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Carbohydrate Polymers 59 (2005) 367–376

Carbohydrate Polymers

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Effect of konjac glucomannan deacetylation on the properties of gels formed from mixtures of kappa carrageenan and konjac glucomannan

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Received 1 July 2004; accepted 15 October 2004

Abstract

Mixtures of konjac glucomannan and kappa carrageeean at temperatures above and below the melting point of the kappa carrageenan helix were studied by rheometry and calorimetry. The objective of the work was to investigate the effect of alkali deacetylation of the konjac glucomannan on the properties of the mixed gels. It was shown that the gel storage modulus, at temperatures above the helix coil transition for kappa carrageenan, increases with increasing deacetylation of the konjac glucomannan. Values were much higher than would be expected for konjac glucomannan in the absence of kappa carrageenan. The ability of kappa carrageenan to promote alkali gel formation of konjac glucomannan was attributed to a segregative interaction between the two biopolymers. It is proposed that the system is single phase but gelation of konjac glucomannan is enhanced since this reduces thermodynamically unfavourable contacts between the two polymers. The coil to helix transition temperature for kappa carrageenan, monitored by calorimetry, generally increased in the presence of konjac glucomannan. An exception was at the highest degree of deacetylation when the major exotherm has a similar peak temperature to kappa carrageenan alone. This may be because at high degrees of deacetylation the konjac glucomannan chains are self-associated to such an extent that they are initially unable to promote the formation of kappa carrageenan helices. The increase in the modulus of mixed gels formed below the kappa carrageenan coil to helix transition temperature also increases with alkali deacetylation. It was postulated that this reflects the increase in strength and extent of these associations with increasing deacetylation. As deacetylation increases the gels become less elastic, as monitored by stress relaxation, which was a further consequence of increasing association.

Keywords: Konjac glucommanan; Carrageenan; Gel; Calorimetry; Segregative interactions

1. Introduction

Konjac glucomannan is becoming an important industrial polysaccharide. It is a neutral polysacchride derived from the tubers of Amorphallus konjac. It consists primarily of a linear chain of β1-4 linked glucose and mannose units with a glucose mannose ratio of approximately 1:1.5 (Bewley & Reid, 1985; Maeda, Shimahara, & Sugiyama, 1980). There has been a suggestion that there are some branches on the C3 on mannose; the length of the branched chains being in the range of 11–16 mannose units (Bewley & Reid, 1985) and 5–10% of the backbone residues being acetylated (Dea et al., 1977; Maekaji, 1978).

In the presence of alkali konjac glucomannan will deacetylate and form a heat stable gel that is the basis of many traditional oriental foods (Kohyama, Iida, & Nishinari, 1993; Maekaji, 1978; Nishinari, Williams, & Phillips, 1992). Gelation has also been reported for non-deacetylated konjac glucomannan but at concentrations of 7% and higher (Hirai, 1954) presumably because in the concentrated systems there are enough molecules with non-acetylated blocks to allow network formation. Alkali gelation is believed to be a consequence of the formation of associations between acetyl free regions of the backbone. NMR studies supported by rheology suggest that there is a time delay between deacetylation and network formation (Williams et al., 2000). In addition to deacetylation, alkali

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doi:10.1016/j.carbpol.2004.10.007

treatment to pHs >11 result in ionisation of hydroxyl groups and increased solvation, promoting solubility. Deacetylation of the unbuffered solution will result in a pH fall, reducing this affect and thus promoting aggregation through the dual mechanisms of charge reduction and loss of acetyl groups. Nishinari and co-workers have shown that gelation rate increases with temperature, molecular weight and concentration of the polysaccharide and the amount of alkali but decreases with increasing initial degree of acetylation (Gao & Nishinari, 2004; Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002; Nishinari et al., 1992; Yoshimura & Nishinari, 1999).

In its non-deacetylated form konjac glucomannan shows a synergistic interaction with both kappa carrageenan and xanthan gum (Dalbe, 1992; Goycoolea, Richardson, Morris, & Gidley, 1995; Williams, Clegg, Langdon, Nishinari, & Piculell, 1993). Using X-ray diffraction Cairns, Miles, and Morris (1988) did not observe any new ordered structure in kappa carrageenan konjac glucomannan mixture. It was concluded that konjac glucomannan was not incorporated into kappa carrageenan crystalline junction zones. Williams et al. (1993) observed the interaction between kappa carrageenan and konjac glucomannan using differential scanning calorimetry (DSC) and electron spin resonance (ESR). It was proposed that konjac glucomannan was adsorbed onto the surface of aggregated kappa carrageenan causing an increase in the transition temperature of the mixed gel. A similar increase in transition temperature has been reported from calorimetric studies by Goycoolea, Foster, Richardson, Morris, and Gidley (1994). Kohyama and coworkers (Kohyama, Iida & Nishinari, 1993; Kohyama, Sano & Nishinari, 1996) studied the effect of konjac glucomannan with different molecular weights on the rheological and thermal properties of mixed (1:1) konjac glucomannan and kappa carrageenan gels. They proposed that there were two crystalline regions in the mixed gel; one region consisting of kappa carrageenan alone and another of associations between konjac glucomannan and kappa carrageenan. The latter junction zone was weaker than the former and was not heat resistant but contributed to gel properties.

