# Effect of locust bean gum and konjac glucomannan oh the conformation and rheology of agarose and ?-carrageenan

**ARTICLE** *in* BIOPOLYMERS · NOVEMBER 1995

Impact Factor: 2.39 · DOI: 10.1002/bip.360360510



**4 AUTHORS**, INCLUDING:

Francisco M Goycoolea
University of Münster
**112** PUBLICATIONS **2,539** CITATIONS
SEE PROFILE

Edwin R. Morris
University College Cork
**222** PUBLICATIONS **10,398** CITATIONS
SEE PROFILE

Mike Gidley
University of Queensland
**333** PUBLICATIONS **9,991** CITATIONS
SEE PROFILE

**F. M. Goycoolea**
**R. K. Richardson**
**E. R. Morris***
*Department of Food Research and Technology*
*Cranfield University*
*Silsoe College*
*Silsoe, Bedford MK45 4DT, England*

**M. J. Gidley**
*Unilever Research*
*Colworth Laboratory*
*Sharnbrook, Bedford MK44 1LQ, England*

# Effect of Locust Bean Gum and Konjac Glucomannan on the Conformation and Rheology of Agarose and κ-Carrageenan

*Mixing with locust bean gum (LBG) induces obvious gel-like character in very dilute solutions of $K^+$ κ-carrageenan (< 0.01% w/w in 100 mM KCl). At higher concentration (0.085%), addition of LBG (0.036%) gives a shoulder on the high-temperature side of the DSC (differential scanning calorimetry) exotherm associated with the carrageenan disorder–order transition, with an accompanying increase in gelation temperature and enhancement in gel strength (storage modulus, G'). On substitution of LBG by konjac glucomannan (KM) the shoulder in DSC converts to a discernable peak. Van't Hoff analysis of optical rotation data indicates that the high-temperature thermal processes could arise from association of LBG or KM chains to the carrageenan double helix as it forms, with the main transition at lower temperature corresponding to ordering of surplus carrageenan. With κ-carrageenan in the nongelling tetramethylammonium salt form, addition of LBG causes no detectable change in DSC; rheological enhancement at high concentration (1% w/w) is limited to development of a very tenuous network, and in dilute solution a decrease in viscosity is observed. Agarose shows only a very slight increase in the disorder–order transition temperature on addition of KM, and it shows no detectable change with LBG. These observations are interpreted as showing that efficient binding of mannan or glucomannan chains requires some aggregation of the algal polysaccharide helices, but that extensive aggregation restricts synergistic interaction by competition with heterotypic association.* © *1995 John Wiley & Sons, Inc.*

## INTRODUCTION

Agarose and κ-carrageenan occur as structural polysaccharides in different species of red seaweed (*Rhodophyceae*).[1] Both have a linear primary structure based on an alternating repeating sequence of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydrogalactopyranose,[2,3] and both exist as coaxial double helices in the solid state,[4,5] with the individual strands having 3-fold symmetry. The 3,6-anhydro units, however, are in the D form in carrageenan and in the L form in agarose,

Received December 7, 1994; accepted February 7, 1995.
* To whom correspondence should be addressed.
Biopolymers, Vol. 36, 643–658 (1995)

CCC 0006-3525/95/050643-16

and this is reflected in a change in helix geometry from right-handed in carrageenan to left-handed in agarose.

Natural carrageenans occur with different degrees and patterns of sulfation.[6,7] The idealized repeating sequence of κ-carrageenan has one sulphate group per disaccharide repeat unit, at O(6) of the D-galactose residue. Carrageenans isolated from certain species of marine algae (notably *Eucheuma cottonii*) approximate closely to this "ideal" structure. Some polysaccharides in the carrageenan series, such as furcellaran, have a lower degree of sulfation. Others are more highly sulphated. In particular, the idealized repeating unit of ι-carrageenan has an additional sulfate group at O(2) of the anhydro sugar, and material approximating closely to this composition is obtained from *E. spinosum*.[8] The neutral repeating sequence of agarose also occurs with varying degrees of sulfation in natural agars, and may also carry *O*-methyl and/or pyruvate ketal groups.[6]

As a further source of structural variation in both carrageenan and agar, the anhydride bridge may be absent in a proportion of the 4-linked residues. This has the effect of changing the geometry of the sugar ring to a form that is sterically incompatible with incorporation in the ordered double-helix structure. The presence of such "kinking" residues is an important factor in development of gel networks.[9,10]

At high temperature, κ-carrageenan and agarose exist in solution as disordered coils, but on cooling they undergo a cooperative conformational transition to a rigid, ordered form.[10] Chiroptical evidence[4,10-14] indicates that the structure adopted under hydrated conditions is the double helix characterized by x-ray fiber diffraction in the condensed phase. Some workers have suggested that the disorder–order transition is preceded and triggered by phase separation or "demixing" in the disordered state,[15] but others have been unable to reproduce their experimental evidence.[16] At sufficiently high concentration and, for carrageenan, under appropriate ionic conditions, conformational ordering is accompanied by gel formation. It is generally accepted that agarose and κ-carrageenan gels are cross-linked by ordered aggregates[17] of double helices, and that "kinking" residues promote formation of a three-dimensional network by limiting the length of the individual helices and thus allowing chains to participate in several different "junction zones."

Ordered assembly of κ-carrageenan into close-packed junctions requires suppression of intermolecular electrostatic repulsion by positive counterions. Large group I cations ($K^+$, $Rb^+$, and $Cs^+$) are the most effective in promoting conformational ordering and gel formation,[18] and it has been demonstrated by nmr that these ions bind directly to the double helix.[19-22] Divalent metal ions, and other monovalent cations ($Li^+$, $Na^+$, $Me_4N^+$), appear to act solely by nonspecific charge screening, with the divalent ions being more effective because of their higher charge.[23] In practical applications as a gelling agent,[1] κ-carrageenan is therefore normally used in the $K^+$ salt form, often in the presence of moderate concentrations of a potassium salt (typically ~ 100 m*M* KCl), conditions that promote extensive helix–helix aggregation.[17] Aggregation of uncharged agarose helices is even more extensive.

One consequence of aggregation is to stabilize the individual helices, with melting of aggregates and associated loss of gel structure on heating occurring at higher temperature than helix formation and gelation on cooling. The extent of aggregation and thermal hysteresis increases as the charge density of the polysaccharide decreases,[24] with a corresponding reduction in the polymer concentration required for gel formation, through the series: ι-carrageenan > κ-carrageenan > furcellaran > agarose. The resulting gels, however, become increasingly more brittle and have an increasing tendency to spontaneous contraction and loss of fluid ("syneresis").

It has been known for many years that these undesirable properties can be reduced, or eliminated, by incorporation of certain plant polysaccharides,[25] notably locust bean gum (LBG), a galactomannan from the seed endosperm of *Ceratonia siliqua*, and konjac mannan (KM), a glucomannan obtained from the tubers of *Amorphophallus konjac* and related species. These materials also reduce the minimum concentration of agarose or κ-carrageenan required to form a continuous network,[26-28] and give a significant enhancement in gel strength at higher concentrations.[28-33] The molecular origin of their "synergistic" action, however, remains controversial.

LBG[25] has a linear backbone of β-D-mannopyranosyl residues linked together through equatorial bonds at positions 1 and 4 (i.e., the same linkage geometry as in cellulose). Solubility is conferred by α-D-galactopyranosyl residues attached at O(6) of a proportion (~ 30%) of the mannose units. Comparison with other galactomannans shows a direct correlation between the fraction of unsubstituted mannose residues present and the magnitude of the

synergistic effects seen with gelling polysaccharides in the agar/carrageenan series.

KM[34] is a copolymer of β-D-mannose and β-D-glucose in the approximate ratio 2:1. As in the galactomannans, the polymer backbone is 1,4-diequatorially linked. A number of different proposals have been made for the arrangement of mannopyranosyl and glucopyranosyl units along the chain, but it is generally agreed that there are no long blocks of either type present. The polymer is solubilized by *O*-acetyl substituents on a proportion of the constituent sugars (with estimates ranging from ~ 5 to ~ 10%). Computer modeling of x-ray diffraction patterns has shown that acetylation at any position of either the glucosyl or mannosyl residues prevents close packing of KM chains.[35]

There have been numerous studies of the effect of LBG, KM and related materials on the failure properties (yield stress and yield strain) of carrageenan and agar gels. The present investigation concentrates on gel rheology under nondestructive conditions (low-amplitude oscillation), interactions in dilute solution, and the temperature course of the disorder–order and order–disorder transitions. A preliminary account of some aspects of the work has been published elsewhere.[36]

## EXPERIMENTAL

### Physical Techniques

Differential scanning calorimetry (DSC) measurements were made using a Setaram microcalorimeter. Values of $\Delta H$ were determined by numerical integration of peak area, using baselines interpolated from higher and lower temperatures by a polynomial curve-fitting procedure. Transition midpoint temperature ($T_m$) was taken as the temperature at which half the total heat change had occurred. Optical rotation was measured at 365 nm on a Perkin-Elmer 241 polarimeter, using jacketed cells of path length 1 or 10 cm, as appropriate. Temperature was controlled by a circulating water bath and measured using a thermocouple in the neck of the cell, but out of the light path. Readings were taken after thermal equilibration at each temperature (typically ~ 5 min). Viscosity measurements were made using concentric cylinder geometry, with inner and outer radii of 5.5 and 6.0 mm, respectively, on a Contraves Low Shear 30 viscometer. The instrument was interfaced with an external drive device to increase and decrease the shear rate linearly between 0 and 100 $s^{-1}$ over a total period of 2 min. Flow curves of shear stress vs shear rate were plotted directly on an X-Y recorder. Low amplitude oscillatory measurements of storage modulus ($G'$), loss modulus ($G''$), and complex dynamic viscosity ($\eta^*$) were made on a sensitive prototype rheometer designed and constructed by one of us (RKR). Most experiments were carried out using cone-and-plate geometry of cone angle 0.05 rad and diameter 5 cm. To circumvent problems of thermal expansion/contraction during heating and cooling scans, the cone was truncated over 45% of its diameter, giving a gap of 0.5 mm between the flat surfaces of the two elements, but retaining constant strain across the outer portion (which constitutes 80% of the total area). For strong gels showing evidence of syneresis and slippage, a specially constructed perforated cylinder geometry was used, in which the sample surrounds and penetrates the moving and stationary elements.[37] In both cases temperature was controlled by a circulating water bath and measured by a thermocouple in direct contact with the stationary element.

