Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Polymers 55 (2004) 343–355
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Effect of polymeric cosolutes on calcium pectinate gelation. Part 1. Galactomannans in comparison with partially depolymerised starches

P. Giannouli[a], R.K. Richardson[b], E.R. Morris[a,*]

[a]*Department of Food and Nutritional Sciences, University College Cork, Cork, Ireland*
[b]*Cranfield University at Silsoe, Silsoe, Bedford MK45 4DT, UK*

Received 4 December 2002; revised 22 September 2003; accepted 29 September 2003

Abstract

The effect of galactomannans on gelation of low methoxy pectin (DE 31; 2.0 wt%; pH ≈ 2.9–3.0) on cooling from 90 to 5 °C in the presence of stoichiometric Ca^{2+} has been characterised by low amplitude oscillatory measurements of G' and G'' for five samples of guar gum of different molecular weights and for a single sample of locust bean gum (LBG). All samples caused an increase in G' and G'' in the solution state at 90 °C and a reduction in final moduli at 5 °C, with cooling curves crossing those for calcium pectinate alone at ~55 °C. The increase in moduli at high temperature is attributed to segregative interactions promoting formation of calcium-mediated 'egg-box' junctions between pectin chains. The loss of gel strength at low temperature is attributed to excessive association of pectin into large aggregated bundles, again driven by segregative interactions with the galactomannan. Both effects increased in magnitude with increasing concentration of galactomannan. At fixed concentration, the effectiveness of the galactomannan samples in promoting self-association of pectin was found to be inversely proportional to their hydrodynamic volume, as characterised by intrinsic viscosity (i.e. with the smallest molecules having the greatest effect). The changes in moduli caused by LBG were closely similar to those observed for guar gum of comparable molecular weight. The effect of galactomannans of high molecular weight was similar in magnitude to that of oxidised starch and substantially greater than that of potato maltodextrin, whose interactions with calcium pectinate have been studied in detail in previous published investigations.

Keywords: Pectin; Gelation; Rheology; Segregation; Guar gum; Locust bean gum

1. Introduction

When gelling hydrocolloids are used to structure food products, they are often required to form networks in the presence of much larger amounts of other biopolymers that occur as natural constituents of ingredients such as meat, fish, milk, fruit and vegetables. It is also not uncommon for two or more different food hydrocolloids to be used together in the same product formulation. Some aspects of the behaviour of biopolymer mixtures are reasonably well understood (Harding, Hill, & Mitchell, 1995); others remain virtually unexplored.

Interactions between two different biopolymers can be classified as 'associative' or 'segregative', depending on whether they are enthalpically more favourable or less favourable than homotypic interactions between like chains of each type (Piculell et al., 1994). Associative interactions (Chilvers & Morris, 1987; Imeson, Ledward, & Mitchell, 1977; Michon, Cuvelier, Launay, Parker, & Takerkart, 1995; Muchin, Wajnermann, & Tolstogusow, 1976; Stainsby, 1980; Tolstoguzov, 1986, 1991; Tolstoguzow & Wajnermann, 1975; Tschumak, Wajnermann, & Tolstogusow, 1976) normally involve electrostatic attraction between polyanions (e.g. sulphated or carboxylated polysaccharides) and polycations (such as proteins below their isoelectric point). Segregative interactions are more common, and arise from the enthalpic advantage of individual molecules being surrounded by others of the same type.

For small molecules, the enthalpic drive to segregation is normally outweighed by the entropic advantage of intimate mixing. For polymers, however, where (at equivalent concentrations) there are far fewer molecules free to move independently, entropy of mixing is much less significant,

* Corresponding author. Tel.: +353-214-903625; fax: +353-214-270001.
E-mail address: ed.morris@ucc.ie (E.R. Morris).

