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Rheological studies of synergistic kappa carrageenan-carob galactomannan gels

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The mechanical properties, Young's modulus and yield stress of different carob galactomannan-kappa carrageenan blends have been examined using an Instron testing machine as a function of the following parameters:

(1) Constant total polysaccharide concentration.
(2) Galactomannan concentration for a given carrageenan concentration.
(3) Carrageenan concentration for a given galactomannan concentration.
(4) Galactomannan molecular weight.
(5) Carrageenan molecular weight.

The properties of the binary gels were compared to the kappa carrageenan gels. From the results, two types of gel structure could occur: a coupled network defined by specific junction zones (and involving unsubstituted mannose residues of the galactomannan chains) and also a gel structure with both polysaccharides associating independently.

INTRODUCTION

Many polysaccharides have found widespread applications in the food industry because they form viscous solutions and gels which are extremely useful in the texturing of foodstuffs. The addition of carob (locust bean gum) galactomannan to certain polysaccharides (Dea & Morrison, 1975; Dea, 1979) is known to modify the gelation behaviour of the latter. In general, this kind of blend leads to improved mechanical properties (Ainsworth & Blanshard, 1978; Cairns *et al.*, 1986; Tako & Nakamura, 1986; Damasio *et al.*, 1990) and to a decrease in the syneresis of gels although, when polysaccharides like agarose or kappa carrageenan are diluted well below the concentration at which they would gel, their mixture with carob galactomannan (which does not form a gel alone) leads to gels.

Different types of structure could exist in these mixed gels. At least three types of gel structures have been defined if both polysaccharides contribute to the network (Cairns *et al.*, 1987):

- separate polymer networks which interlace and form an interpenetrating network;
- a phase-separated network;
- the binding of one polysaccharide to the other to form a coupled network defined by specific junction zones.

Until recently, the model most accepted for gelation of the mixture (Dea, 1979) postulated interaction between the helical structure of the carrageenan or agarose and the unsubstituted mannan regions of galactomannan chains. However no evidence for intermolecular binding was found in studies (Cairns *et al.*, 1987) of tara or locust bean gum mixed with kappa carrageenan. These findings would suggest that the most likely model for such gels consists of a galactomannan solution contained within a carrageenan network.

Kappa carrageenan is characterized by an alternating disaccharide unit of $(1\rightarrow3)$ linked β-D-galactose-4-sulphate and $(1\rightarrow4)$ linked 3,6-anhydro-α-D-galactose. This polysaccharide undergoes a coil-helix conformational transition upon temperature reduction in the presence of ions such as potassium which affects the solubility of the polymer and thus promotes aggregation (Rochas & Rinaudo, 1984). The ordered conformation has been described as a single helix (Smidsrod, 1980), a double-helix (Morris *et al.*, 1980), or a helical dimer (Rochas & Landry, 1987).

Galactomannans are polysaccharides which consist

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of linear chains of (1→4) linked β-D-mannopyranosyl residues, to which are attached (1→6) linked α-D-galactopyranosyl groups as single unit side chains. The proportion of α-D-galactopyranose units in the polymer varies from 15% to 49% depending on the species from which the gum is extracted (Dea & Morrison, 1975). The D-galactose distribution in locust bean gum has been shown to be non-regular, with a high proportion of substituted couplets, smaller amounts of triplets, and an absence of long substituted blocks (McCleary *et al.*, 1985).

In this paper, some rheological properties of kappa carrageenan–locust bean gum mixed gels have been studied.

EXPERIMENTAL

Materials and methods

The samples of kappa carrageenan, (M_w = 690 000), carob (M_w = 880 000 galactose/mannose ratio: G/M = 23/77) and guar galactomannans used, were purchased from Sanofi Bio-Industries (France). Because of the amount of gum necessary for the experiments, the commercial gum has been used without any purification for preparation of the gels. Partial acid hydrolysis (Grasdalen & Painter, 1980; Bociek *et al.*, 1981), that is mild enough not to affect the structure of the polysaccharide, was used to obtain galactomannans of different molecular weights. Carob gum molecular weights were estimated from the Mark-Houwink equation proposed by Robinson *et al.* (1982) and modified by Fernandes *et al.*, (1991). The different fractions of carrageenan were prepared previously and their molecular weights were determined (Rochas *et al.*, 1990*a*.