### Materials

Potassium chloride was AnalaR grade from BDH (Poole, Dorset, UK). Tetramethylammonium chloride and iodide were from Aldrich. Distilled deionized water was used throughout. A sample of konjac glucomannan from Senn Chemicals AG was kindly supplied by Dr. V. J. Morris (AFRC Institute of Food Research, Norwich, UK) and was from the same batch as the material used in the x-ray diffraction studies of Cairns et al.[38] The powder was allowed to swell overnight in water (at ~ 2% w/w) and was dissolved by autoclaving for 20 min at 120°C. The solution was clarified by centrifugation and dialysed for 2 days against four changes of water, and the final concentration was determined by freeze drying a weighed aliquot. A stock solution of locust bean gum (Meypro fleur M-175 from Meyhall) was prepared in the same way, but with initial dispersion in water in place of the preswelling step. Agarose was kindly supplied by the Marine Colloids Division of FMC (Seakem LE agarose, batch number 60425) and was dissolved in water by stirring on a boiling water bath for 15 min to give a 1% w/w stock solution.

The κ-carrageenan sample used was X6960 from Hercules. The content of ι-carrageenan sequences present (i.e., 2 sulphate groups per disaccharide) was 8%, as determined by the $^1$H-nmr method described by Welti.[39] Potassium and tetramethylammonium salt forms were prepared by cation exchange on Amberlite IR-120 from BDH. The resin was first converted to the $H^+$ form by elution with HCl, and then to the $K^+$ or $Me_4N^+$ form using the appropriate chloride salts. Ion exchange of the carrageenan sample was carried out at high temperature to maintain the polymer in the disordered coil form. A short column of resin in the required cationic form was prepared in a separating funnel and held in an atmosphere of steam under aluminium foil over a boiling water bath. The resin was preheated by elution with hot water (~ 95°C), and the carrageenan solution was introduced at a concentration of ~ 3% w/w, left on the

column for 30 min, and expelled by gentle pressure from a pipette bulb. The solution was then dialyzed against water, and the final concentration again determined by freeze drying a weighed aliquot.

## Preparation of Mixed Solutions

In investigation of the interactions of κ-carrageenan with LBG or KM, all solutions were dialyzed to equilibrium against the same salt solution to ensure that observed changes in physical properties were not due to changes in ionic strength on mixing. Dialysis was carried out at ~ 80°C, to maintain the carrageenan in the disordered state. Two salt solutions were used: 100 m*M* KCl and 150 m*M* $Me_4NI$. The KCl solution was changed once, about midway through a 24 h dialysis period. With $Me_4N^+$, which does not bind specifically to carrageenan,[18,23] the dialysis period was extended to 48 h and the salt solution was changed 3 times. In both cases the final dialysate was used for all subsequent dilutions. Mixtures were prepared by accurate weighing at ambient temperature, and were then heated to fully disorder the algal polysaccharide constituent (for ~ 15 min at 90°C in the case of carrageenan, and at boiling point for agarose). For oscillatory studies of gel formation, the heated mixtures were loaded onto the rheometer with the temperature pre-set at or near the same values (90 and 98°C, respectively).

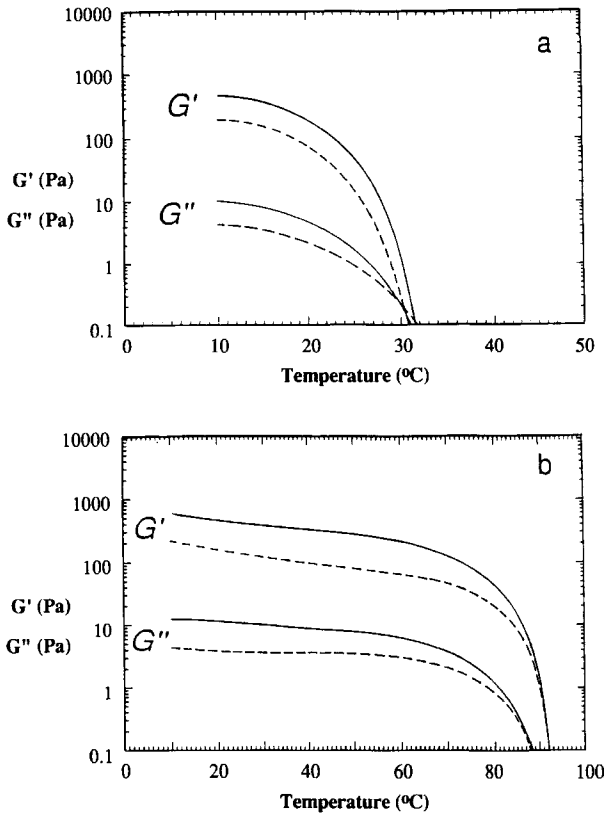


**FIGURE 1** Temperature-dependence of $G'$ and $G''$ (10 rad $s^{-1}$; 2% strain) for agarose (0.2% w/w in water) in the presence (——) and absence (- - -) of 0.08% w/w LBG, on (a) cooling and (b) heating at 1 deg/min.

## RESULTS

### Agarose in Water

Figure 1(a) shows the temperature course of gel formation on cooling (1 deg/min) for agarose (0.2% w/w) in the presence and absence of LBG (0.08% w/w), as monitored by low-amplitude oscillatory measurements of $G'$ and $G''$. The final moduli are significantly higher for the mixed system, but there is no detectable change in gelation temperature (i.e., the onset of the steep rise in moduli). The melting temperature is also unaffected by the presence of LBG [Figure 1(b)], and is about 60°C higher than the temperature of gelation.

Similar results were obtained (Figure 2) using KM in place of LBG, but with indications of a slight elevation of transition temperature. This was confirmed by DSC. Figure 3 shows the heat changes accompanying conformational ordering and gel formation for the same solutions as in Figure 2 (0.2% agarose alone and in combination with 0.08% KM) and for mixtures incorporating two higher concentrations of KM, 0.2 and 0.4% (i.e., equal to and double that of the agarose component). In all cases the presence of KM has no discernable effect (Table I) on the transition enthalpy ($\Delta H$), and the overall form of the DSC traces is also unaffected. There is, however, a small but consistent increase in transition-midpoint temperature ($T_m$; Table I), by ~ 1.5°C.

The mechanical spectra (variation of $G'$, $G''$, and $\eta^*$ with frequency, $\omega$) recorded on completion of the cooling scans in Figures 1(a) and 2(a) are shown in Figure 4. The spectrum obtained for 0.2% agarose alone is typical[40] of a polysaccharide gel at a concentration well above the minimum critical gelling concentration ($c_0$), with $G' \gg G''$ and little frequency dependence in either modulus. The presence of LBG or KM causes an overall shift to higher values, but with no significant change in spectral form. The enhancement in moduli is slightly greater with LBG than with KM.

### $Me_4N^+$ κ-Carrageenan in 150 m*M* $Me_4NI$

Most investigations of the synergistic interactions of κ-carrageenan have concentrated on the $K^+$ salt

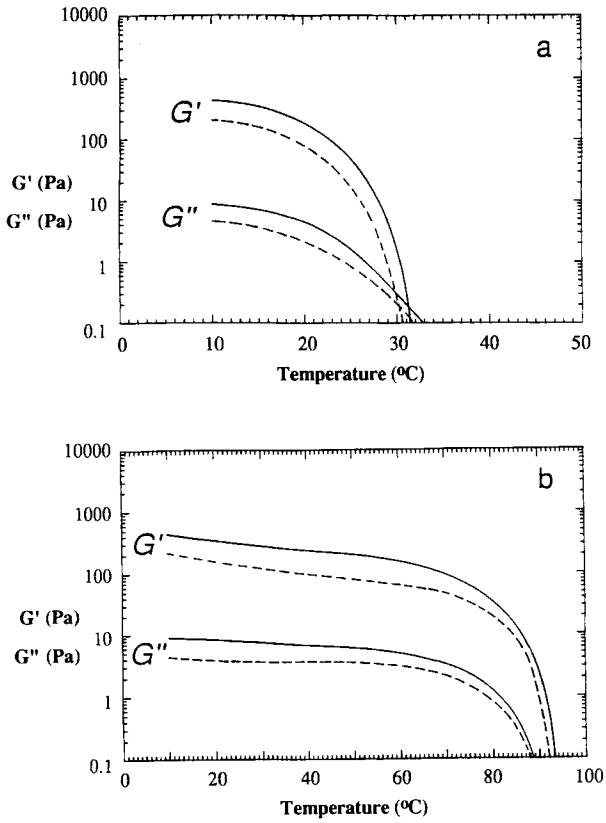


**FIGURE 2** Variation of $G'$ and $G'$ on (a) cooling and (b) heating for agarose in the presence (——) and absence (– – –) of KM; concentrations and conditions as in Figure 1.