0144-8617/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2003.09.013

and mixtures often resolve spontaneously into two separate phases, each enriched in one constituent and depleted in the other (see, for example, Grinberg and Tolstoguzov (1972, 1997), Morawetz (1965), Morris (1986), Morris (1990), Picullel, Bergfeldt, and Nilsson (1995), Piculell, Nilsson, Falck, and Tjerneld (1991), Suchkov, Grinberg, and Tolstoguzov (1981), Tolstoguzov (1986, 1988, 1991), Zasypkin, Braudo, and Tolstoguzov (1997)). The composition of the co-existing phases obtained from different starting compositions can be defined by a curve known as the 'binodal', which represents the boundary between the monophasic and biphasic states.

The effect of segregative interactions can, however, also be seen at concentrations below the binodal, where the system remains single-phase. In mixtures where one biopolymer can undergo a transition from an expanded coil conformation to a more compact ordered form, the presence of a second polymer within the same phase may cause a large increase in the rate and/or extent of conformational ordering (Kasapis & Morris, 1994; Tolstoguzov et al., 1974). In some cases one component may drive the other out of solution. For example, mixtures of gelatin and maltodextrins prepared at concentrations in the 'single-phase' region and held at a temperature above the onset of conformational ordering and gelation of either component showed massive precipitation of maltodextrin (Kasapis, Morris, Norton, & Gidley, 1993), with the fraction precipitated increasing in direct proportion to the protein concentration. However, in comparison with the extensive studies that have been made of phase-separation phenomena, there has been little research on the effect of segregative interactions on gelation from single-phase biopolymer mixtures.

One recent investigation was by Picout, Richardson, Rolin, Abeysekera, and Morris (2000a), who was found that the networks formed when pectin is cooled in the presence of Ca^{2+} can be strengthened, weakened, or remain unaffected by progressive incorporation of oxidised starch, depending on the degree of esterification (DE) of the pectin and on the concentration of Ca^{2+}. Strengthening was observed only at comparatively low concentrations of Ca^{2+}, and attributed to segregative interactions between the two polymers promoting conversion of the pectin component from the expanded coil state to the more compact ordered structure that forms the crosslinks of the gel network. At each DE there was a critical Ca^{2+} concentration above which gel strength, as characterised by storage modulus (G'), was decreased, rather than increased, by the presence of oxidised starch. The transition from enhancement to depletion was accompanied by a change in gel structure (as visualised by light microscopy) from homogeneous to grossly heterogeneous, and moved to progressively higher Ca^{2+} concentration with increasing DE; however, it invariably occurred at concentrations below stoichiometric equivalence to the unesterified carboxyl groups of the polymer (which is the value used in the present investigation). The reduction in final gel strength became progressively greater as the starch concentration was increased, and was found to arise from a sharp drop in modulus during cooling, indicating abrupt collapse of the developing network.

A quantitative analysis (Picout, Richardson, & Morris, 2000b), based on the concept that increasing concentrations of starch cause progressive association of pectin into large aggregates whose contribution to gel strength is negligible, gave good agreement with experimental moduli. The same analysis also gave close agreement (Picout, Richardson, & Morris, 2000d) between observed and fitted moduli for calcium pectinate gels formed in the presence of potato maltodextrin (Picout, Richardson, & Morris, 2000c), where the reductions in gel strength were smaller, and drops in moduli during cooling were observed only at high concentrations of maltodextrin.

The oxidised starch used by Picout et al. (2000a) was from waxy maize, and can therefore be regarded as consisting essentially of partially depolymerised amylopectin. In addition to branched fragments of amylopectin, the maltodextrin used by Picout et al. (2000c) also has a high content of linear sequences released by enzymic debranching of amylopectin and partial hydrolysis of amylose. The purpose of the investigations reported here, and in the two following papers (Giannouli, Richardson, & Morris, 2004a, b) was to determine whether the ability to cause collapse of calcium pectinate networks is unique to densely branched materials such as amylopectin, or indeed to partially depolymerised starches, or if it extends to other types of biopolymer.

In the present work we have examined the effect of locust bean gum (LBG) and a range of samples of guar gum of different molecular weights, in comparison with oxidised starch (Picout et al., 2000a) and potato maltodextrin (Picout et al., 2000c). Results from similar studies using dextrans and inulin are presented in the following paper (Giannouli et al., 2004a). The third paper of the series (Giannouli et al., 2004b) reports results obtained using gum arabic (in water and in 0.1 M NaCl) and gives a unified summary of the overall findings.