Homogeneous gels of well defined geometry have been prepared. The following preparation procedure has been chosen:

1. dispersion of each polymer in hot water, under stirring;
2. mixing of the two hot polymer (95°C) solutions;
3. introduction of the hot mixture into a cylindrical mould (17 mm average diameter) and standing at room temperature. After 24 h the gel was cut into small cylinders (diameter = 17 mm and height = 17 mm).

Elastic modulus and yield stress measurements were obtained through the use of compression between parallel plates at room temperature on an Instron 4301 testing machine.

^{13}C NMR spectra were obtained at 75 MHz on a AC 300 Bruker spectrometer, equipped with a process controller Aspect 3000 computer and a variable temperature control.

RESULTS AND DISCUSSION

Mechanical properties of mixed gels for a 10 g/litre total concentration of polysaccharide

The mechanical measurements (elastic modulus and yield stress) recorded for a constant total concentration of polysaccharide of 10 g/litre and two salt concentrations (0·05 M and 0·1 M KCl) were in agreement with previous results (Cairns *et al.*, 1986; Damasio *et al.*, 1990). When the galactomannan concentration increased, the elastic modulus and the yield stress (Figs 1 and 2) increased rapidly until the proportion of galactomannan in the blend reached 35% and then decreased continuously. Syneresis decreased when the galactomannan concentration was increased. The difference between the elastic modulus of the blend and the kappa carrageenan elastic modulus gives the degree of synergy as a function of the kappa carrageenan concentration (Fig. 3). Using this approach synergy was maximum for a 50/50 galactomannan–kappa carrageenan blend in 0·1 M KCl, and a 40/60 blend in 0·05 M KCl. This phenomenon was not apparent from the representation used for Fig. 1 in which the maximum found (35/65) was not sensitive to the salt concentration. The representation used in Fig. 3 was not employed for the yield stress, because the fracture came at a deform-

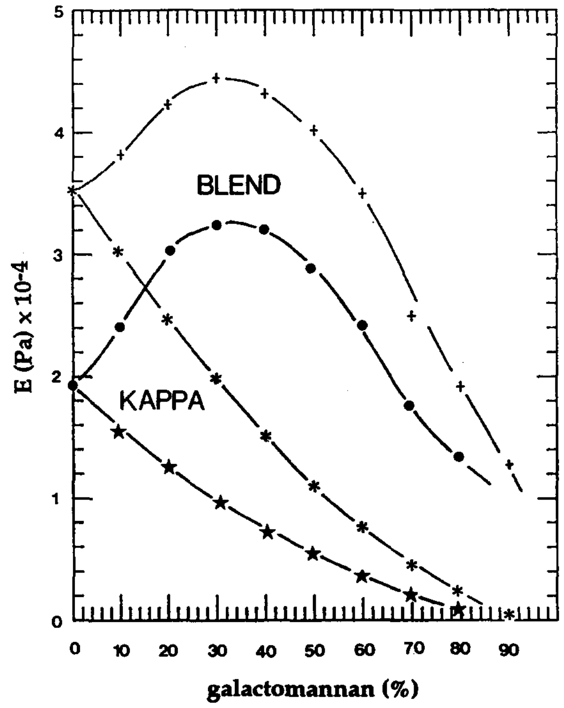

Fig. 1. Dependence of elastic modulus of ratio of galactomannan to carrageenan in blend and also on carrageenan concentration. ●, +; Carrageenan–carob blend, total polymer concentration of 10 g/litre 0·1 M KCl (+), or 0·05 M KCl (●). ★, * Carrageenan alone (carrageenan concentration is the same as in the equivalent blend) in 0·1 M KCl (*), or 0·05 M KCl (★).