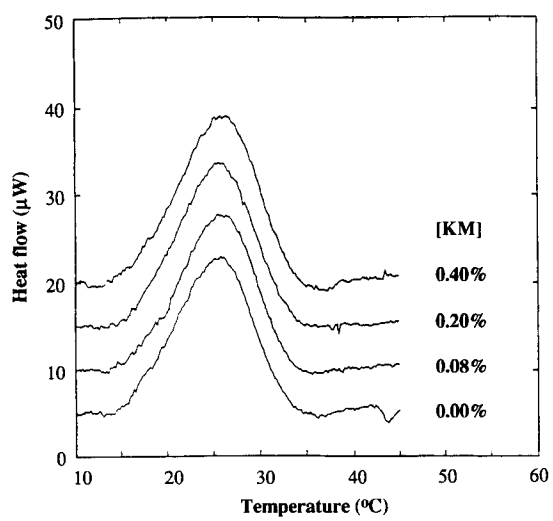


**FIGURE 3** DSC cooling scans (0.3 deg/min) for agarose (0.2% w/w in water) alone, and in the presence of KM at the concentrations (% w/w) shown.

**Table 1 Transition Enthalpy ($\Delta H$) and Midpoint Temperature ($T_m$) from DSC Cooling Scans[a] for Agarose and κ-Carrageenan, Alone and in the Presence of LBG or KM**

| System | $\Delta H$ (J g$^{-1}$) | $T_m$ (°C) |
|---|---|---|
| Agarose (0.2% w/w in water) | | |
| Alone | 18.8 | 23.5 |
| With 0.08% KM | 17.7 | 25.2 |
| With 0.20% KM | 19.6 | 25.0 |
| With 0.40% KM | 17.8 | 25.2 |
| $Me_4N^+$ κ-carrageenan (1.0% w/w in 150 m*M* $Me_4NI$) | | |
| Alone | 29.4 | 41.1 |
| With 0.43% LBG | 29.2 | 40.8 |
| $K^+$ κ-carrageenan (0.085% w/w in 100 m*M* KCl) | | |
| Alone | 29.0 | 49.7 |
| With 0.037% LBG | 40.4 | 50.1 |
| With 0.037% KM | 39.5 | double peak |

[a] $Me_4N^+$ carrageenan was scanned at 0.5 deg/min; all other scans were at 0.3 deg/min; $T_m$ values are reported without adjustment for scan rate.

form which, as discussed previously, displays appreciable helix–helix aggregation, with accompanying thermal hysteresis between gel formation and melting. In the presence of tetramethylammonium as sole counterion, however, conformational ordering may occur without hysteresis or gelation.[41] The transition temperature is strongly affected by nature of the counteranion in the tetramethylammonium salt used, and in particular increases systematically through the halide series: $F^-$ < $Cl^-$ < $Br^-$ < $I^-$. In the experiments reported here, 150 m*M* $Me_4NI$ was used to position the disorder–order transition in a convenient temperature range for investigation.

Figure 5(a) shows the changes in small-deformation moduli on cooling observed for 1% w/w $Me_4N^+$ κ-carrageenan under these ionic conditions, alone, and in the presence of 0.43% w/w LBG. In the absence of galactomannan, the rheological response remains solution-like ($G'' > G'$), but there is a sharp increase in $G''$ on cooling below ~ 50°C, coincident with the temperature course of conformational ordering as monitored by optical rotation [Figure 5(b)]. $G'$ shows a corresponding increase, but does not become large enough for reliable measurement until substantially lower temperature (~ 40°C).

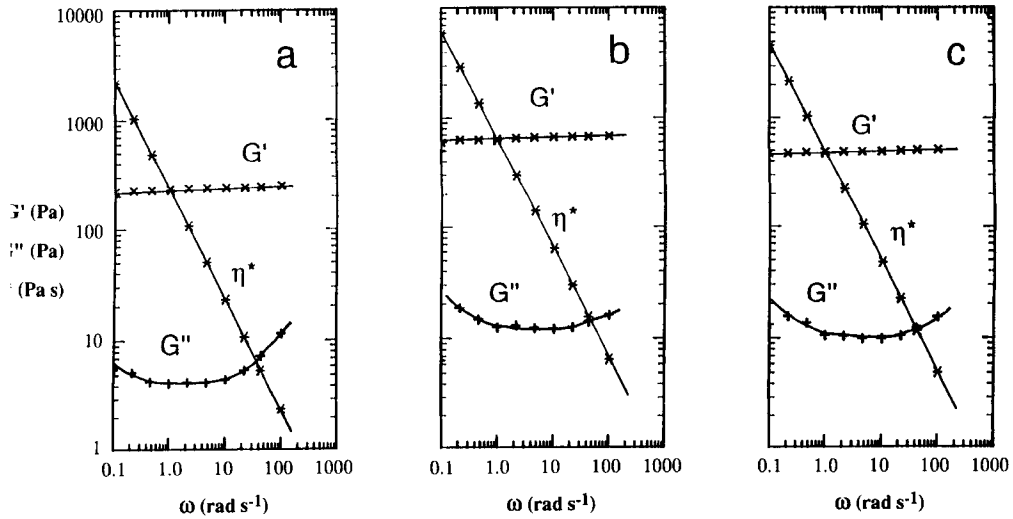


**FIGURE 4** Mechanical spectra (7°C; 2% strain) for agarose (0.2% w/w in water) (a) alone, (b) with 0.08% LBG, and (c) with 0.08% KM.

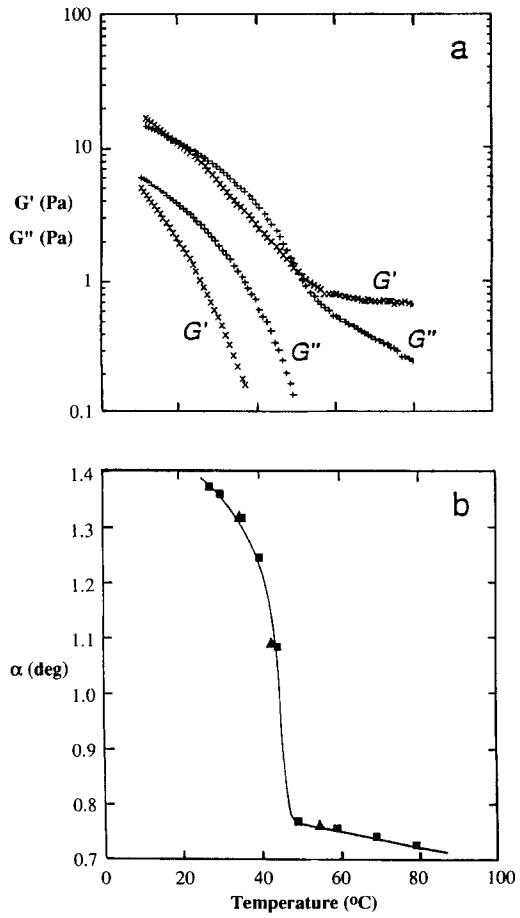


**FIGURE 5** Temperature dependence of (a) $G'$ and $G''$ on cooling (1 deg/min) for $Me_4N^+$ κ-carrageenan (1% w/w in 150 m$M$ $Me_4NI$) alone (lower values) and in the presence of 0.43% LBG (higher values), and (b) optical rotation (wavelength 365 nm; path length 10 cm) for $Me_4N^+$ κ-carrageenan (0.5% w/w in 150 m$M$ $Me_4NI$) on heating (▲) and cooling (■).

In the presence of LBG, the two moduli become roughly comparable in magnitude, and both increase sharply over the temperature range of the conformational transition. There is also a signifi-

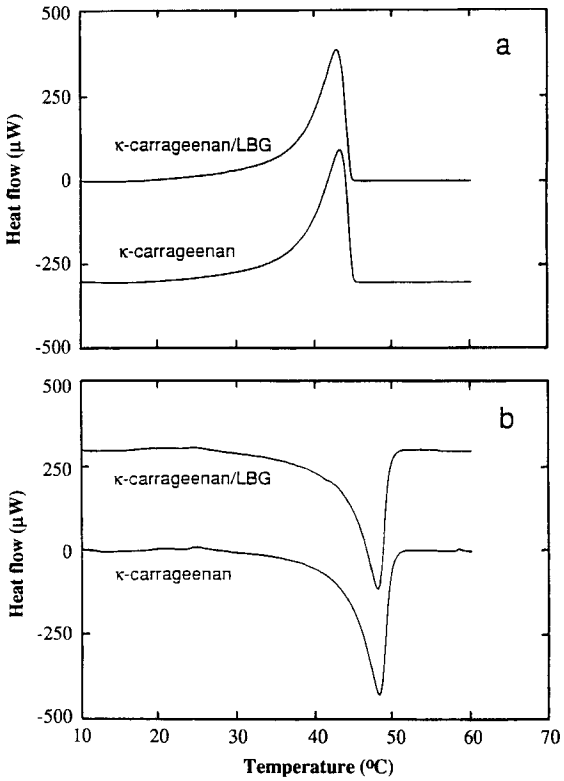


**FIGURE 6** DSC scans for $Me_4N^+$ κ-carrageenan (1% w/w in 150 m$M$ $Me_4NI$) alone, and in the presence of 0.43% LBG on (a) cooling and (b) heating at 0.5 deg/min.