2. Materials and methods

Low methoxy pectin (DE 31%; free acid form) was kindly supplied by the Copenhagen Pectin Division of Hercules. The calcium source used for gel formation was calcium chloride dihydrate (AnalaR grade from BDH). LBG was from Meyhall (Meypro fleur M-175). The guar gum samples used were Meyprogat M7, M30, M60, M90 and M150, also from Meyhall. Oxidised starch (C*Set 06598) and potato maltodextrin (C*deLight MD01970) were kindly supplied by Cerestar, and were identical to the samples used by Picout et al. (2000a) and Picout et al. (2000c), respectively. Distilled deionised water was used throughout.

Pectin concentration was held fixed at 2.0 wt%. No adjustment was made to the natural pH of the pectin samples at this concentration (pH ≈ 2.9–3.0). Ca^{2+} concentration was also held fixed, at stoichiometric equivalence to the unesterified carboxyl groups of the polymer. For preparation of mixtures, the individual polymers were dissolved separately, the required amount of calcium chloride was incorporated in the pectin solution by dropwise addition as a dilute solution, and the solution of the other polymer was then added. Both mixing procedures were carried out with continuous stirring at 90 °C. The volume of calcium chloride solution was held fixed at 10% of the total volume; the relative volumes of the individual polymer solutions were varied as necessary to allow mixtures of the required final composition to be obtained from starting solutions prepared at experimentally tractable concentrations. Solutions of calcium pectinate alone were prepared in the same way, but omitting the second polymer.

Small-deformation measurements of storage modulus (G'), loss modulus (G'') and complex dynamic viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$, where ω is frequency of oscillation in rad s^{-1}) were made using cone-and-plate geometry (50 mm diameter; 0.05 rad cone angle) on a sensitive prototype rheometer designed and constructed by one of us (R.K.R.). To minimise problems of thermal expansion/contraction during heating and cooling, the cone was truncated over 40% of its diameter, giving a gap of 0.5 mm between the flat surfaces of the two elements, but keeping strain constant at a fixed, maximum, value across the outer portion (which constitutes 84% of the total area). Temperature was controlled by a Haake circulating water bath and measured by a thermocouple attached to the stationary element. Samples were loaded onto the rheometer in the solution state at 90 °C and their periphery was coated with light silicone oil to minimise evaporation. They were then cooled to 5 °C at 1 °C min^{-1}, with measurement of G' and G'' at 10 rad s^{-1} and 0.5% strain. A mechanical spectrum (frequency-dependence of G', G'' and η^*) was then recorded (at 0.5% strain).

Measurements of dilute solution viscosity, for determination of intrinsic viscosity, were made at 20 °C on a Contraves Low Shear 30 rotational viscometer, using cup-and-bob geometry with inner and outer radii of 5.5 and 6.0 mm, respectively. Optical rotation was measured at 20 °C on a Perkin–Elmer 241 polarimeter, using a cell of pathlength 1 cm.

3. Results

3.1. Characterisation of cosolutes

At the outset of this investigation, it seemed reasonable to expect that the effect of different polymeric cosolutes on formation of calcium pectinate gels might in some way be related to their molecular size. This was characterised by measurement of intrinsic viscosity, $[\eta]$, using the following standard relationships (Bohdanecký & Kovár, 1982).