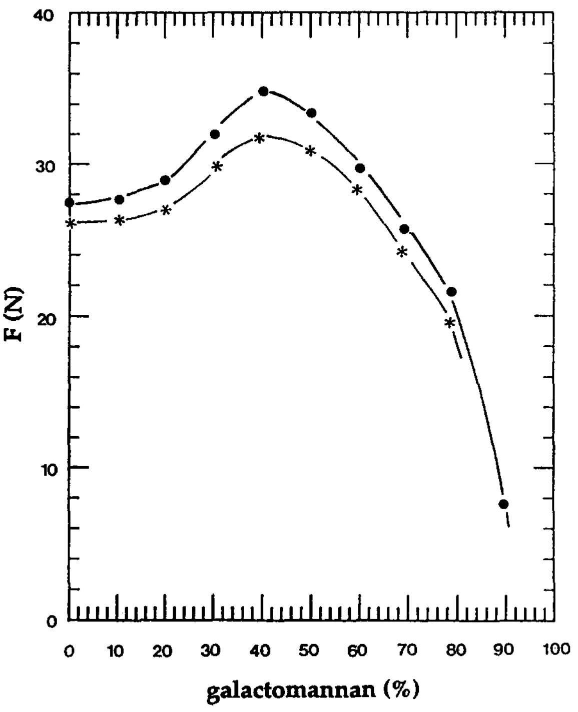

Fig. 2. Plot of yield stress versus % galactomannan in kappa carrageenan-carob blend at a polymer concentration of 10 g/litre. *, In 0·05 M KCl; ●, in 0·1 M KCl.

ation which was not the same for the blend and for the kappa carrageenan alone.

Mechanical properties of mixed gels as a function of galactomannan concentration, at a constant kappa carrageenan concentration of 4 g/litre

This study was carried out with a set of samples having a constant kappa carrageenan concentration (4 g/litre), the galactomannan concentration varying between 0 and 15 g/litre. Different salt concentrations were used. When the galactomannan concentration increased, the rigidity of the gel increased, while syneresis decreased. The curves of the elastic modulus (Fig. 4) and yield stress (Fig. 5) versus galactomannan concentration show an increase with increasing galactomannan concentration up to 8 g/litre. At high galactomannan concentrations the elastic modulus remained constant whereas the yield stress showed a slight decrease.

Several explanations could be employed to explain the shape of the curves: specific interactions could occur between the kappa carrageenan chains and the galactomannan chains, resulting in the formation of a carrageenan-galactomannan complex. An alternative explanation could involve a gel structure with both polysaccharides influencing each other (a kappa carrageenan network and self associated galactomannan chains). Thus the results shown in Fig. 4 could be explained as follows.

At low galactomannan concentrations the elastic

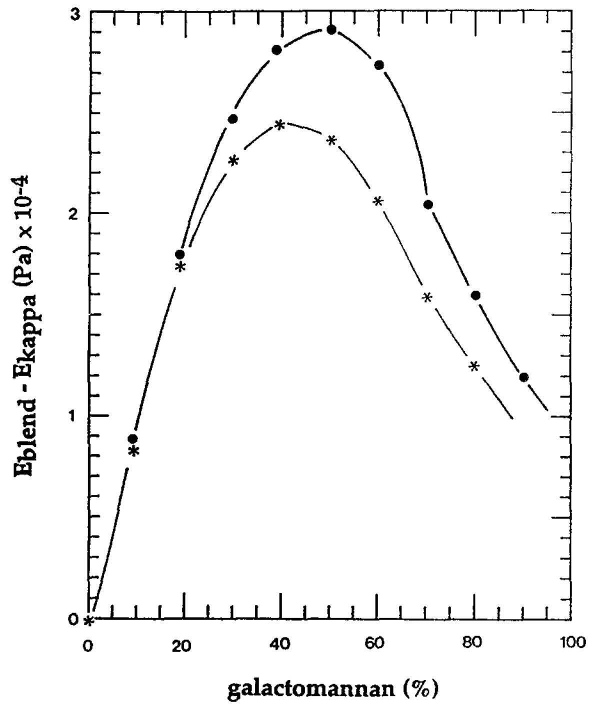

Fig. 3. Difference between elastic modulus of blend and carrageenan alone. ●, In 0·1 M KCl; *, in 0·05 M KCl.