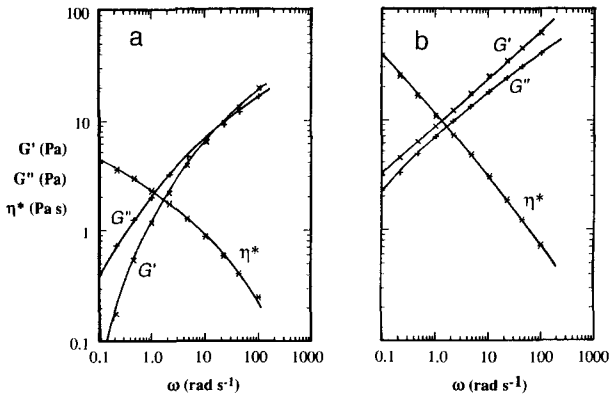


**FIGURE 7** Mechanical spectra (8°C; 2% strain) for $Me_4N^+$ κ-carrageenan (1% w/w in 150 m*M* $Me_4NI$) (a) alone and (b) in the presence of 0.43% LBG.

cant increase in their absolute values in comparison with those for carrageenan alone. As shown in Figure 6, however, there is no detectable change in the DSC exotherms accompanying the disorder–order transition on cooling, or in the corresponding endotherms obtained on heating. Both agree well with the temperature course of conformational change from optical rotation [Figure 5(b)] when allowance is made for thermal lag in the calorimeter, and the enthalpy values ($\Delta H$) and transition temperatures ($T_m$) obtained in the presence and absence of LBG agree closely (Table I).

Figure 7 shows mechanical spectra recorded on completion of the cooling scans in Figure 5(a). The spectrum for carrageenan alone [Figure 7(a)] is typical of a polymer solution, despite the polysaccharide being present in an ordered form rather than as a mobile coil. With LBG present [Figure 7(b)] the rheological response has some gel-like characteristics: $G'$ exceeds $G''$ throughout the accessible frequency range, the relative magnitude of the two moduli ($\tan \delta = G''/G'$) remains roughly constant, and $\log \eta^*$ shows an approximately linear increase with decreasing $\log \omega$, rather than leveling out to a "Newtonian plateau" at low frequency [as seen for carrageenan alone in Figure 7(a)]. The frequency dependence of $G'$ and $G''$, however, is much greater than for a normal polysaccharide gel, and the separation of the two moduli is much lower. Similar behavior is observed for single-polymer geling systems close to the gel point,[42,43] and is characteristic of a very sparingly cross-linked network.

These indications of interaction between LBG and a nongelling preparation of κ-carrageenan were explored further by measurements of dilute-solution viscosity. The approach adopted was to prepare solutions of the two polysaccharides (dialyzed together against 150 m*M* $Me_4NI$) to approximately the same, low viscosity (~ 2.4 times that of the solvent—i.e., at $\eta_{sp} \approx 1.4$), and to mix them together in various proportions. In the absence of any interaction between the polymers, the viscosity of the mixed solutions would be expected to remain close to the common viscosity of the individual starting solutions. Similar experiments have been carried out for mixed solutions of LBG and xanthan (which, at higher concentrations, form strong gels), and show a massive increase in viscosity (by about an order of magnitude).[44] For LBG in combination with $Me_4N^+$ κ-carrageenan, by contrast, there is a significant reduction (Figure 8), indicating that there is an interaction between the two polymers, but that it leads to a contraction in molecular dimensions, rather than to formation of extended clusters of chains.

## $K^+$ κ-Carrageenan in 100 m*M* KCl

An attempt was made to carry out an analogous viscometric study with the carrageenan in the $K^+$ salt form. Two solutions of LBG (equilibrated against 100 m*M* KCl) were prepared to specific vis-

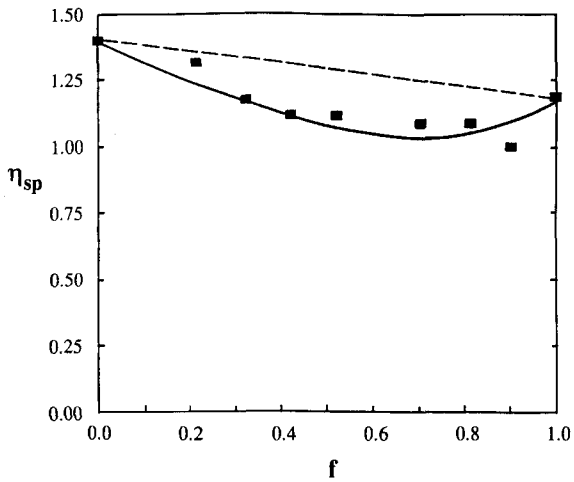


**FIGURE 8** Variation in specific viscosity (25°C) on mixing solutions of $Me_4N^+$ κ-carrageenan (0.085% w/w) and LBG (0.078% w/w) in various proportions. Both solutions were equilibrated against 150 m*M* $Me_4NI$; "f" denotes the fraction of LBG solution in each mixture. Solvent and solution viscosities can be measured to within ±1%, giving a maximum error of ~ 2% in $\eta_{rel}$ and ~ 3% in $\eta_{sp}$ (i.e., ±0.04 at $\eta_{sp} \approx 1.4$). The observed reductions are therefore well beyond experimental error.

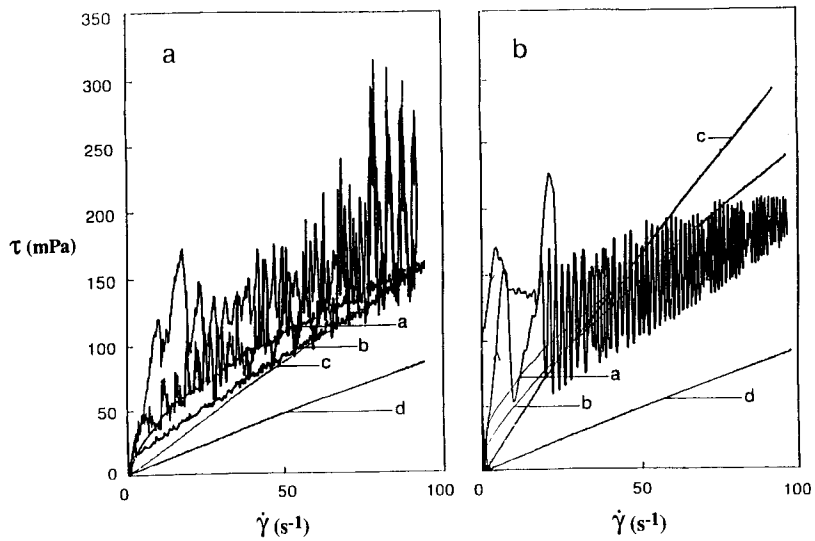


**FIGURE 9** Flow curves (25°C) of shear stress ($\tau$) vs shear rate ($\dot{\gamma}$) for solutions of $K^+$ κ-carrageenan and LBG, both equilibrated against 100 m*M* KCl, and for mixtures incorporating 70% carrageenan solution and 30% LBG solution. The curves labeled "a" and "b" were recorded for the carrageenan solution on, respectively, increasing and decreasing the shear rate; curves "c" and "d" are for, respectively, the LBG solution and the KCl dialysate (with close superposition on acceleration and deceleration); the erratic traces were recorded for the mixed solutions as shear rate was increased and decreased. The concentrations (w/w) of the individual solutions were (a) 0.010% carrageenan and 0.075% LBG; (b) 0.025% carrageenan and 0.157% LBG.

cosities of ~ 0.9 and ~ 2.4, the polymer concentrations used being 0.075 and 0.157% w/w, respectively. The concentrations of carrageenan required to give approximately the same viscosities toward the center of the shear-rate range used (i.e., at ~ 50 $s^{-1}$) were ~ 0.010 and ~ 0.025% w/w. However, even at these extremely low concentrations, the carrageenan solutions showed appreciable shear-thinning (Figure 9) and significant divergence between the flow curves obtained on increasing and decreasing the applied shear rate (i.e., thixotropy). On mixing with LBG, the flow curves became extremely erratic, with sharp peaks and troughs in shear stress, indicating the presence of a dynamic network breaking under the imposed deformation and then immediately reforming before again being broken.

Because of this complicated rheological response to unidirectional shear, the original intention of following changes in viscosity in response to changes in composition of the mixed solutions was obviously impossible. Instead, the solutions were characterized by small-deformation oscillatory measurements. Figure 10 shows the mechanical spectra obtained for the carrageenan solution from Figure 9(b), alone, and in two representative mixtures with LBG. In all cases they have obvious gel-like character ($G' \gg G''$; linear variation of log $\eta^*$ with log $\omega$; little frequency dependence of $G'$), confirming the indications of network structure from viscometry.

The measured values of $G'$ show a systematic variation with composition (Figure 11), rising to a maximum when the LBG solution constitutes about 14% of the total volume [corresponding to the spectrum shown in Figure 10(c)], and falling again to a value close to that for carrageenan alone when the two solutions are mixed in equal proportions [Figures 10(a) and 10(b)]. Figure 11 does not, however, give a true reflection of the relative amounts of each polymer present, since the polysaccharide concentrations required to give comparable viscosity were grossly different (0.157% LBG; 0.025% carrageenan). Figure 12 shows the same data plotted against the concentration of LBG expressed as a percentage of the total polysaccharide content of each solution. $G'$ reaches its maximum value when the two polymers are present in equal amounts, falls steeply on slight reduction in galactomannan content, and shows an approximately linear decrease at higher proportions of LBG.