In the patent literature Vernon, Cheney, and Stares (1980) disclose the preparation of a heat stable gel from konjac glucomannan and kappa carrageenan mixtures at pHs and konjac glucomannan concentrations lower than would be expected for the formation of thermo-irreversible gels by deacetylaton of konjac glucomannan alone. For example, a thermo-irreversible gel of kappa carrageenan and konjac glucomannan at 1% total concentration could be prepared by initially heating to 130 °C for 50 min at pH 7.0 (Vernon et al., 1980). A possible interpretation is that, under these severe heating conditions, the konjac glucomannan deacetylates at neutral pH and such konjac glucomannan gels can form at low concentrations in the presence of kappa carrageenan.

There has been little fundamental work carried out on the gelation mechanism of deacetylated konjac glucomannan when it is incorporated with kappa carrageenan at low concentrations. The objective of the work reported in this paper was to explore the effect of alkali deacetylation of konjac glucomannan on the properties of mixed gels formed with kappa carrageenan. These gels were compared with mixed gels formed under conditions where konjac glucomannan does not deacetylate and also with kappa carrageenan gels formed in the absence of the konjac glucomannan.

2. Materials and methods

2.1. Materials

Konjac glucomannan No.3 (K3) was kindly supplied by Siam Konjac, Thailand. Kappa carrageenan was purchased from Sigma Aldrich Ltd, Poole, UK. All chemical reagents were supplied by Fischer Scientific UK Ltd, Loughborough, UK and were of reagent grade.

2.2. Sample preparation

Phosphate buffers, pH 6 and pH 8 were prepared by dissolving $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4 and NaCl in distilled water. The pH 8 buffer was adjusted to pH 10 with 0.5 N NaOH. KCl was added to all buffers to give 30 mM K^+. Solutions of kappa carrageenan were prepared, at a range of concentrations, at pH 6 and pH 10. Mixtures of kappa carrageenan and konjac glucomannan (1:1) were prepared at 0.6% (w/w) total polysaccharide concentration in phosphate buffer at pH 6, 8 and 10. The appropriate amount of polysaccharide (dry basis) was dispersed in the phosphate buffer in a media-glass bottle at room temperature by stirring with a magnetic stirrer for 1 min. The samples in the screw capped bottles were then heated to 85 °C in a water bath with continuous magnetic stirring and held for 10 min after the internal temperature reached 85 °C.

For DSC measurement and rheological testing, 15 ml of hot solution were poured into a glass tube that was then capped. Samples were left to cool at room temperature for at least 1 h. Some samples were stored overnight at 6 °C. Before measurement the samples were re-melted by putting the screw capped tubes in a water bath until the internal sample temperature reached 85 °C.

For stress relaxation measurements the gel was prepared by pouring 7 ml of the re-melted sample into small cylindrical perspex moulds of 40 mm in height and 20 mm in diameter. These were covered with paraffin oil to limit evaporation. The sample was held at room temperature for 3 h and cut to a height of 15 mm before measurement at ambient temperature. In some cases sealed samples were incubated in an oven at 65 °C for 8 h or 90 °C for 2 h prior to holding at room temperature.

2.3. Differential scanning calorimetry (DSC)

Measurements were performed using a Micro DSC (Setarum, France) fitted with two 1 cm^3 cells; sample and reference. About 800 mg of the re-melted sample were weighed precisely and the same quantity of buffer (within <0.5 mg) was used as a reference. Each sample was heated from 25 to 90 °C to ensure the same thermal history and then cooled down to 10 °C to enable gelation to take place. After the initial heating and cooling some samples were heated to and held at 65 °C for 8 h, or 90 °C and held for 2 h. After holding the sample was cooled down to 10 °C and heated to 90 °C. In all cases the scanning rate was 1 °C min^{-1}. The instrument baseline was determined with buffer alone in both the sample and reference cell. This baseline was subtracted from the results obtained for the sample and the enthalpy was then normalised to the sample weight.

2.4. Rheological measurement

2.4.1. Dynamic testing

Small deformation shear oscillatory testing was performed using a controlled stress rheometer (CVO 50, Bohlin, UK). The measurements were carried out in ribbed concentric cylinder geometry, inner cylinder diameter 14 mm at a frequency of 1 Hz and a strain of 2%, which was within the linear viscoelastic region. Samples were covered with paraffin oil to limit evaporation. Data was recorded during cooling and heating at a rate of 1 °C min^{-1}. Some samples were cooled down from 90 to 25 °C, heated to 65 °C and held for 8 h, or to 90 °C and held for 2 h prior to measuring during to cooling to 25 °C and reheating to 90 °C.

2.4.2. Young's modulus and stress relaxation

The stress relaxation in compression was measured using a TA-XT2 (Stable Micro Systems, UK) fitted with a 5 kg load cell. The gels (20 mm diameter and 15 mm high) were compressed to 5% strain at a rate of 10 mm s^{-1}, with a 60 mm diameter perspex probe.

Young's modulus (E) was estimated from the initial slope of the force deformation response (dF/dL) using the expression

$$E = \frac{dF}{dL}\frac{L_0}{A_0} \qquad (1)$$

Where A_0 is the initial gel cross sectional area and L_0 the initial gel height.

The force required to maintain the 5% strain for 300 s was recorded. The response was fitted to the relationship:

$$\frac{F(0)t}{F(0)-F(t)} = k_1 + k_2 t \qquad (2)$$

where $F(0)$ is the maximum force and $F(t)$ is the force at time t and k_1 and k_2 are constants (Peleg & Normand, 1983).

2.5. Degree of acetylation and the amount of acetyl release

Acetyl substitution of the konjac glucomannan powder was measured using an enzymic assay from Boehringer Mannheim (R-Biopharm Cat. No. 0148261). The amount of acetyl groups in the gels was calculated after measuring the amount of free acetic acid released.