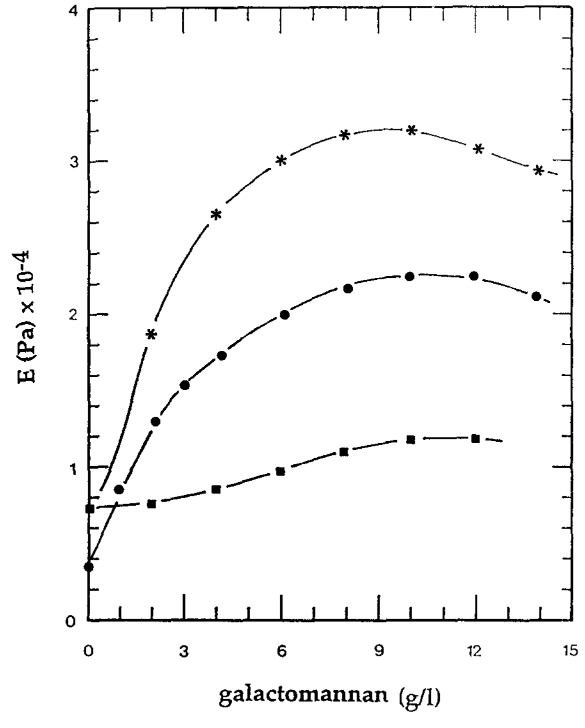

Fig. 4. Elastic modulus of kappa carrageenan (4 g/litre)–galactomannan blends as a function of galactomannan concentration. *, Carob in 0·1 M KCl; ●, carob in 0·05 M KCl; ■, guar in 0·1 M KCl.

modulus recorded was the sum of the kappa carrageenan modulus and of a blend modulus resulting from intermolecular binding. The latter corresponds to the synergistic component. When the galactomannan

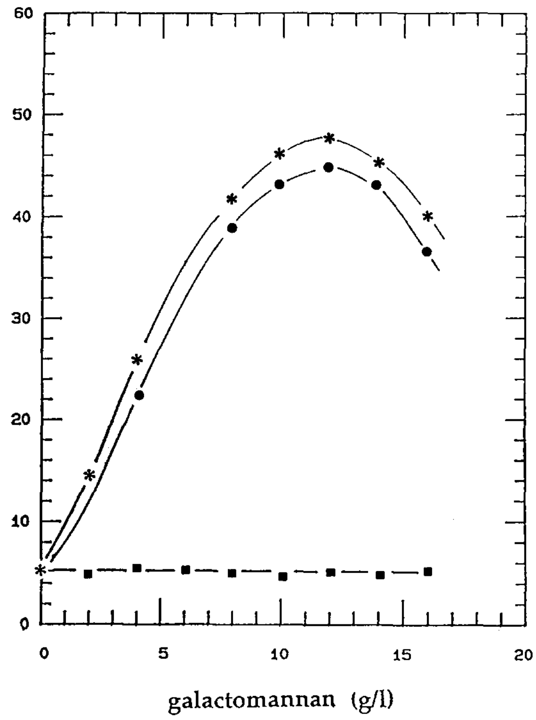

Fig. 5. Yield stress of kappa carrageenan (4 g/litre)–galactomannan blend, as a function of galactomannan concentration. *, Carob in 0·1 M KCl; ●, carob in 0·05 M KCl; ■, guar in 0·1 M KCl.

concentration increased the relative importance of the complex increases and consequently the mechanical properties of the blend increase.

At high galactomannan concentrations all the possible regions of the galactomannan (whose elastic modulus is zero) were complexed, so the increase in galactomannan concentration did not modify the blend modulus (in this region a small decrease in the modulus was observed at high galactomannan concentrations). This decrease in the blend modulus could be explained by a more 'dispersed' network compared to the one obtained for the same number of kappa carrageenan chains but with a smaller number of galactomannan chains. The effects observed for this last region could also be due to the poor solubility of the galactomannan at high concentration of polysaccharide. Nevertheless whatever the cause of this slight decrease this effect is not important. The only important fact is that the elastic modulus of the blend rises up to a certain galactomannan concentration.

The results could also be explained by an interpenetrating network model. In the first part of the curves the mutual influence of both polysaccharide networks is effective while it stabilizes over a certain galactomannan concentration.

A comparison of the synergistic effect was made between galactomannans of different origins. We found (see Figs 4 and 5) that the best effect was observed for carob gum (characterized by a galactose/mannose ratio of 23/77) compared to guar gum (G/M of 38/62). This is in agreement with previous findings concerning the degree of interaction of galactose-depleted guar galactomannan samples with xanthan or agarose (McCleary *et al.*, 1984) which was found to increase as the galactose content decreased.

Mechanical properties of mixed gels as a function of kappa carrageenan concentration, at constant galactomannan concentration

The variation in the elastic modulus of the blend with kappa carrageenan concentration was studied at several constant concentrations of galactomannan (Fig. 6). As previously when the kappa carrageenan concentration was increased, the rigidity of the gel increased.

The curves showed two trends (Fig. 6). At low carrageenan concentrations the slope of the curve of elastic modulus versus carrageenan concentration was almost 1 which is substantially lower than the usual value of 2 for kappa carrageenan alone. For higher concentrations the slope was near 2, similar to the value obtained for carrageenan alone.