In the mixing experiments described above, the

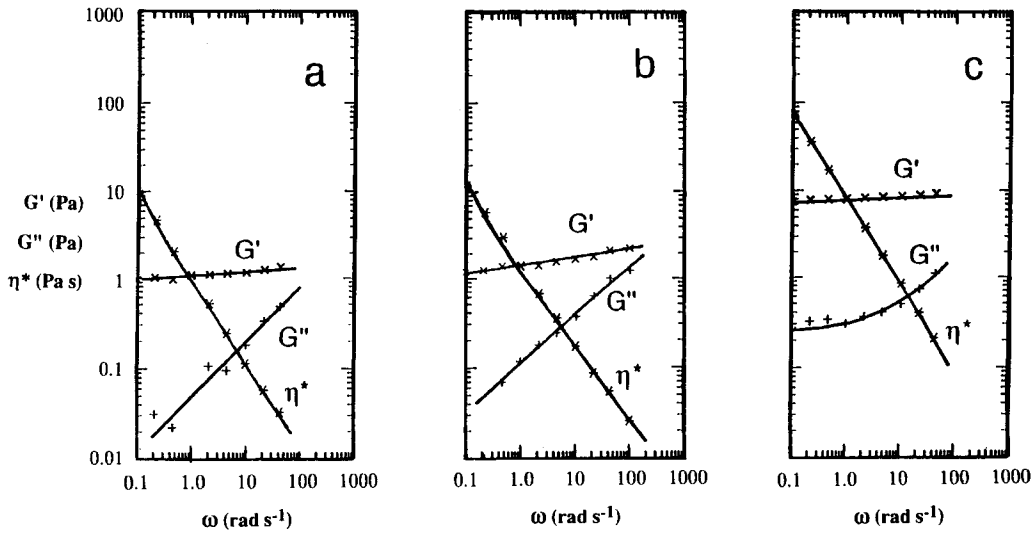


**FIGURE 10** Mechanical spectra (25°C; 2% strain) for mixtures of the solutions from Figure 9(b): 0.025% $K^+$ carrageenan and 0.157% LBG, both equilibrated against 100 m*M* KCl. The fraction of LBG solution present was (a) 0 (i.e., carrageenan alone), (b) 50%, and (c) 13.8% (the ratio giving the maximum enhancement of *G'*; see Figure 11).

concentrations of both polysaccharides are varying simultaneously. For investigation of the temperature course of gelation and melting, the concentration of carrageenan was held constant and the effect of addition of LBG or KM was examined, as in the studies of agarose presented in Figures 1–3.

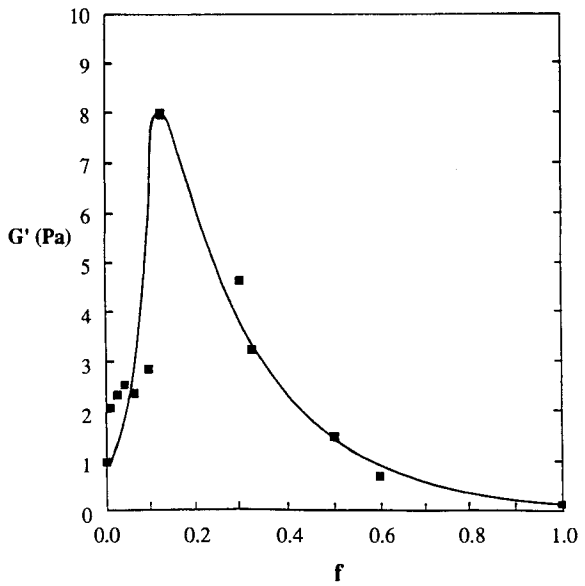


**FIGURE 11** Variation of *G'* (25°C; 10 rad $s^{-1}$; 2% strain) with mixing ratio for the solutions from Figure 9(b): 0.025% $K^+$ κ-carrageenan and 0.157% LBG, both equilibrated against 100 m*M* KCl; "**f**" denotes the proportion of LBG solution present.

Figure 13 shows the variation in *G'* on heating and cooling observed for $K^+$ κ-carrageenan (0.085% w/w in 100 m*M* KCl), alone, and in the presence of 0.036% w/w LBG. As found with agarose (Figure 1), the galactomannan causes an appreciable enhancement in rigidity. In contrast to the agarose system, however, there is also a significant increase in melting temperature [Figure 13(b)] and

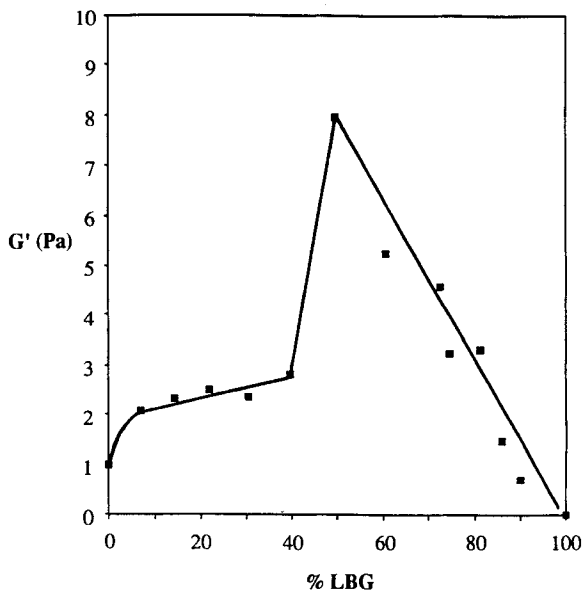


**FIGURE 12** *G'* values from Figure 11, plotted against LBG content expressed as a percentage of the total polysaccharide concentration.

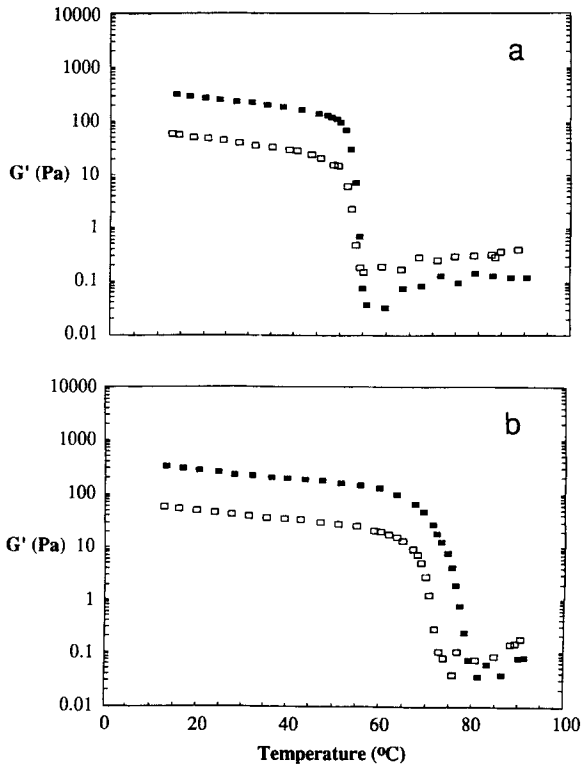


**FIGURE 13** Temperature dependence of $G'$ (10 rad $s^{-1}$; 2% strain) on (a) cooling and (b) heating (1 deg/min) for $K^+$ κ-carrageenan (0.085% w/w in 100 m$M$ KCl) in the presence (■) and absence (□) of 0.036% w/w LBG.

a smaller, but discernible, increase in setting temperature on cooling [Figure 13(a)]. Figure 14 shows cooling curves recorded for the same mixing ratio ( ~ 7:3 carrageenan : LBG), but with the concentration of carrageenan increased [Figure 14(a)] or decreased [Figure 14(b)] by about a factor of 4.3. The enhancement in modulus on addition of LBG becomes greater as the overall polymer concentration is reduced, and the elevation of setting temperature is clearly evident. Closely similar results were obtained (Figure 15) using KM in place of LBG (at the same concentrations as in Figure 13).

Figure 16 shows DSC cooling scans for the same solutions (i.e., 0.085% $K^+$ carrageenan in 100 m$M$ KCl, alone, and with 0.036% LBG or KM). The corresponding heating scans were poorly resolved, since the melting transition is less sharp than the setting process. However, traces obtained at higher concentration, but with the same mixing ratio of carrageenan : KM, are shown in Figure 15, and coincide closely with the temperature course of gel melting for carrageenan alone and in combination with KM. In both cases the DSC heating scans show a single endothermic transition. In the cooling direction, however, the presence of LBG induces a shoulder on the high-temperature side of the single exotherm observed for carrageenan alone, and with KM present the transition is split into two distinct peaks (Figure 16). There is also a significant increase in the overall enthalpy change (Table I), by ~ 40% in the presence of LBG and by ~ 35% with KM.

Bimodal DSC cooling scans have been reported previously by Williams et al.[45] for mixtures of κ-carrageenan and KM at higher (5×) total polymer concentration and lower salt (50 m$M$ KCl). As shown in Figure 17, it was found that as the proportion of KM was increased, the higher temperature peak eventually became the only observable transition. Such behavior is, of course, consistent with a binding process in which the peak at higher temperature arises from association between carrageenan and KM, and the second peak at lower temperature corresponds to ordering of surplus carrageenan.