Table 1
Thermal properties from DSC and rheological properties of k-carrageenan at various concentrations compared with a k-carrageenan and konjac glucomannan mixture (1:1) 0.6% total polysaccharide at pH 6

Sample	DSC				Rheology			
	Cooling		Heating		T_{gel}[a] (°C)	T_m[b] (°C)	G'25 °C (kPa)	E (kPa)
	T_{gel} (°C)	Enthalpy (J/g Car)	T_m (°C)	Enthalpy (J/g Car)				
Car[c] 0.3%	35	37	n.d.[d]	n.d.	34	52	1.6	5.6
							1.9[e]	*7.1*
Car 0.4%	35	37	n.d.	n.d.	35	52	3.4	8.1
							1.6	*5.3*
Car 0.5%	36	38	n.d.	n.d.	37	53	5.9	14.2
							2.2	*2.6*
Car 0.6%	36	38	56	32	37	54	8.2	19.4
							1.1	*4*
Car 0.7%	37	38	56	32	38	54	11.3	24.3
							2.8	*5.2*
CK[f] 0.6%	37	36	59	28	37	57	7.1	23.2
							2.5	*5.2*

[a] Determined as the temperature when G' markedly increased from baseline.
[b] Determined as the temperature when G' decreased to baseline.
[c] Car, k-carrageenan.
[d] n.d., not determined.
[e] Coefficient of variation (%).
[f] CK, k-carrageenan and konjac glucomannan mixture (1:1).

Table 2
Percentage of acetyl release in carrageenan and konjac glucomannan mixture (1:1) 0.6% total polysaccharide concentration at various pHs before and after holding at different levels

Treatment	% release[a]		
	pH 6	pH 8	pH 10
Before holding	n.f.[b]	35 *1.77*[c]	77 *3.87*
After holding at 65 °C for 8 h	n.f.	71 *3.11*	87 *2.68*
After holding at 90 °C for 2 h	n.f.	81 *2.96*	89 *3.12*

[a] Based on the substitution of acetyl group in konjac glucomannan.
[b] n.f., not found (below the detection limit).
[c] Coefficient of variation (%).

3. Results and discussion

3.1. Kappa carrageenan and mixed gels at pH 6.0

Table 1 compares the melting and setting parameters obtained from calorimetry and rheological measurement for kappa carrageenan at a range of concentrations and for the 0.6% mixed gel (1:1) at pH 6.0. Under these conditions deacetylation would not be expected to occur (see results in Table 2). For kappa carrageenan alone both techniques reveal a slight increase in melting (T_m) and setting temperatures (T_{gel}) with polysaccharide concentration. The melting temperature is approximately 17° higher than the setting temperature. T_{gel} and T_m for the kappa carrageenan and konjac glucomannan mixture (0.3%/0.3%) is higher than found for 0.3% kappa carrageenan. The temperature increase is greater for the melting transition. The enthalpy of the transition, when expressed per g of kappa carrageenan, is consistent with the value of ∼36 J/g reported by Williams et al. (1993) for this polysaccharide in the concentration range 0.3–0.6% prepared in 50 mM KCl. In contrast to these previously reported results we observed only a small decrease in enthalpy or increase in setting temperature for the kappa carrageenan and konjac glucomannan mixture (1:1) compared with 0.6% kappa carrageenan alone. These differences between kappa carrageenan alone and the mixture were more noticeable in the heating response.

Rheologically determined melting and setting points were close to those obtained calorimetrically (Table 1). Although the incorporation of konjac glucomannan decreased rather than increased the enthalpy change due to the kappa carrageenan setting and melting transition, it made a major contribution to the rheology of the gel. The addition of 0.3% konjac glucomannan to 0.3% kappa carrageenan more than doubled the G' value obtained on cooling to 25 °C and showed a large relative increase in the Young's modulus (E) obtained from the compression experiments.

The determination of moduli from oscillatory measurements on kappa carrageenan gels has recently been discussed by Chen, Liao, and Dunstan (2002) who have shown that slippage can cause anomalously low values. For a perfectly elastic material E obtained from the initial slope of the compression response should equal $3G'$ (where G' is the storage modulus obtained from oscillatory rheometry). Often a dependence of the modulus with an exponent close to the second power of the concentration is reported for polysaccharide gels, although it is recognised that a much more severe dependency at low concentrations would be predicted (Clark & Ross-Murphy, 1985). Fig. 1 shows plots of E and $3G'$ obtained at 1 Hz against the square of the kappa carrageenan concentration (c^2) for kappa carrageenan gels. Reasonable agreement with a c^2 dependence is obtained particularly for the G' data, however, at higher