As for the preceding experiment, at least two

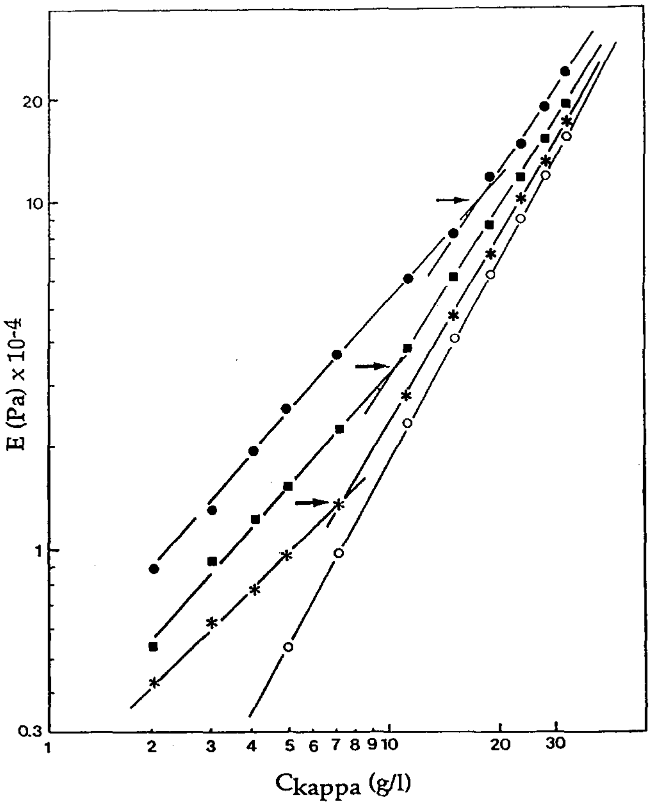

Fig. 6. Plot of elastic modulus versus kappa carrageenan concentration in 0·05 M KCl at different constant carob concentrations. O, Carrageenan alone; *, carrageenan–carob (1 g/litre) blend; ■, carrageenan–carob (2 g/litre) blend; ●, carrageenan–carob (6 g/litre) blend.

hypotheses could explain the shape of the curves. The first part of the curves (at low kappa carrageenan concentration) was characterized by an elastic modulus which corresponded to the free galactomannan chains and to the associated kappa carrageenan-galactomannan chains or to the kappa carrageenan chains and to the self associated galactomannan chains. The elastic modulus recorded was governed by the modulus of the complex formed. It increased when the complex concentration increased. In the second part, all the galactomannan chains participated in the complex. As the carrageenan concentration increased, the modulus of the blend was dominated by the modulus of the carrageenan.

The point defined by the intersection of the two linear regions of the log-log plot of concentration versus elastic modulus (Fig. 6) depended on the galactomannan level. It could reveal the realization of all the potential interactions, but it was not possible to distinguish if the interactions were galactomannan-galactomannan or galactomannan-kappa carrageenan.

Role of the molecular weight on the mechanical properties of the mixed gel

This role for the carrageenan alone was determined previously (Rochas *et al.*, 1990*a*). For concentrations higher than the overlapping concentration the elastic modulus (E) increased steadily with the molecular weight (M_w). Beyond a M_w value around 2×10^5 the modulus remained constant and independent of the molecular weight. Also for carrageenan alone, the intercept of the curve E versus M_w (Fig. 7) with the M_w axis indicated the lowest molecular weight required to form a gel (i.e. $E > 0$).

The influence of the molecular weight on the elastic modulus of the mixed gel was determined in 0·1 M KCl.

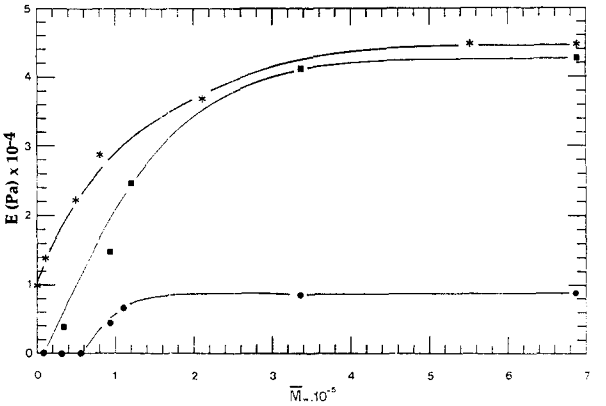

Fig. 7. Elastic modulus versus weight average molecular weight in 0·1 M KCl of: ■, different molecular weight fractions of kappa carrageenan (5 g/litre) mixed with carob (6 g/litre); ●, different molecular weight fractions of kappa carrageenan alone (5 g/litre); *, different molecular weight fractions of carob (6 g/litre) mixed with kappa carrageenan (5 g/litre).

