G' is followed by a relatively rapid decrease. The phase angle is increased from 1 to 17°, which indicates a change in the viscoelastic character of the sample. The large decrease in G' as the gel ages suggests that the network is structurally changed after gel formation.

It should be observed from Fig. 7 that the mixture of κ-carrageenan with 1.2% LBG35 shows a different rheological behaviour. G' initially increases and reaches a maximum, followed by a relatively slow decrease in gel strength with time. The phase angle is increased from a initial value of 3 to 6°.

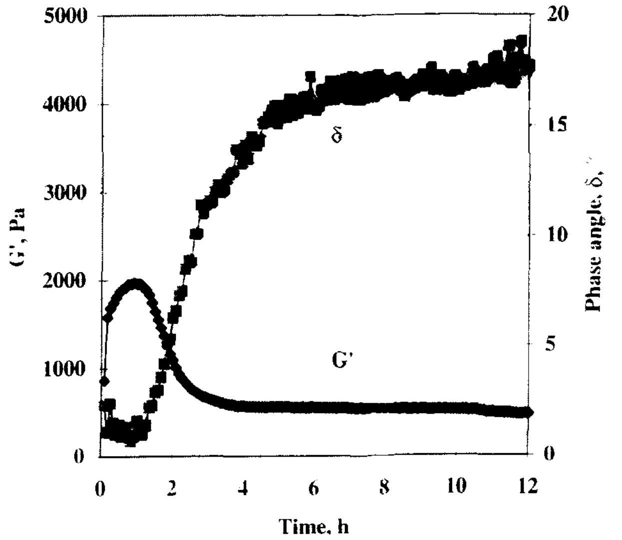

Fig. 6. Storage modulus (G') and phase angle (δ) as function of time for a mixture of 0.4% κ-carrageenan and 0.4% LBG35 in 0.10 M KCl at 48°C.

The modulus G' for the blends of κ-carrageenan and LBG80 is presented in Fig. 8 and Fig. 9. Figure 8 shows the rheological properties of the blend of κ-carrageenan and 0.1% LBG80. At this relatively low level of added LBG80, ageing of the network leads to a continuous decrease in gel strength and an increase in phase angle from 1 to 5°. As can be seen in Fig. 9, the mixture containing 0.2% w/w LBG80 shows an initial increase in G'. As the gel ages, G' is relatively constant for 10 h, after which a slight decrease can be noted. The phase angle is increased from 2 to 4°.

The results of mixtures of κ-carrageenan and either of the LBGs in 0.10 M KCl show that gel strength is retained as the galactomannan content is increased. The effect is most pronounced for the mixture containing LBG35, although the mixtures containing LBG80 showed the same behaviour at much lower LBG concentrations. The results suggest that LBG has a stabilising effect on the network structure of κ-carrageenan–LBG gels. This effect seems to be dependent on the M:G ratio and LBG content.

The existence of an initial maximum in the viscoelastic spectra of pure κ-carrageenan during cooling is controversial. The maximum in G' has been suggested to result from a rearrangement of the network due to aggregation (Hermansson, 1989; Hermansson *et al.*, 1991) or by a change in sample volume and subsequent slippage (Richardson & Goycoolea, 1994). Recent results from axial dynamic mechanical analysis, a technique where the experimental design is set up to compensate for any effects of change in volume, show a maximum in the spectra of potassium–κ-carrageenan in 0.10 M KCl (Stading, pers. comm.). On the basis of the rheology and microscopy results presented, as well as results from previous studies (Hermansson, 1989; Hermansson *et al.*, 1991; Stading & Hermansson, 1993), we interpret the decrease in G' for κ-carrageenan–LBG gels to be caused by κ-carrageenan secondary aggregation and rearrangement after network formation. It should be noted that, for the mixtures, the kinetics of the rearrangement after gel formation seems to be a relative slow process. The decrease in G' for the mixtures with κ-carrageenan in excess, occurs over a longer timescale compared to previously published results for pure κ-carrageenan gel aged at a temperature just below the transition temperature (Hermansson *et al.*, 1991).

If the stabilising effect is the result of restrictions in κ-carrageenan aggregation, it seems reasonable that the effect should also be dependent on KCl concentration.