$$\eta_{rel} = \eta/\eta_s \quad (1)$$

$$\eta_{sp} = (\eta - \eta_s)/\eta_s = \eta_{rel} - 1 \quad (2)$$

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad (3)$$

$$(\ln \eta_{rel})/c = [\eta] + k''[\eta]^2 c \quad (4)$$

where η and η_s are the viscosities of the solution and solvent, respectively, η_{rel} and η_{sp} are the (dimensionless) parameters of relative and specific viscosity, c is concentration and k' and k'' are constants. Eqs. (3) and (4), which are known, respectively, as the Huggins and Kraemer equations, are valid only for solution viscosities up to about twice that of water (i.e. $\eta_{rel} \approx 2$), beyond which higher-order terms (c^2, c^3, etc.) are no longer negligible. Experimental measurements of solution viscosity (at 20 °C) were therefore confined to the approximate range $\eta_{rel} = 1.2–2.0$, the lower limit being imposed by the increasing error in values of η_{sp} ($\eta_{rel} - 1$) as η_{rel} approaches 1. Within this range, plots of η_{sp}/c against c (Huggins plot) and $(\ln \eta_{rel})/c$ against c (Kraemer plot) should both be linear, extrapolating to a common intercept of $[\eta]$ as $c \rightarrow 0$, and with slopes of $k'[\eta]^2$ and $k''[\eta]^2$, respectively.

Since $\eta_{rel} = \eta_{sp} + 1$ then $\ln \eta_{rel}$ (Eq. (4)) may be expressed (Eq. (5)) as a logarithmic expansion of $\ln(1 + \eta_{sp})$.

$$\ln(1+\eta_{sp}) = \eta_{sp} - (\eta_{sp}^2)/2 + (\eta_{sp}^3)/3 - (\eta_{sp}^4)/4 + \cdots \quad (5)$$

For low values of η_{sp}, where powers higher than squared terms are negligible (i.e. where $\ln(1+\eta_{sp}) = \eta_{sp} - 0.5\eta_{sp}^2$), it follows from Eqs. (3)–(5) that $k'' = k' - 0.5$. Thus only

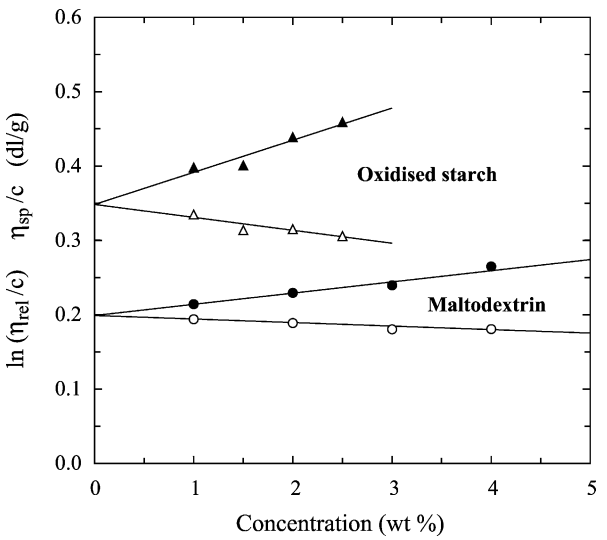
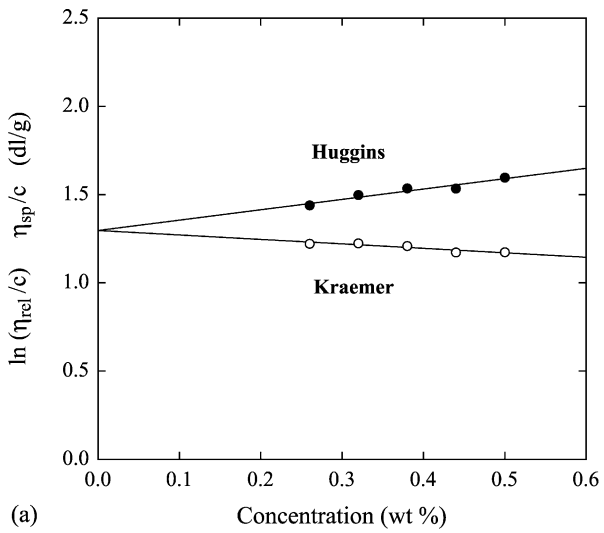