Two cases were investigated. In a first set of experiments, different fractions of carrageenan were tested with the carob and in a second set carob samples with different molecular weights were tested with carrageenan.

The dependence of the elastic modulus on the carrageenan molecular weight (Fig. 7) was different in the presence of galactomannan. In this condition it appeared that the lowest carrageenan molecular weight required to form a gel was drastically decreased from 60 000 (for carrageenan alone) to 30 000. In fact, from the general shape of the curve (Fig. 7) it can even be postulated that the lowest carrageenan molecular weight required to form a gel was probably substantially lower than 30 000 mol. wt. From this curve, the galactomannan appeared to be a carrageenan binding agent or an agent able to induce self association phenomena. The curve of the modulus versus galactomannan molecular weights (Fig. 7) showed the same trends, with a plateau near a molecular weight of 450 000.

Dialysis of the gels in water solution

In order to determine if the reinforcement of the structure of mixed gels was obtained by specific junction zones or by a self association phenomenon, we have studied the immersion of the gels under stirring.

Kappa carrageenan gels (10 g/litre) and kappa carrageenan (10 g/litre)-carob (6 g/litre) mixed gels were prepared in 0·1 M KCl. In these ionic conditions, at room temperature, the conformational transition of kappa carrageenan was promoted and a rigid gel was obtained in both cases. Ten pieces of gels (40 ml) formed as described under Materials and methods, were immersed in water (1 litre) at room temperature. To avoid erosion as a result of magnetic stirring, these gels were enclosed in a net (porosity 100 μm). With kappa carrageenan, after 5 h, the gel completely dissolved and the sample freely diffused in the solvent. After this time, due to the final salt concentration ($3{\cdot}8 \times 10^{-3}$ M KCl) of the solution, the polymer should be a coil (Rochas & Landry, 1987) at room temperature. This was confirmed by optical rotation measurements. The gel therefore dissolved as a result of the helix-coil transition.

With the mixed gels after 90 h, the gels still existed (an increase of about 50% in volume was observed for the gel during the course of the experiment). The gel was separated from the supernatant by centrifugation. After freeze-drying and weighing of the two components it was observed that 25% of the total polysaccharide content was found in the supernatant.

From ^{13}C NMR carried out at 353 K, to destroy the interactions, and in the conditions of quantitative analysis it was shown that the relative composition of carob-carrageenan was almost similar for the two phases (estimated from the area of C-1 signals corresponding to both polysaccharides). Also the supernatant and the remaining gel had G/M ratios of the same order (25/75), as determined by signal reconstitution (Rochas *et al*., 1990*b*).

In comparison with the gel sample, the solubility of the galactomannan in the supernatant appeared very high. By NMR it was also shown that the resolution of the corresponding spectra was much better for the latter. Both observations could indicate that the carob diffusing from the gel was mainly low molecular weight material. Also as previously described, under these ionic conditions obtained after the dialysis step, the carrageenan should be in the coil state and should move freely in the solvent. This was not the case and the remaining gel, analysed by ^{13}C NMR at 298 K, did not show any carrageenan signal. This signal disappearance is classically attributed to a rigid helical structure (Morris *et al*., 1977). We can then postulate that the carob chains stabilize the carrageenan helix even at a temperature and a concentration at which the helix should not exist.

CONCLUSION

These experiments and in particular the last experiment involving immersion of the gels in aqueous solution have shown that the synergistic properties of kappa carrageenan–carob galactomannan gels could be described by a gel structure implying a coupled network defined by specific junction zones. The intermolecular binding between the polysaccharides occurred via unsubstituted mannose units (Rochas *et al*., 1990*b*). However the hypothesis of a self-association of the galactomannan chains promoted by the presence of kappa carrageenan chains, lowering the water activity and the solubility of the galactomannan and reciprocally stabilizing the carrageenan network, could not be completely rejected. We suggest that both gelation processes should be considered in a complementary fashion. But more information is required to understand their exact role.

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