Since the onset of the heat changes for the mixed systems appears (Figures 16 and 17) to lie outside the DSC envelope for carrageenan alone, Williams

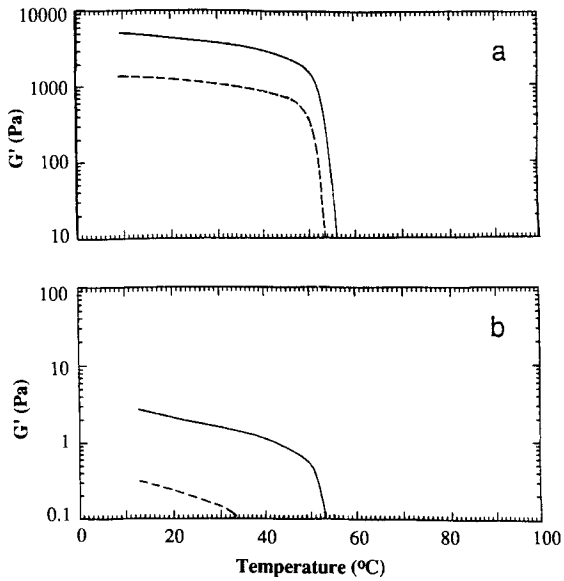


**FIGURE 14** Variation of $G'$ (10 rad $s^{-1}$; 2% strain) on cooling (1 deg/min) for $K^+$ κ-carrageenan in 100 m$M$ KCl in the presence (——) and absence (- - -) of LBG. The polysaccharide concentrations (w/w) used were (a) 0.37% carrageenan and 0.17% LBG; (b) 0.020% carrageenan and 0.008% LBG. The curves in (a) were recorded using perforated geometry to overcome problems of slippage.

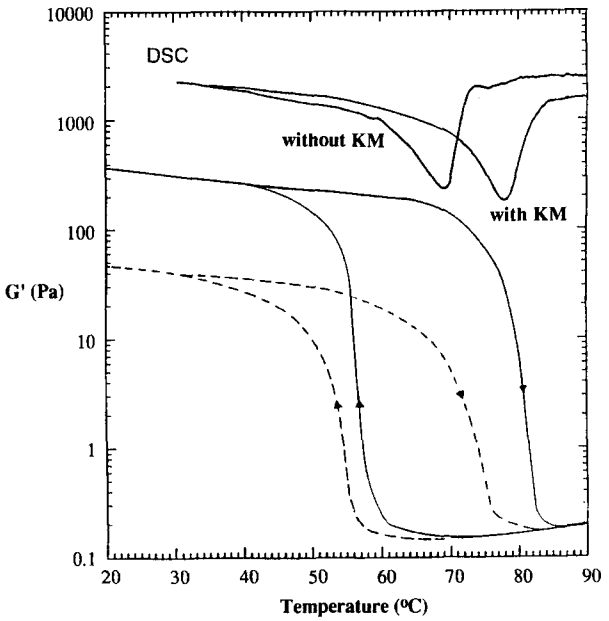


**FIGURE 15** Temperature dependence of $G'$ (10 rad $s^{-1}$; 2% strain) on cooling and heating (1 deg/min) for $K^+$ κ-carrageenan (0.085% w/w in 100 m$M$ KCl) in the presence (——) and absence (- - -) of 0.036% w/w KM. The upper curves are a direct reproduction of DSC heating scans (0.3 deg/min) for $K^+$ κ-carrageenan (1.15% w/w in 100 m$M$ KCl) in the presence and absence of 0.49% w/w KM (i.e., with the same ionic conditions and mixing ratio as in the rheological experiments, but at higher overall polymer concentration).

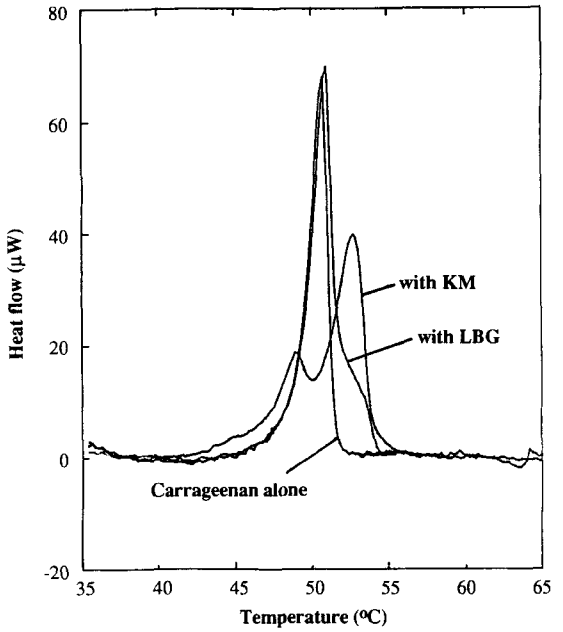


**FIGURE 16** DSC cooling scans (0.3 deg/min; baselines subtracted) for $K^+$ κ-carrageenan (0.085% w/w in 100 m$M$ KCl), alone, and in the presence of 0.036% w/w LBG or KM.

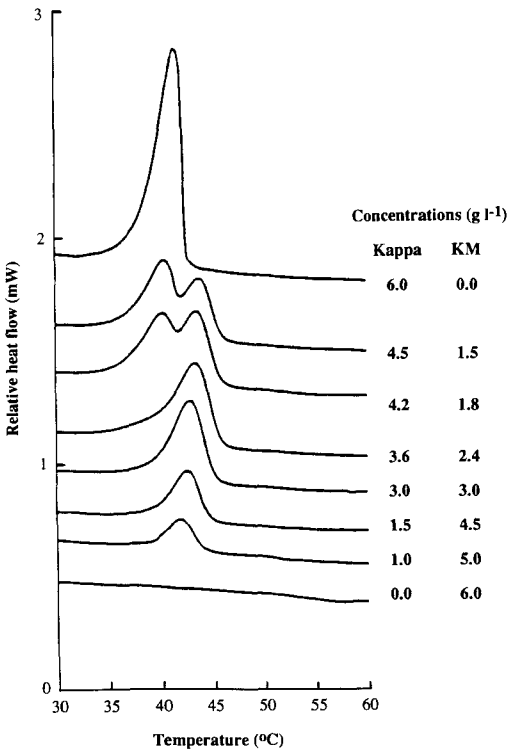


**FIGURE 17** DSC cooling scans (1 deg/min) for mixtures of κ-carrageenan and KM at a total polysaccharide concentration of 0.6% in 50 m$M$ KCl; from Williams et al.,[45] with permission.

et al. proposed[45] that the plant polysaccharide attaches to the carrageenan coil, rather than, as suggested in earlier studies, to the double helix. However, it is extremely difficult to pinpoint the precise onset of DSC transitions, because of the uncertainty in the position of the baseline. As a further complication, the shape of the peaks may be distorted somewhat by heat-transfer processes within the cell. In the present work these difficulties have been circumvented by using static measurements of optical rotation to quantify the temperature course of conformational ordering.

Figure 18 shows optical rotation values for $K^+$ κ-carrageenan, recorded at narrow intervals of temperature on cooling from well above to well below $T_m$. The linear regions at high and low temperature were fitted by least-squares regression analysis to give the "all-coil" and "all-helix" values at intermediate temperatures, and these were used to derive values for the equilibrium constant ($K$) between disordered and ordered sequences across the temperature range of the transition. For two coils in dynamic equilibrium with a double helix, $K$ is

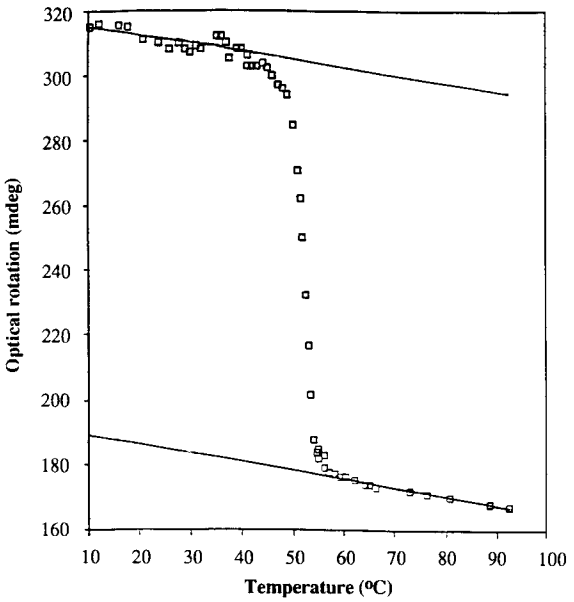


**FIGURE 18** Temperature dependence of optical rotation (wavelength 365 nm; path length 1 cm) on cooling for $K^+$ κ-carrageenan (1.5% w/w in 100 m*M* KCl). The solid lines are from least-squares regression analysis of the linear regions at high and low temperature.

given by $2f^2/(1-f)$, where $f$ is the fraction of disorder (determined directly from the position of the experimental points in Figure 18 between the extrapolated values for coil and helix) and $(1-f)$ is the helix fraction $f_H$. As shown in Figure 19, the resulting values gave an acceptably linear van't Hoff plot of ln $K$ vs $1/T$ (where $T$ is absolute temperature) which was then used to derive $f_H$ as a function of temperature above $T_m$ (Figure 20).

As shown in Figure 16, the onset of thermal change on cooling κ-carrageenan in the presence of KM or LBG occurs about 5°C above $T_m$ for carrageenan alone, where $f_H \approx 0.25\%$ (Figure 20). The formula weight per disaccharide for $K^+$ κ-carrageenan is 424. Thus for a chain with a typical molecular weight of $\sim 5 \times 10^5$, a helix fraction of 0.25% would correspond to three ordered disaccharide units, or in other words to one full turn of the (3-fold) helix structure. An alternative interpretation of the DSC results might therefore be that the plant polysaccharides bind to helical sequences as they form, and thus displace the coil–helix equilibrium to higher temperatures.