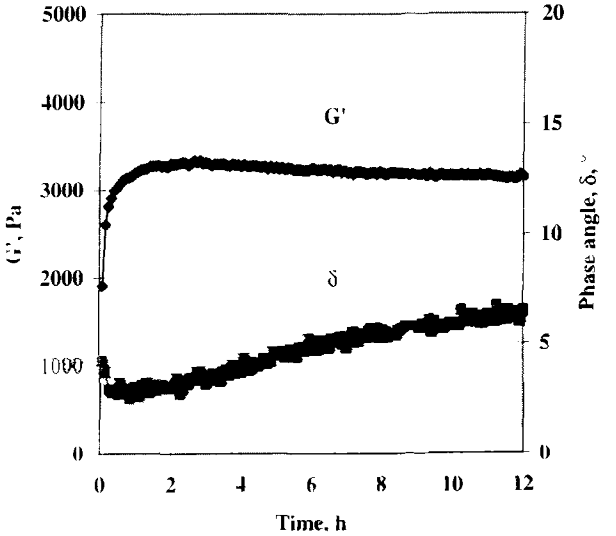

Fig. 7. Storage modulus (G') and phase angle (δ) as function of time for a mixture of 0.4% κ-carrageenan and 1.2% LBG35 in 0.10 M KCl at 48°C.

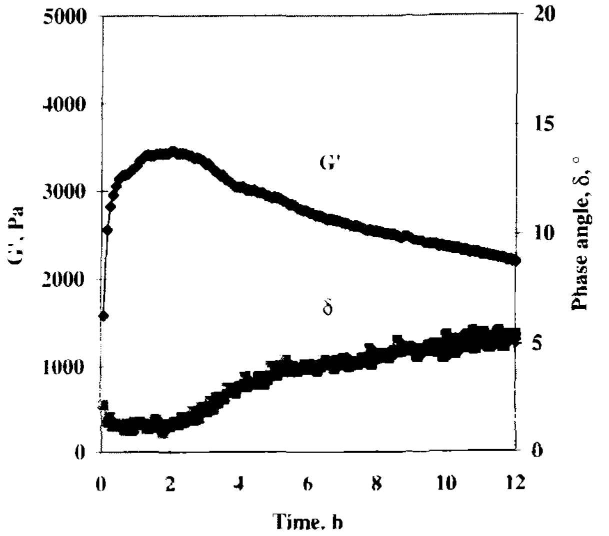

Fig. 8. Storage modulus (G') and phase angle (δ) as function of time for a mixture of 0.4% κ-carrageenan and 0.1% LBG80 in 0.10 M KCl at 48°C.

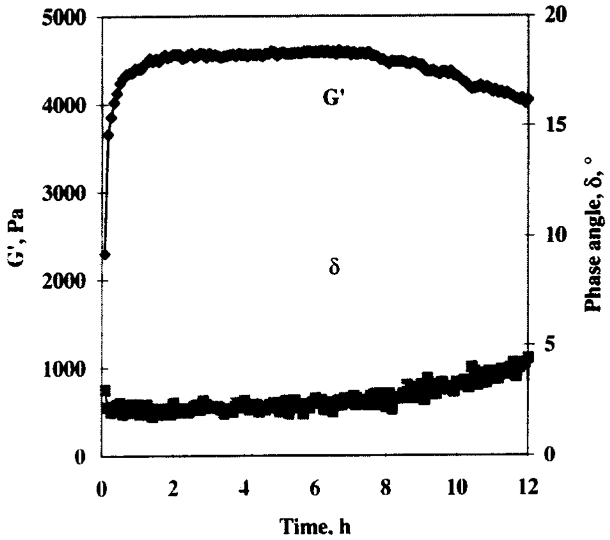

Fig. 9. Storage modulus (G') and phase angle (δ) as function of time for a mixture of 0.4% κ-carrageenan and 0.2% LBG80 in 0.10 M KCl at 48°C.

At the 0.050 M KCl concentration, the driving force for κ-carrageenan aggregation is reduced, which ought to lead to enhanced stabilising effects of the LBGs.