Fig. 1. Determination of intrinsic viscosity (20 °C) from Huggins and Kraemer plots of, respectively, η_{sp}/c (filled symbols) and $(\ln \eta_{rel})/c$ (open symbols) against concentration (c) for potato maltodextrin (circles) and oxidised starch (triangles). As described in the text, only two variable parameters were used to fit both plots for each sample.

two parameters, $[\eta]$ and k', are needed to define both the Huggins and Kraemer plots for each polymer sample. These were varied, using the 'Solver' routine in Microsoft Excel, to minimise the combined root-mean-square deviation between observed and fitted values of η_{sp}/c (Eq. (3)) and $(\ln \eta_{rel})/c$ (Eq. (4)), and the resulting value of $[\eta]$ was taken as the intrinsic viscosity of the sample.

This procedure gave values of $[\eta] = 0.20$ dl g^{-1} for the maltodextrin sample and $[\eta] = 0.35$ dl g^{-1} for the oxidised starch. The standard of agreement between the fitted lines (defined jointly by only two variable parameters) and experimental data is shown in Fig. 1. Fig. 2(a) shows Huggins and Kraemer plots for the lowest viscosity grade of guar gum studied (M7). The corresponding plots for LBG and a representative example of the higher viscosity guar gums (M90) are presented in Fig. 2(b). The intrinsic viscosity obtained for the LBG sample was $[\eta] = 15.8$ dl g^{-1}; the values of $[\eta]$ derived for the various samples of guar gum are listed in Table 1.



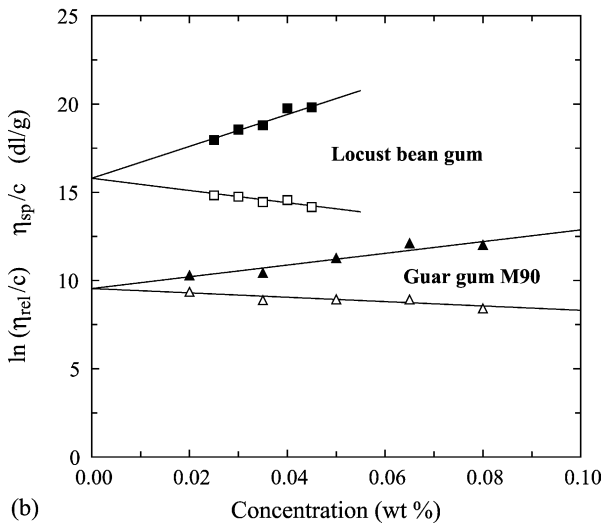


Fig. 2. Determination of intrinsic viscosity (20 °C) from Huggins and Kraemer plots of, respectively, η_{sp}/c (filled symbols) and $(\ln \eta_{rel})/c$ (open symbols) against concentration (c) for (a) guar gum M7 (circles) and (b) guar gum M90 (triangles) and locust bean gum (squares). As described in the text, only two variable parameters were used to fit both plots for each sample.

Table 1
Molecular weight and composition of guar gum samples

Sample	$[\eta]$ (dl g^{-1})	M_r (kDa)	$[\alpha]_D$	M/G
M7	1.30	60	38.8	2.21
M30	3.85	280	40.9	2.14
M60	6.11	538	48.7	1.89
M90	9.55	1013	51.8	1.80
M150	14.25	1786	55.5	1.70

Molecular weight (relative molecular mass, M_r) was derived from intrinsic viscosity, $[\eta]$, using the Mark–Houwink equation proposed by Picout and Ross-Murphy (2002); the ratio of mannose to galactose (M/G) was calculated from specific rotation at 589 nm ($[\alpha]_D$) by the relationship proposed by Morris (1990).

The molecular weight (relative molecular mass, M_r) of each galactomannan sample was calculated from the measured value of intrinsic viscosity using the Mark–Houwink relationship (Eq. (6)) derived by Picout and Ross-Murphy (2002).

$$\log[\eta] = 0.706 \log M_r - 3.26 \quad (6)$$

The calculated molecular weights for the five samples of guar gum are reported in Table 1. The value of M_r corresponding (Eq. (6)) to the measured intrinsic viscosity of 15.8 dl g^{-1} for the LBG sample is 2067 kDa.