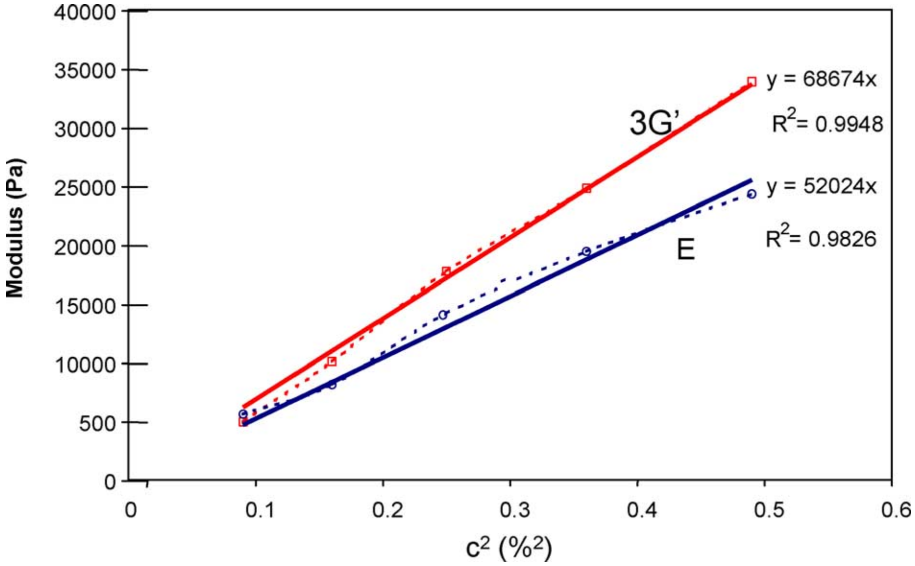

Fig. 1. Comparisons of storage modulus from oscillatory rheometry (G') and initial slope of compressed gels (E) of kappa carrageenan gels. Plot of $3G'$ (□) and E (O) against square of concentration (c^2) The solid line is the best fit to modulus $\propto c^2$.

concentrations $3G'$ is greater than E. It is possible that this difference could be explained by the viscoelasticity of the gel and the different time scales of measurement. Although G' for gels is generally found to be frequency-independent, the stress relaxation modulus, which will be relevant to the determination of Young's modulus, is always time-dependent (Mitchell, 1980). This time dependence is observed for all the gels in this study. The higher values for $3G'$ compared to E do not suggest problems with slip effects in oscillatory measurements when the ribbed concentric cylinder geometry is used.

3.2. Effect of alkali treatment

Konjac and konjac/carrageenan gels were prepared at pH 6.0, 8.0 and 10.0 and additional holding times of 8 h at 65 °C and 2 h at 90 °C were used. As controls these holding times and pH treatments were applied to 0.3 and 0.6% kappa carrageenan alone. It was not possible to prepare a homogenous gel with 0.3% konjac glucomannan alone even for the most severe alkali treatments used as a precipitate was obtained. This is in agreement with Case, Knopp, Hamann, and Schwartz (1992) who reported a critical concentration for konjac glucomamman gelation of 0.4% and precipitation on alkali treatments of konjac glucomannan at concentrations of 0.3% and below.

Table 2 displays the effect of these treatments on the acetyl release and hence deacetylation of the konjac glucomannan. Whereas there appears to be no deacetylation at pH 6.0 for the most severe treatment (pH 10 at 90 °C for 2 h) nearly 90% of the acetyl groups originally present were lost.

The thermal transitions accompanying cooling and then heating were measured rheologically and by calorimetry. The rheological data shown in Figs. 2 and 3 reveals a transition on heating and cooling for the deacetylated samples prepared at pH 10.0 and at pH 8.0 at comparable temperatures to that observed for the non-deacetylated

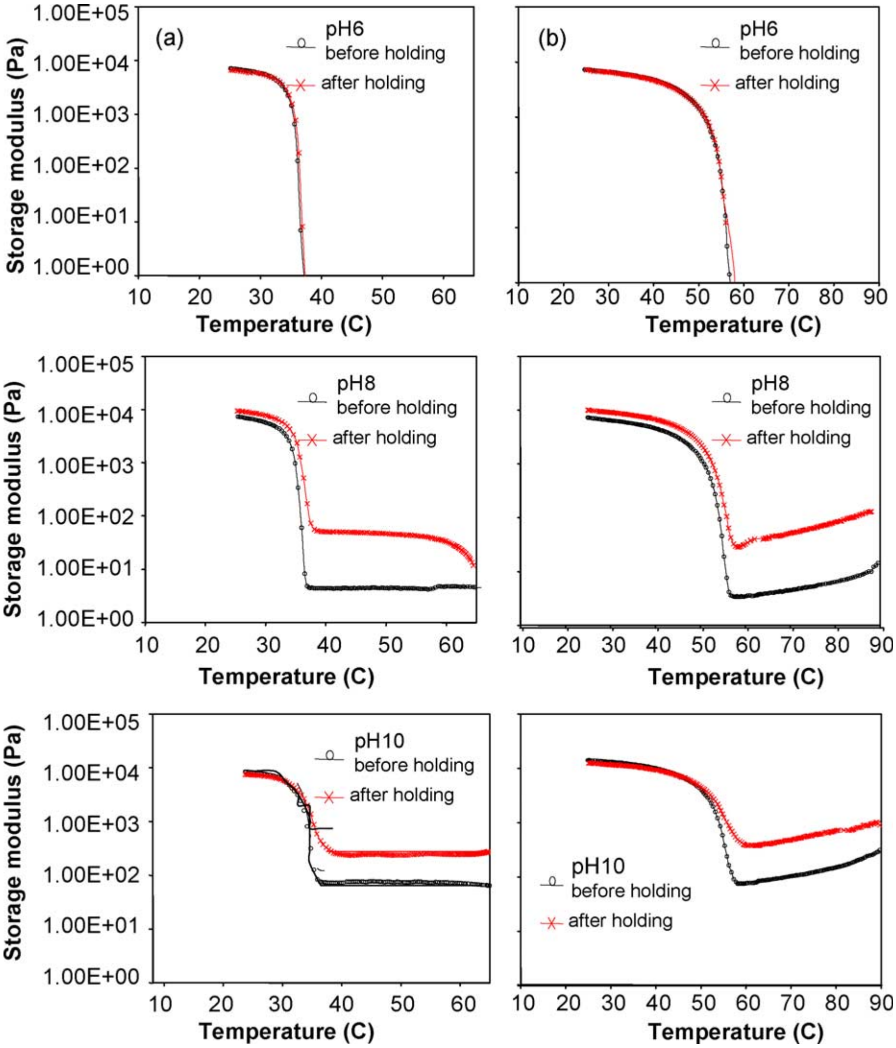

Fig. 2. Storage modulus (1 Hz) when cooling (a) and heating (b) kappa-carrageenan and konjac glucomannan mixture (1:1) 0.6% total polysaccharide at various pH before and after holding at 65 °C for 8 h.