Viscoelastic measurements were also performed at 0.050 M KCl to investigate whether any differences in stabilising effect could be observed between κ-carrageenan–LBG mixtures in 0.10 and 0.050 M KCl. 0.4% κ-carrageenan was mixed with 0.4 or 1.2% LBG35 and 0.05% or 0.1% LBG80. The mixtures were treated as above with the difference that the measurements were conducted at 38°C in 0.050 M KCl and compared with the experimental temperature, 48°C, in 0.10 M KCl. The use of different measuring temperatures is related to the differences in coil–helix transition temperature. The viscoelastic properties of κ-carrageenan–LBG mixtures in 0.050 and 0.10 M KCl are summarised in Table 1.

The viscoelastic measurements show the expected results. The mixtures containing 0.4% and 1.2% LBG35 in 0.050 M KCl showed an increased ability to retain the gel strength during a 12 h ageing period as compared to mixtures in 0.10 M KCl (Table 1).

The same behaviour was observed for the mixtures with LBG80, although the concentrations were considerably lower than for the LBG35 blends. Addition of 0.1% LBG80 to the mixture led to a gel with stable viscoelastic properties, as compared to an addition of 0.2% LBG80 in 0.10 M KCl. When the LBG80 concentration was reduced to as low as 0.05%, the stabilising effect was again lost, i.e. a decrease in G' was observed during the 12 h ageing period (Table 1). It can be concluded that the stabilising effect of LBG on the network is enhanced when the KCl concentration is decreased from 0.10 to 0.050 M.

Stading and Hermansson (1993) presented results of viscoelastic measurements on potassium–κ-carrageenan mixed with LBG (M:G~3) at a total polymer concentration of 1.0%. At KCl concentrations in the range of 0.050–0.10 M and at a relatively high κ-carrageenan content, their results showed an initial maximum in G'. As the LBG content was increased, the maximum vanished. It was also observed that, as the KCl concentration was increased from 0.050 to 0.10 M, the ratio for maximal synergy was shifted to an increased LBG content (Stading & Hermansson, 1993). This is in agreement with the results presented in this study and indicates that a sufficient amount of LBG must be present to obtain maximal synergy and, as the KCl concentration increases, the amount of LBG must also be increased.

Fernandes *et al.* (1991) presented results of viscoelastic measurements of mixtures of κ-carrageenan and LBGs of different origins. They found that decreased galactose substitution of the galactomannan enhanced the synergism obtained for κ-carrageenan–LBG mixtures. They also found that the addition of KCl and differences in M:G ratio did not affect the optimum mixing ratio for maximal synergism. Our results indicate that both potassium ions and the mixing ratio exert a strong effect on the the rheology and microstructure of the mixed gels.

The results presented here have shown that the viscoelastic properties of κ-carrageenan and LBG are dependent on the M:G ratio of the galactomannan as well as the factors affecting self-association of potassium κ-carrageenan, i.e. potassium chloride concentration. In the presence of excess amounts of κ-carrageenan, i.e. when properties of the mixture are governed by the κ-carrageenan, it seems that the initial gel formation is followed by a rearrangement of the network, probably caused by self-association of κ-carrageenan, leading to a decrease in gel strength.

Rheological properties in relation to microstructure

There are similarities in rheological behaviour between the pure potassium–κ-carrageenan and mixtures

Table 1. Viscoelastic properties of κ-carrageenan–LBG mixtures

Polymer contents	$G'_{(\max)}$ Pa	$G'_{(12\ \mathrm{h})}$ Pa	δ^b
in 0.10 M KCl			
0.4% LBG35[a]	1970	470	17
1.2% LBG35[a]	3340	3140	7
0.1% LBG80[a]	3470	2230	5
0.2% LBG80[a]	4620	4080	4
in 0.050 M KCl			
0.4% LBG35[a]	1040	660	8
1.2% LBG35[a]	1120	1030	2
0.05% LBG8[a]	720	410	4
0.1% LBG80[a]	2130	2110	1

[a] All mixtures contain 0.4% κ-carrageenan.
[b] Phase angle after 12 h.

containing low amounts of LBG. Following the coil–helix transition of κ-carrageenan, a strong initial increase in gel strength and a decrease as the gel cures are observed for both systems.

Hermansson *et al.* visualised the microstructure of κ-carrageenan during gelation and correlated the structure to measured rheological properties. They found that a transient structure was formed after the coil–helix transition. This structure consisted of associated κ-carrageenan helices and generated the sharp increase in G'. As the gelation process proceeded, the κ-carrageenan helices became aligned and supermolecular strands were formed, which caused the gel strength to rapidly decrease. When the gels were further cooled, a slow increase in G' was observed. It was suggested that the final gel consisted of a mixed structure of fine and coarse helical κ-carrageenan superstrands. The structure and rheology of the potassium–κ-carrageenan gels were dependent on the cooling rate and the salt concentration (Hermansson *et al.*, 1991).