## DISCUSSION

The concept of formation of a "coupled network" by direct binding of galactomannans and related plant polysaccharides to the agarose or carrageenan double helix was first proposed more than 20 years ago by Dea et al.,[26] and seems consistent with the results from all subsequent investigations. The only apparent inconsistency is that x-ray fiber diffraction studies of κ-carrageenan in mixed specimens with LBG[46] or KM[38] have yielded patterns closely similar to those seen for carrageenan alone, with no indication of additional diffraction intensities that could be attributed to heterotypic junctions. This has been interpreted as arguing against the coupled network model, but should perhaps more properly be regarded as a null experiment.

The appearance of new diffractions, such as those obtained by the same group for LBG[47] or KM[48] in combination with xanthan, must obviously be regarded as compelling evidence for molecular association (although with the general caveat, applicable to all such studies, that the drying and orientation processes used in sample preparation could, in principle, induce formation of structures that were not present under hydrated conditions). The absence of any such diffractions, however, does not necessarily imply the absence of mixed associations. There are many examples of polymers that are known to adopt ordered structures, but that have resisted protracted attempts to obtain discernable fiber-diffraction patterns. Indeed it could be argued that failure to obtain alignment of comparatively flexible mannan or glucomannan chains attached to a network of rigid, aggregated helices is exactly the behavior that would be anticipated from the coupled network model. There are, however, more general ways in which

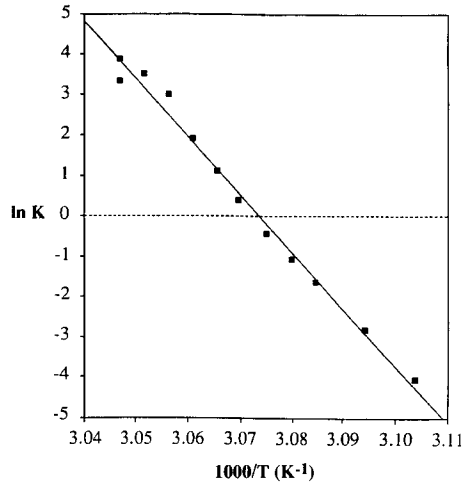


**FIGURE 19** Second-order van't Hoff plot of the optical rotation data from Figure 18.

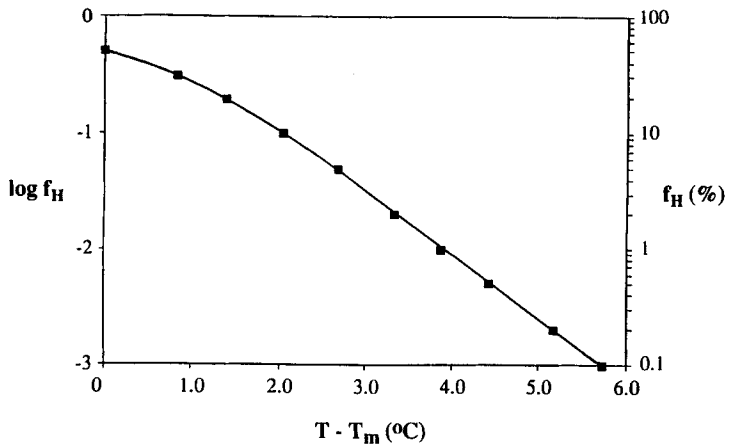


**FIGURE 20** Decrease in helix fraction ($f_H$) for $K^+$ κ-carrageenan with increasing temperature ($T$) above the transition-midpoint temperature ($T_m$), calculated from the van't Hoff plot in Figure 19.

two polymers present in the same system can interact with one another,[49] and these must be considered as a possible alternative explanation of synergistic phenomena.

Enthalpic interactions between chains of two different polymers are normally less favorable than interactions between chains of the same type, thus promoting mutual exclusion of the two materials. Exclusion effects can often cause large changes in physical properties. In particular, if one component is capable of undergoing a disorder–order transition, the presence of a second polymer may promote conversion to the more compact ordered form. When both materials are present at sufficiently high concentration, the system may resolve spontaneously into two separate phases, each containing most of one component and little of the other. Since the concentration of the individual polymers within their respective phases exceeds their original concentration across the whole system, phase separation in biopolymer gels can lead to substantial enhancement of mechanical properties.

It is firmly established, from independent studies by many different groups, that the synergistic activity of galactomannans increases with decreasing galactose content. This is entirely consistent with the original proposal by Dea et al.,[26] which envisaged unsubstituted regions of the mannan backbone binding to the algal polysaccharide helix. In later publications[50–52] the same concept has been extended to include regions with one side devoid of galactose substituents when the mannan chain is fixed in the normal $2_1$ ordered conformation.[53] As would be expected from the coupled network model, nmr studies of LBG in mixed gels with κ-carrageenan[54] or agarose[31] show that a substantial proportion of the galactomannan loses conformational mobility, and that the immobilized sequences have a very low content of galactose.

However, it is also known that under forcing conditions (such as freezing and thawing a concentrated solution), galactomannans can themselves form cross-linked networks.[50] Self-association also increases with decreasing galactose content, again suggesting the involvement of unsubstituted sequences or sides.[52] Thus synergistic interactions could alternatively be explained by an exclusion mechanism, with the presence of the algal polysaccharide promoting formation of a galactomannan (or glucomannan) network.[55]

In most mixed polymer systems exclusion effects become significant only at comparatively high concentrations.[49] Thus the evidence presented in Figures 9–12 of a strong interaction between $K^+$ κ-carrageenan and LBG in very dilute solution seems far more consistent with direct binding between the two polymers. In particular, it is extremely unlikely that self-association of LBG could make any significant contribution to overall rheology under these conditions, since concentrations about 2 orders of magnitude higher are required for network formation by LBG alone.[50] It could, however, be argued that, because κ-carrageenan in the $K^+$ salt form is poised on the verge of insolubility, the presence of even very low concentrations of another polymer could be sufficient to cause a substantial increase in the extent of helix–helix aggregation, with consequent increase in gel-like character.

The most compelling indication of formation of a coupled network is the DSC evidence presented in Figure 16, which, as discussed previously, is entirely consistent with direct binding of LBG or KM to the carrageenan helix as it forms. The observed splitting of the thermal transition could, in principle, also be explained by phase separation. In terms of this interpretation, the carrageenan-rich phase would attract a higher concentration of $K^+$ counterions, and therefore give a conformational transition at higher temperature than the residual carrageenan in the other phase. However, studies by scanning electron microscopy (EM), using atomic mapping of sulphur and potassium to locate carrageenan and fluorescein-labeling to detect galactomannan, gave no indication of phase separation at 1 $\mu$m resolution,[47] and a recent investigation[56] using impact freezing ("slamming"), freeze-fracture, and rotary shadowing to obtain replicas for scanning transmission EM showed homogenous dispersion to a resolution of 100 nm (which is about one tenth of the end-to-end length of a fully extended polysaccharide chain of typical molecular weight). Thus the only interpretation that seems consistent with both EM and DSC is the coupled network model as originally proposed.

The results of the present investigation, however, indicate that binding of galactomannan to the carrageenan helix is not, in itself, sufficient to induce gelation. As shown in Figure 13, addition of LBG to a low concentration (0.085% w/w) of $K^+$ $\kappa$-carrageenan gives a substantial increase in modulus and stabilizes the gel network to higher temperature. At the same concentration, but with the carrageenan in the $Me_4N^+$ salt form, mixing with LBG causes a reduction in solution viscosity (Figure 8). At much higher concentration (1% w/w; Figure 7) addition of LBG induces some gel-like character, but the system remains fluid. These observations demonstrate that there is an interaction between the two polymers, but that it does not result in formation of a true gel.

As found in previous studies,[41] conformational ordering of $Me_4N^+$ $\kappa$-carrageenan under the conditions used in the present work occurs without hysteresis [Figure 5(b)] or gelation, indicating that the helices, once formed, do not aggregate.[17] It would therefore appear that LBG attaches less effectively to the isolated helices of $Me_4N^+$ $\kappa$-carrageenan than to the aggregates induced by $K^+$ ions, and/or that the intermolecular network developed by helix–helix aggregation is an essential component of the synergistic gels. The experimental evidence indicates that both of these factors are involved.

As shown in Figure 6 and Table I, the presence of LBG has no discernable effect on the thermal stability of the $Me_4N^+$ $\kappa$-carrageenan helix, in contrast to the significant stabilisation observed (Figure 16) for the $K^+$ salt form, arguing for weaker binding. The reduction in viscosity observed (Figure 8) on mixing $Me_4N^+$ $\kappa$-carrageenan and LBG in dilute solution indicates that attachment of the galactomannan causes consolidation and contraction of small bundles of carrageenan chains linked together through double helices. In the $K^+$ salt form, by contrast, even at very much lower concentration of carrageenan [Figure 9(a)], the galactomannan appears to generate a continuous network by linking together large microgel particles cross-linked through helix aggregates.