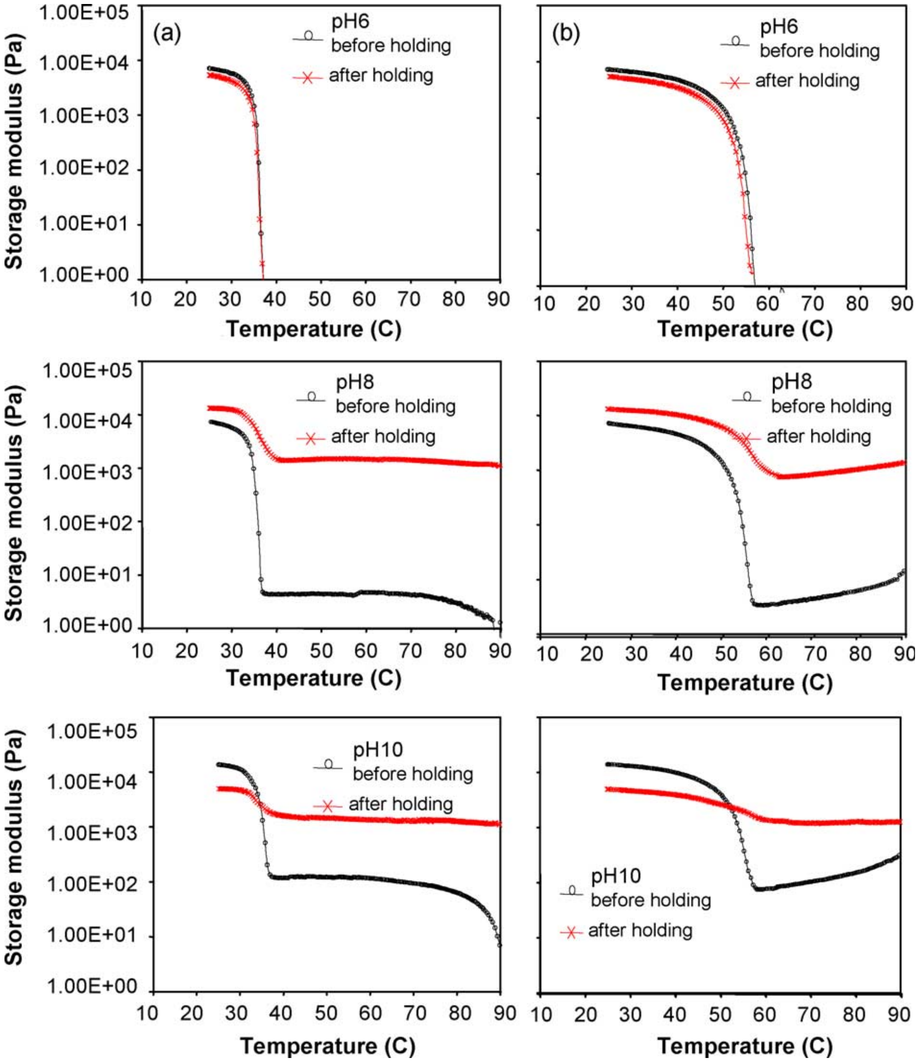

Fig. 3. Storage modulus (1 Hz) when cooling (a) and heating (b) of k-carrageenan and konjac glucomannan (1:1) 0.6% total polysaccharide at various pH before and after holding at 90 °C for 2 h.

samples. This can be associated with the melting and formation of kappa carrageenan helices. For these deacetylated samples the modulus at temperatures above the kappa carrageenan transition increases with the extent of deacetylation. The konjac glucomannan concentration in these systems is only 0.3%. It is not possible for this alone to account for the moduli obtained above the kappa carrageenan melting temperature since, as discussed previously, alkali treatment of 0.3% konjac glucomannan did not give a gel. Yoshimura and Nishinari (1999) and Williams et al. (2000) reported moduli of ~10 Pa after alkali gelation of 1% konjac glucomannan and ~1 Pa for 0.5% konjac glucomannan. For the most extensively deacatylated samples moduli in excess of 10^3 Pa above the kappa carrageenan melting temperature are observed in our work. This data is consistent with the hypothesis that the thermo irreversible gels described in the patent literature formed at low concentrations (~0.2%) of konjac glucomannan in the presence of kappa carrageenan result because the glucomannan deacetylates during autoclaving (Vernon et al., 1980).