The composition of the guar gum samples, as characterised by the ratio of mannose to galactose (M/G), was estimated from optical rotation measurements (at 20 °C) using the relationship (Eq. (7)) proposed by Morris (1990).

$$\text{M/G} = (235 - [\alpha]_D)/(50 + [\alpha]_D) \quad (7)$$

where $[\alpha]_D$ is the specific rotation (Eq. (8)) measured at the sodium D line (589 nm):

$$[\alpha]_D = \alpha/(c \times l) \quad (8)$$

where α is the observed optical rotation (in degrees), c is concentration (in g ml^{-1}) and l is pathlength (in dm). Eq. (7) was derived initially from studies of a range of galactomannans by vacuum ultraviolet circular dichroism (Buffington, Stevens, Morris, & Rees, 1980), but was found to agree well (Morris, 1990) with published data from conventional measurements of D-line optical rotation.

To improve the precision of the $[\alpha]_D$ values, optical rotation measurements (1 cm pathlength; polymer concentration 1.0% w/v) were made using the emission lines from a mercury lamp at wavelengths (λ) of 365, 436, 546 and 578 nm and the values of optical rotation at 589 nm were then derived from linear Drude plots of $1/\alpha$ versus λ^2. The Drude plots obtained for each of the five samples of guar gum are shown in Fig. 3, and the resulting values of $[\alpha]_D$ and hence of M/G (from Eq. (7)) are listed in Table 1.

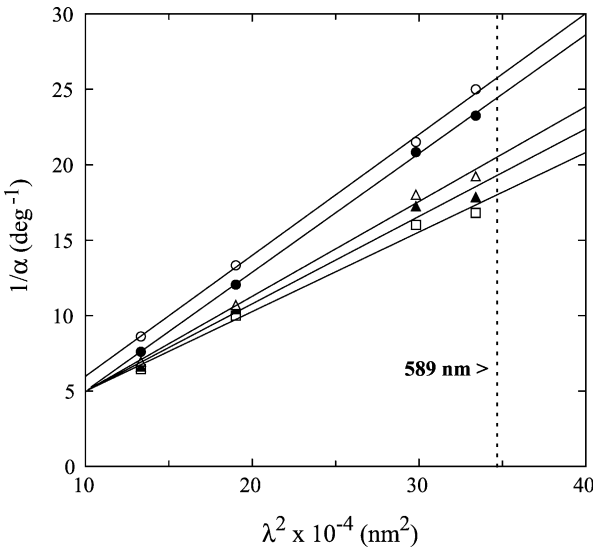


Fig. 3. Drude plots used to determine optical rotation (α) at 589 nm (sodium D line) from measurements (at 20 °C) using mercury emission lines at lower wavelengths (λ), for guar gum samples M7 (○), M30 (●), M60 (△), M90 (▲) and M150 (□).

There is a systematic increase (Table 1) in M/G ratio (i.e. decrease in galactose content) on going from the sample of highest molecular weight (M150) to the sample of lowest molecular weight (M7). This is probably caused by some loss of galactose sidechains during partial hydrolysis of native guar gum to produce grades of lower viscosity. LBG, which, as described above, has much higher molecular weight than M7, but a much lower content of galactose, was included in the investigation to distinguish the effect of molecular size from the effect of composition.

3.2. Gelation of pectin on cooling with Ca^{2+}

Fig. 4 illustrates the changes in G' (Fig. 4(a)) and G'' (Fig. 4(b)) observed on cooling preparations of low methoxy pectin (DE 31; 2.0 wt%) with stoichiometric Ca^{2+} but without incorporation of any other polymer. Pectin 'blanks' were recorded periodically throughout the course of the investigation, and the traces shown in Fig. 4 are representative of the experimental scatter observed. The bold solid lines in Fig. 4 are average values, ignoring occasional 'outliers', and are used for purposes of comparison with the cooling curves obtained for mixtures with the various polymeric cosolutes.