With agarose, where aggregation is even more extensive than in $K^+$ $\kappa$-carrageenan, addition of LBG or KM again appears to have the effect of augmenting the algal polysaccharide network. LBG causes no detectable change in the temperature course of gelation or melting (Figure 1), but KM does appear (Figure 2 and Table I) to induce ordering at slightly higher temperature than for agarose alone. A likely interpretation of why the changes are smaller than those observed (Figure 16) for $K^+$ $\kappa$-carrageenan is that formation of heterotypic junctions occurs in competition with self-association of the algal polysaccharide and therefore becomes less apparent with increasing ease of helix–helix aggregation. Consistent with this proposal, carrageenans with lower sulphate content (and therefore greater tendency to aggregate formation) than the $\kappa$ fraction show a smaller increase in gel strength on addition of LBG.[30]

In summary, the results of the present investigation agree well with the coupled network model of Dea et al.,[26] but with the following qualifications and extensions.

1. Efficient binding requires some aggregation of the algal polysaccharide helices, a possibility that was considered explicitly by Dea et al., and that is fully consistent with the reported absence of detectable synergism for $\iota$-carrageenan.[7]
2. Extensive aggregation restricts synergistic interaction by competition with heterotypic association.
3. Heterotypic junctions augment, rather than replace, the algal polysaccharide network.
4. Galactomannan or glucomannan chains promote conformational ordering of carrageenan or agarose by binding to the double

helix as it forms, thus suppressing the back-reaction in the coil–helix transition.

We thank Dr. I. T. Norton and Dr. T. J. Foster (Unilever Research), Dr. V. J. Morris (AFRC, Norwich), and Dr. I. C. M. Dea (Leatherhead Food RA) for helpful discussions. We also thank CONACYT (Mexico) and Unilever Research for studentship support to one of us (FMG).

## REFERENCES

1. Whistler, R. L. & BeMiller, J. N. (1993) *Industrial Gums,* 3rd ed., Academic Press, San Diego, CA, USA.
2. Araki, C. & Arai, K. (1967) *Bull. Chem. Soc. Jpn.* **40,** 1452–1456.
3. Anderson, N. S., Dolan, T. C. S., Penman, A., Rees, D. A., Mueller, G. P., Stancioff, D. J. & Stanley, N. F. (1968) *J. Chem. Soc.* (C), 602–606.
4. Arnott, S., Fulmer, A., Scott, W. E., Dea, I. C. M., Moorhouse, R. & Rees, D. A. (1974) *J. Mol. Biol.* **90,** 269–284.
5. Millane, R. P., Chandrasekaran, R., Arnott, S. & Dea, I. C. M. (1988) *Carbohydr. Res.* **182,** 1–17.
6. Painter, T. J. (1983) in *The Polysaccharides,* Aspinall, G. O., Ed., Vol. 2, Academic Press, Orlando, FL, pp. 195–285.
7. Moriano, A. L. (1977) in *Food Colloids,* Graham, H. D., Ed., AVI, Westport, CT, pp. 347–381.
8. Anderson, N. S., Dolan, T. C. S. & Rees, D. A. (1973) *J. Chem. Soc. Perkin Trans. I,* 2173–2176.
9. Rees, D. A. (1972) *Biochem. J.* **126,** 257–273.
10. Rees, D. A., Morris, E. R., Thom, D. & Madden, J. K. (1982) in *The Polysaccharides,* Aspinall, G. O., Ed., Vol. 1, Academic Press, New York, pp. 195–290.
11. Rees, D. A., Scott, W. E. & Williamson, F. B. (1970) *Nature* (London) **227,** 390–392.
12. Liang, J. N., Stevens, E. S., Morris, E. R. & Rees, D. A. (1979) *Biopolymers* **18,** 327–333.
13. Stevens, E. S. & Morris, E. R. (1990) *Carbohydr. Polym.* **12,** 219–224.
14. Arndt, E. R. & Stevens, E. S. (1994) *Biopolymers* **34,** 1527–1534.
15. Plashchina, I. G., Muratalieva, I. R., Braudo, E. E. & Tolstoguzov, V. B. (1986) *Carbohydr. Polym.* **6,** 15–34.
16. Hermansson, A.-M. (1989) *Carbohydr. Polym.* **10,** 163–181.
17. Morris, E. R. & Norton, I. T. (1983) in *Aggregation Processes in Solution,* Wyn-Jones, E. & Gormally, J., Eds., Elsevier, Amsterdam, pp. 549–593.
18. Rochas, C. & Rinaudo, M. (1980) *Biopolymers* **19,** 1675–1687.
19. Grasdalen, H. & Smidsrød, O. (1981) *Macromolecules* **14,** 229–231.
20. Belton, P. S., Morris, V. J. & Tanner, S. F. (1985) *Int. J. Biol. Macromol.* **7,** 53–56.
21. Belton, P. S., Morris, V. J. & Tanner, S. F. (1986) *Macromolecules* **19,** 1618–1621.
22. Picullel, L., Nilsson, S. & Ström, P. (1989) *Carbohydr. Res.* **188,** 121–135.
23. Nilsson, S., Picullel, L. & Jönsson, B. (1989) *Macromolecules* **22,** 2367–2375.
24. Dea, I. C. M. (1989) *Pure Appl. Chem.* **61,** 1315–1322.
25. Dea, I. C. M. & Morrison, A. (1975) *Adv. Carbohydr. Chem. Biochem.* **31,** 241–312.
26. Dea, I. C. M., McKinnon, A. A. & Rees, D. A. (1972) *J. Mol. Biol.* **68,** 153–172.
27. Dea, I. C. M. & Rees, D. A. (1987) *Carbohydr. Polym.* **7,** 183–224.
28. Turquois, T., Rochas, C. & Taravel, F. R. (1992) *Carbohydr. Polym.* **17,** 263–268.
29. Cairns, P., Morris, V. J., Miles, M. J. & Brownsey, G. J. (1986) *Food Hydrocolloids* **1,** 89–93.
30. Sewall, C. J. (1992) *J. Appl. Phycol.* **4,** 347–351.
31. Turquois, T., Taravel, F. R. & Rochas, C. (1993) *Carbohydr. Res.* **238,** 27–38.
32. Kohyama, K., Iida, H. & Nishinari, K. (1993) *Food Hydrocolloids* **7,** 213–226.
33. Stading, M. & Hermansson, A. M. (1993) *Carbohydr. Polym.* **22,** 49–56.
34. Nishinari, K., Williams, P. A. & Phillips, G. O. (1992) *Food Hydrocolloids* **6,** 199–222.
35. Millane, R. P., Hendrixson, T. L., Morris, V. J. & Cairns, P. (1992) in *Gums and Stabilisers for the Food Industry 6,* Phillips, G. O., Williams, P. A. & Wedlock, D. J., Eds., IRL Press, Oxford, UK, pp. 531–534.
36. Goycoolea, F. M., Foster, T. J., Richardson, R. K., Morris, E. R. & Gidley, M. J. (1994) in *Gums and Stabilisers for the Food Industry 7,* Phillips, G. O., Williams, P. A. & Wedlock, D. J., Eds., IRL Press, Oxford, UK, pp. 333–344.
37. Richardson, R. K. & Goycoolea, F. M. (1994) *Carbohydr. Polym.* **24,** 223–225.
38. Cairns, P., Miles, M. J. & Morris, V. J. (1988) *Carbohydr. Polym.* **8,** 99–104.
39. Welti, D. (1977) *J. Chem. Res.* (M), 3566–3587.
40. Clark, A. H. & Ross-Murphy, S. B. (1987) *Adv. Polym. Sci.* **83,** 57–192.
41. Norton, I. T., Morris, E. R. & Rees, D. A. (1984) *Carbohydr. Res.* **134,** 89–101.
42. Durand, D., Delsanti, M., Adam, M. & Luck, J. M. (1987) *Europhys. Lett.* **3,** 297–301.
43. te Nijenhuis, K. & Winter, H. H. (1989) *Macromolecules* **22,** 411–414.
44. Foster, T. J. & Morris, E. R. (1994) in *Gums and Stabilisers for the Food Industry 7,* Phillips, G. O., Williams, P. A. & Wedlock, D. J., Eds., IRL Press, Oxford, UK, pp. 281–289.
45. Williams, P. A., Clegg, S. M., Langdon, M. J., Nishinari, K. & Phillips, G. O. (1992) in *Gums and Sta-*

*bilisers for the Food Industry 6,* Phillips, G. O., Williams, P. A. & Wedlock, D. J., Eds., IRL Press, Oxford, UK, pp. 209–216.
46. Miles, M. J., Morris, V. J. & Carroll, V. (1984) *Macromolecules* **17,** 2443–2445.
47. Cairns, P., Miles, M. J., Morris, V. J. & Brownsey, G. J. (1987) *Carbohydr. Res.* **160,** 411–423.
48. Brownsey, G. J., Cairns, P., Miles, M. J. & Morris, V. J. (1988) *Carbohydr. Res.* **176,** 329–334.
49. Tolstoguzov, V. B. (1991) *Food Hydrocolloids* **4,** 429–468.
50. Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A. & Price, J. (1977) *Carbohydr. Res.* **57,** 249–272.
51. McCleary, B. V. (1979) *Carbohydr. Res.* **71,** 205–230.
52. Dea, I. C. M., Clark, A. H. & McCleary, B. V. (1986) *Food Hydrocolloids* **1,** 129–140.
53. Song, B. K., Winter, W. T. & Taravel, F. R. (1989) *Macromolecules* **22,** 2641–2644.
54. Rochas, C., Taravel, F. R. & Turquois, T. (1990) *Int. J. Biol. Macromol.* **12,** 353–358.
55. Fernandes, P. B., Gonçalves, M. P. & Doublier, J. L. (1992) *Carbohyr. Polym.* **19,** 261–269.
56. Brigham, J. E., Gidley, M. J., Hoffman, R. A. & Smith, C. G. (1994) *Food Hydrocolloids* **8,** 331–344.