A possible mechanism for forming gels at low konjac glucomannan concentrations in the presence of kappa carrageenan is segregative phase separation. Phase separation would be encouraged by deacetylation that promotes konjac glucomannan association. To explain the high moduli obtained above the carrageenan melting temperature it is necessary to assume that the konjac glucomannan rich phase is continuous. If this is not the case, a precipitate rather than a gel would be formed. The moduli of such mixed systems has been discussed in terms of the blending laws proposed by Takayanagi, Harima, and Iwata (1963). Where the continuous phase has a higher modulus than the included phase the modulus of the phase separated system will approach that predicted by the isostrain model. If the modulus of the included phase is very much lower than the continuous phase, which will be the case suggested here, then the modulus of the mixed system will equal ΦG where

Φ is the volume fraction occupied by the continuous phase and G is the modulus of that phase. The maximum values found for G' prior to carrageenan gelation was $\sim 2\times 10^3$ Pa (Fig. 3b). Values of the modulus $\sim 10^4$ Pa have been previously reported for alkali gels prepared from 2% konjac glucomannan (Huang et al., 2002). If it is assumed that, under the most severe alkaline treatment used, segregative phase separation occurs to the extent where there is no konjac glucomannan in the carrageenan domain. At concentrations of konjac glucomannan above 2%, if G is proportional to the square of the concentration then a modulus of 2×10^3 Pa would only be achieved if the phase volume of the konjac glucomannan was reduced to ~ 0.11 and its concentration in that phase increased from 0.3 to 2.7%. If a similar argument is applied to the data of Yoshimura and Nishinari (1999), who obtained a plateau value of G' of 2300 Pa for alkali gelation of a 2% solution of a high molecular weight konjac glucomannan and an exponent of 2.55 for the relationship between modulus and concentration, then an even lower volume for the konjac phase (~ 0.05) is obtained. The maximum phase volume that could be occupied by carrageenan inclusions if they remained spherical would be 0.68 corresponding to a volume for the konjac phase of 0.32. It is, therefore, difficult to explain our results using the idea of segregative phase separation.

It appears, therefore, that random coils of kappa carrageenan promote konjac glucomannan gelation when the two polymers are in a single phase. The thermodynamic driving force for this is the reduction of enthalpically unfavourable contacts between the two polymers. This reduction will be promoted by the polymers adopting more compact conformations. The latter can be achieved through association resulting in gelation. The effect of a non-gelling polysaccharide on gelation of a second polymer within a single phase has been previously reported. For example, Tolstogusov et al. (1974) demonstrated that the incorporation of dextran increased the rate of conformational ordering and gelation of gelatin and more recently it has been shown that oxidised starch promoted the gelation of low methoxyl pectin (Picout, Richardson, & Morris, 2000; Picout, Richardson, Rolin, Abeysekera, & Morris, 2000). Enhancement occurred when the system remained homogenous but depletion in gel strength occurred when the system became inhomogeneous. This phenomenon of promotion of gelation in biopolymer mixtures in a single phase has been reported in a recent series of papers (Giannouli, Richardson, & Morris, 2004a–c).

The next point of interest is whether deacetylation of konjac glucomannan network influenced the subsequent kappa carrageenan helix formation and gelation event. Calorimetry does not reveal significant differences in the position or magnitude of the cooling exotherm nor heating endotherm on konjac glucomannan deacetylation, with the exception of the sample that had been held for 90 °C at pH 10 for 2 h (Table 3, Fig. 4). When this was cooled it showed a bimodal peak (Fig. 4a). The majority of the endotherm corresponds to the temperature observed for kappa carrageenan alone, rather than the higher temperature found for the other kappa carrageenan konjac glucomannan mixtures. This finding was similar to that reported by Williams et al. (1993) for mixtures of kappa carrageenan and konjac glucomannan where the ratio of kappa carrageenan to konjac glucomannan was higher than 2:1. From the dependence of the two transitions on the cation ion content Williams et al. (1993) suggested that both events, observed as the bimodal peak, were due to the formation of kappa carrageenan helices. These workers postulated that

Table 3
Transition temperature and enthalpy required for k-carrageenan compared with k-carrageenan and konjac glucomannan mixture (1:1) before and after holding at 65 °C for 8 h or 90 °C for 2 h

Sample	Before holding				After holding			
	Cooling		Heating		Cooling		Heating	
	T_{gel} (°C)	Enthalpy (J/g Car)	T_m (°C)	Enthalpy (J/g Car)	T_{gel} (°C)	Enthalpy (J/g Car)	T_m (°C)	Enthalpy (J/g Car)
Car[a] 0.3% pH 10 hold at 90 °C	33	38	n.d.[b]	n.d.	33	34	n.d.	n.d.
Car 0.6% pH 6 hold at 90 °C	36	38	56	32	36	38	55	30
Car 0.6% pH 10 hold at 90 °C	35	39	54	36	35	39	54	33
CK[c] 0.6% pH 6 hold at 65 °C	37	36	59	24	37	34	59	29
CK 0.6% pH 8 hold at 65 °C	36	34	58	24	36	32	59	29
CK 0.6% pH 10 hold at 65 °C	36	33	58	25	36	32	59	30
CK 0.6% pH 6 hold at 90 °C	37	36	59	28	37	35	59	31
CK 0.6% pH 8 hold at 90 °C	36	34	58	27	36	35	59	29
CK 0.6% pH 10 hold at 90 °C	36	33	58	27	33[d] 37[e]	30[d] 7[e]	61	30

[a] Car, k-carrageenan.

[b] n.d., not determined.

[c] CK, k-carrageenan and konjac glucomannan mixture (1:1).

[d] Big peak.

[e] Small peak.