The results presented in this paper indicate that there is a competitive situation between κ-carrageenan self-association and an aggregate-inhibiting process caused by LBG. The inhibiting process may very well originate from an association of mannan backbone to the κ-carrageenan helices, although this could not be confirmed by the microscopy technique used in this study. It seems as though the stoichiometry of the stabilising process is dependent on the degree of substitution of the mannan chain as well as the factors inducing κ-carrageenan aggregation, such as salt concentration and temperature. The micrographs in Figs 3 and 5 suggest that as the galactomannan is in excess, the aggregation of κ-carrageenan is hindered. This would lead to a strong, more homogeneous network of fine strands. The network is stabilised by LBG, and no pronounced decrease in gel strength is observed. It is also possible that, as κ-carrageenan is in excess and at a higher concentration, the rheological behaviour is governed by a mixed network formed by a network of κ-carrageenan superstrands and a fine network, stabilised by LBG, characterised by a lower degree of aggregation owing to the effects of the galactomannan.

Our results are in agreement with the model proposed for κ-carrageenan–glucomannnan and κ-carrageenan–galactomannan gels by Williams and co-workers (Williams *et al.*, 1993; Piculell *et al.*, 1994). On the basis of results of a DSC and ESP study on κ-carrageenan and konjac mannan, Williams *et al.* suggested that the glucomannan affects the self-aggregation of κ-carrageenan through an association of konjac mannan on the κ-carrageenan helices. They conclude that the κ-carrageenan–konjac mannan association competes with κ-carrageenan self-aggregation, so that there is less κ-carrageenan self-association in the mixture. When the amount of κ-carrageenan is in excess, this would lead to a mixed network of κ-carrageenan strands with konjac mannan attached to the surface and self-aggregated κ-carrageenan strands, while, when the glucomannan is in excess, the coarse aggregation of κ-carrageenan helices is completely hindered. This suggests a network structure of κ-carrageenan helices with konjac mannan attached to the surface. They were uncertain of the nature of the κ-carrageenan–konjac mannan aggregate, but suggested that a core of aggregated κ-carrageenan helices with mannan chains attached to the surface is a possibility. According to Piculell *et al.*, 'the ^{113}Cs-NMR results indicate that the mode of association of the κ-carrageenan helices also changes in the mixtures', i.e. the presence of galactomannan changes the dynamics of ions bound to the κ-carrageenan helices (Piculell *et al.*, 1994). As in our observations, their results suggest that ion-induced self-association of κ-carrageenan helices can be hindered by the presence of a galactomannan (see Figs 3 and 5).

Mixtures of different ion forms of κ-carrageenan and caseinate or casein fractions show similar impedimentary effects on the self-aggregation of κ-carrageenan. Microscopy results indicate that, when casein is added to a κ-carrageenan sample, the association process of κ-carrageenan is hampered. The results of viscoelastic measurements also suggest that a structural rearrangement occurs after the coil–helix transition of κ-carrageenan (Hermansson, 1995). At suitable physiochemical conditions, the presence of a second biopolymer, such as konjac mannan, LBG or casein in κ-carrageenan solutions seems to affect the behaviour of the polyelectrolyte κ-carrageenan.

It can be concluded that the addition of LBG to dilute κ-carrageenan solutions results in restrictions to the aggregation process of κ-carrageenan. The rheological properties during gelation of κ-carrageenan–LBG gels in 0.050 and 0.10 M KCl show preserved stability as LBG is added in appropriate amounts. The effect of LBG is dependent on salt concentration and the degree of substitution of the galactomannan, where the polymer with the lowest degree of substitution is more effective. The synergistic effects observed for κ-carrageenan–LBG mixtures may originate from a modified κ-carrageenan network which is stabilised by mannan chains attached to the surface of fine κ-carrageenan strands. The dimensions of the fine strands of the synergistic gels vary slightly, but are much smaller than the coarse supermolecular strands typical for potassium–κ-carrageenan in 0.50–0.10 M KCl.

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