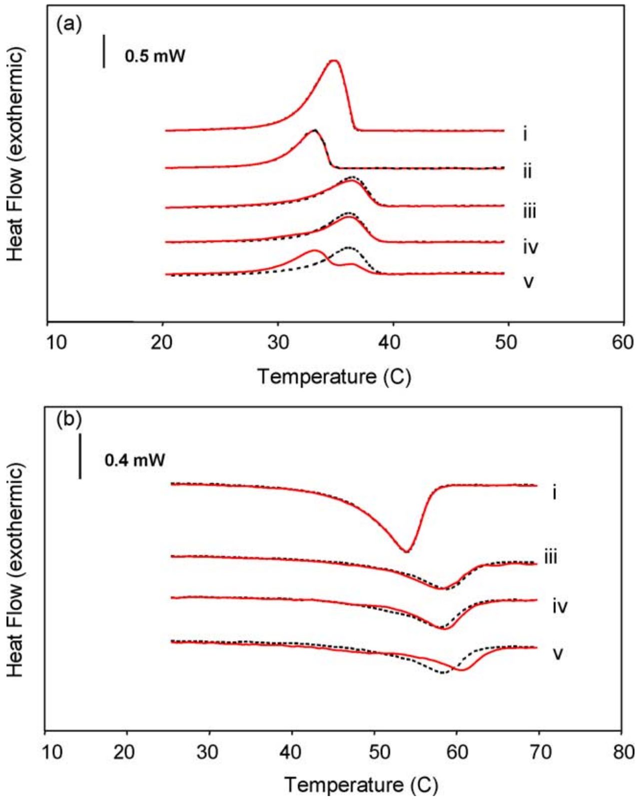

Fig. 4. DSC cooling (a) and heating (b) curve before (dot line) and after (solid line) holding at 90 °C for 2 h: (i) k-carrageenan 0.6% pH 10; (ii) k-carrageenan 0.3% pH 10; (iii) k-carrageenan and konjac glucomannan mixture (1:1) 0.6% pH 6; (iv) k-carrageenan and konjac glucomannan mixture (1:1) 0.6% pH 8; (v) k-carrageenan and konjac glucomannan mixture (1:1) 0.6% pH 10.

the higher temperature event could be related to some association between kappa carrageenan and konjac glucomannan, perhaps consisting of a core of kappa carrageenan helices onto which konjac glucomannan adsorbs. At high kappa carrageenan konjac glucomannan ratios there would be free kappa carrageenan that could associate in the absence of konjac glucomannan. The possibility that the bimodal peak was due to phase separation seems unlikely since a bimodal peak has also been observed for these mixed systems at a total polysaccharide concentration as low as 0.12% (Goycoolea et al., 1994; Morris, 1995). The order of events and the reason for the higher setting temperature for the system containing the konjac glucomannan associates was not clear. A possibility was that the increase in setting temperature is a general effect based on the enhanced helix stability inferred by the addition of a polymer to helix forming systems (Williams et al., 1993). At low konjac glucomannan to kappa carrageenan ratios all the konjac glucomannan would associate with the helical carrageenan leaving some remaining carrageenan that would set at the lower temperature characteristic of this polysaccharide alone.

Within the context of these arguments an interpretation for our data is possibly that on holding at pH 10 and 90 °C deacetylated konjac glucomannan associates to such an extent that there are few free flexible regions left. Hence promotion of carrageenan association by the segregative induced mechanism does not occur, although this mechanism can be used to explain the gelation of konjac glucomannan in the presence of random coil kappa carrageenan. Essentially konjac glucomannan gelation/association, after almost complete deactylation, removes the influence of this polysaccharide on the initial carrageenan helix formation event resulting in an exotherm at a temperature normally found for carrageenan alone or very high ratios of carrageenan to konjac glucomannan.

On reheating the pH 10 sample held at 90 °C a single endotherm was observed that had a single high temperature endotherm as found for the other mixed systems, although its temperature was somewhat higher. It is interesting that Williams et al. (1993) also observed a higher melting temperature for the main peak for the system with the highest ratio of kappa carrageenan to konjac glucomannan studied (6:1) that after initial cooling showed a large low temperature carrageeenan peak. This may suggest that after kappa carrageenan helix formation there is an association between the kappa carrageenan and the konjac glucomannan. Perhaps the initially ‘naked’ bundles of kappa carrageenan helices collect konjac glucomannan as the system matures. These complexes are slightly more stable than kappa carrageenan konjac glucomannan associates that have formed at higher temperatures. Another feature of this high pH data is the temperature when the pH 10 kappa carrageenan control sets. It is slightly lower than was observed at the lower pHs. This may reflect the higher charges on the hydroxyl groups. It should be emphasised that the above interpretations are inevitable speculative. Recent advances in atomic force microscopy (Ikeda, Morris, & Nishinari, 2001) may resolve some of the controversy surrounding the structure of mixed gels of this type.

The prior deacetylation of the konjac glucomannan has an affect on the texture of the mixed gel measured at ambient temperatures. This can be seen from the G' value at ambient temperature (Figs. 2 and 3), Young’s modulus from the compression experiments and the stress relaxation parameters k_1 and k_2 (Table 4). Deacetylation results in some increase in the initial modulus except under the most severe conditions (pH 10 held at 90 °C). Since deactylation reduces the solubility of konjac glucomannan, presumably this promotes adsorption onto kappa carrageenan helix surfaces as well as self-association between segments of konjac glucomannan molecules. Hence deactylation would be expected to enhance gel strength. The decrease observed in the modulus after holding at pH 10 could be due to some polysaccharide degradation resulting from holding under these conditions.

Eq. (2) is a simple way of modelling the stress relaxation response. The parameter k_1 is the reciprocal of the initial rate of decay of the stress normalised to the stress at zero time. This model would predict that at long times the stress

Table 4
Rheological properties of k-carrageenan compared with k-carrageenan and konjac glucomannan mixture (1:1) at various pHs before and after holding at 65 °C for 8 h or holding at 90 °C for 2 h

Sample	Before holding				After holding at 65 °C 8 h				After holding at 90 °C 2 h			
	G'25 °C (kPa)	E (kPa)	k_1 (sec)	k_2	G'25 °C (kPa)	E (kPa)	k_1 (sec)	k_2	G'25 °C (kPa)	E (kPa)	k_1 (sec)	k_2
Car[a] 0.3% pH 6	1.6	5.6	40	1.2	1.5	5.5	34	1.1	1.4	4.9	32	1.1
	1.9[b]	*7.1*	±*1.2*[c]	±*0.01*	*5.1*	*7.8*	±*1.0*	±*0.01*	*3.2*	*8.3*	±*0.98*	±*0.01*
Car 0.6% pH 6	8.3	19.4	50	1.4	7.7	20.2	50	1.4	5.0	20.4	48	1.4
	1.1	*4*	±*1.5*	±*0.01*	*5.4*	*3.6*	±*1.5*	±*0.01*	*1.9*	*5.2*	±*1.5*	±*0.01*
Car 0.6% pH 10	5.8	16.3	60	1.5	5.5	16.4	58	1.5	4.6	16.4	59	1.5
	4.2	*3.9*	±*2*	±*0.01*	*6.4*	*3.5*	±*1.9*	±*0.01*	*3.1*	*3.8*	±*2*	±*0.01*
CK[d] pH 6	7.1	23.2	41	1.4	7.0	24.3	42	1.4	5.4	23.7	41	1.3
	2.5	*5.2*	±*1.4*	±*0.01*	*5.5*	*6.6*	±*1.4*	±*0.01*	*1.8*	*8.4*	±*1.3*	±*0.01*
CK pH 8	7.3	20.8	41	1.4	9.4	26.3	35	1.3	13.2	33.7	25	1.2
	6.4	*6.1*	±*1.4*	±*0.01*	*5.4*	*6.2*	±*1.1*	±*0.01*	*6.8*	*6.5*	±*0.7*	±*0.00*
CK pH 10	13.7	37.0	21	1.1	12.2	38.0	15	1.1	5.0	27.1	14	1.1
	3.2	*5.1*	±*0.5*	±*0.00*	*5.0*	*6.6*	±*0.3*	±*0.00*	*1.7*	*5*	±*0.3*	±*0.00*

[a] Car, k-carrageenan.

[b] Coefficient of variation (%).

[c] Error.

[d] CK, k-carrageenan and konjac glucomannan mixture (1:1).

will have decayed to $(1-1/k_2)$ of its initial value. For a perfectly elastic material, i.e. no decay of the stress, k_1 and k_2 would both go to infinity. Where there is no residual stress, as in a viscoelastic liquid, k_2 would be one. It has been reported that for a mixed kappa carrageenan locust bean gum gel these parameters increase markedly from the values obtained for kappa carrageenan alone indicating that the mixed gel is more elastic (Winwood, Jones, & Mitchell, 1986). This is consistent with the generally accepted view of the influence of locust bean gum on gel structure. An increase in k_1 is not observed with mixed gels formed from non-deacetylated konjac glucomannan and kappa carrageenan when compared with kappa carrageenan alone (Table 3). It was suggested that the textural change on addition of locust bean gum to kappa carrageenan was due to the incorporation of relatively long flexible chains of the galactomannan connecting the kappa carrageenan junction zones. Long flexible chains would deform on application of a stress rather than transmit the stress to the junction zones resulting in movement or breakage that was responsible for the viscoelastic behaviour observed in the stress relaxation experiments. It can be argued that konjac glucomannan is less likely to cause this mechanism, since its molecular weight is lower than locust bean gum and also because it is more homogeneous. Locust bean gum has blocks of galactose substituents and arguably few areas where the unsubstituted portion of the mannose chain is sufficiently long to allow stable associations with the kappa carrageenan junction zones to form. Increasing deacetylation of the konjac glucomannan results in a marked decrease in the k_1 and k_2 for the mixed gels. Highly aggregated gels such as agarose, where the major proportion of the chains are in junction zones, result in this type of viscoleastic behaviour. Whereas gels such as gelatin, which have long flexible network chains outside the junction zones that can store energy entropically, show more elastic behaviour with high values of k_1 and k_2 (Winwood et al., 1986). The decrease in k_1 and k_2 with increasing deacetylation is consistent with more of the konjac glucomannan chain associating onto the kappa carrageenan helices as discussed above.

4. Conclusions

Deacetylation of konjac glucomannan in the presence of kappa carrageenan results in the formation of a gel at temperatures above the melting temperature of kappa carrageenan and at concentrations much lower than expected for konjac glucomannan alone. It is suggested that this is a consequence of segregative association. On cooling kappa carrageenan helix formation is promoted by the presence of the konjac glucomannan as has been observed for non-deacetylated konjac glucomannan. The extent of segregatively induced self-association and the degree of subsequent association between the two biopolymers, following kappa carrageenan helix formation, increases with the degree of deacetylation of konjac glucomannan and hence the gel modulus increases with deacetylation both above and below the temperature where carrageeenan helix formation occurs.

Acknowledgements

Valuable discussions with Professor E.R. Morris are gratefully acknowledged.

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