At the loading temperature of 90 °C, the preparations of pectin in the absence of cosolute, although pourable, already have substantial gel-like character, with G' (Fig. 4(a)) exceeding G'' (Fig. 4(b)) by about a factor of 5. On cooling below ~65 °C, there is a sharp, sigmoidal rise in both moduli, but the increase in G' is greater than for G'', with the final divergence on completion of cooling to 5 °C being more than an order of magnitude. The mechanical spectra recorded for the calcium pectinate gels at 5 °C (Fig. 5(a)) have the form typical (Ross-Murphy, 1984) of an extensively crosslinked

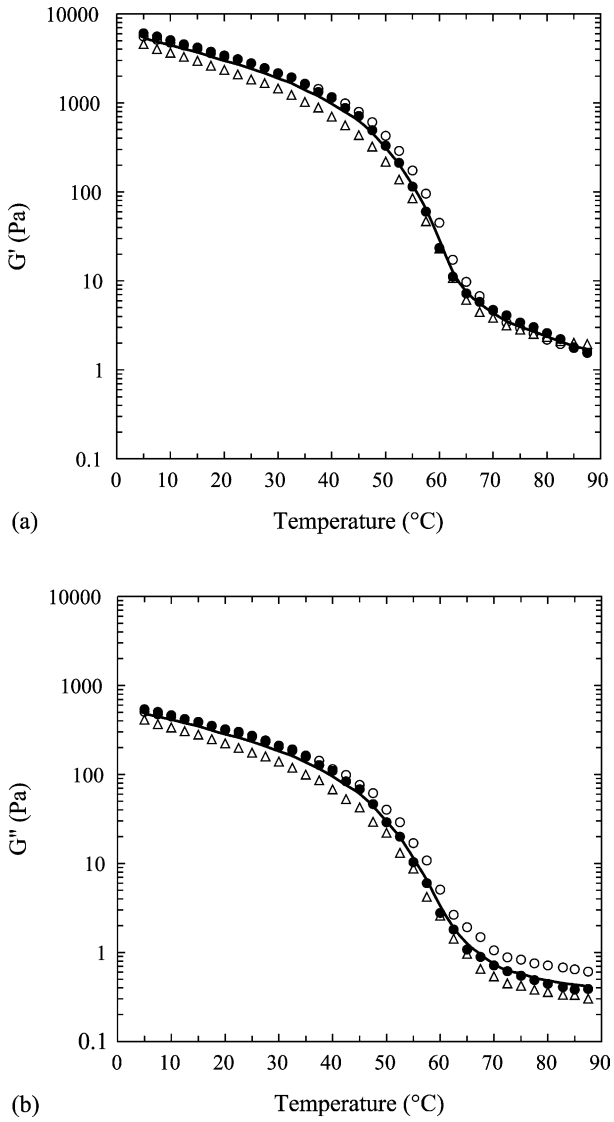


Fig. 4. Changes in (a) G' and (b) G'', measured at 10 rad s^{-1} and 0.5% strain, for 2.0 wt% low methoxy pectin (DE 30; pH ~ 2.9–3.0) on cooling from 90 to 5 °C at 1 °C min^{-1} in the presence of stoichiometric Ca^{2+}. Different symbols show representative curves recorded at different times throughout the course of the investigation. The solid lines show average values from all such curves (omitting 'outliers').

network, with $G' \gg G''$, little frequency-dependence of either modulus, and a steep linear reduction in log η^* with increasing log ω, with a slope close to -1.

In the investigation of pectin–maltodextrin mixtures by Picout et al. (2000c), samples were prepared by two different procedures, identified as 'Method 1' and 'Method 2'. In Method 1, the maltodextrin was dispersed in cold water and dissolved by stirring at 90 °C. The calcium salt was then dissolved in the maltodextrin solution, and the pectin was slowly added, with continuous stirring until a clear solution was obtained. In Method 2, the individual polymers were dissolved separately, the required amount of calcium chloride was incorporated in the pectin solution by dropwise addition as a dilute solution, and the maltodextrin

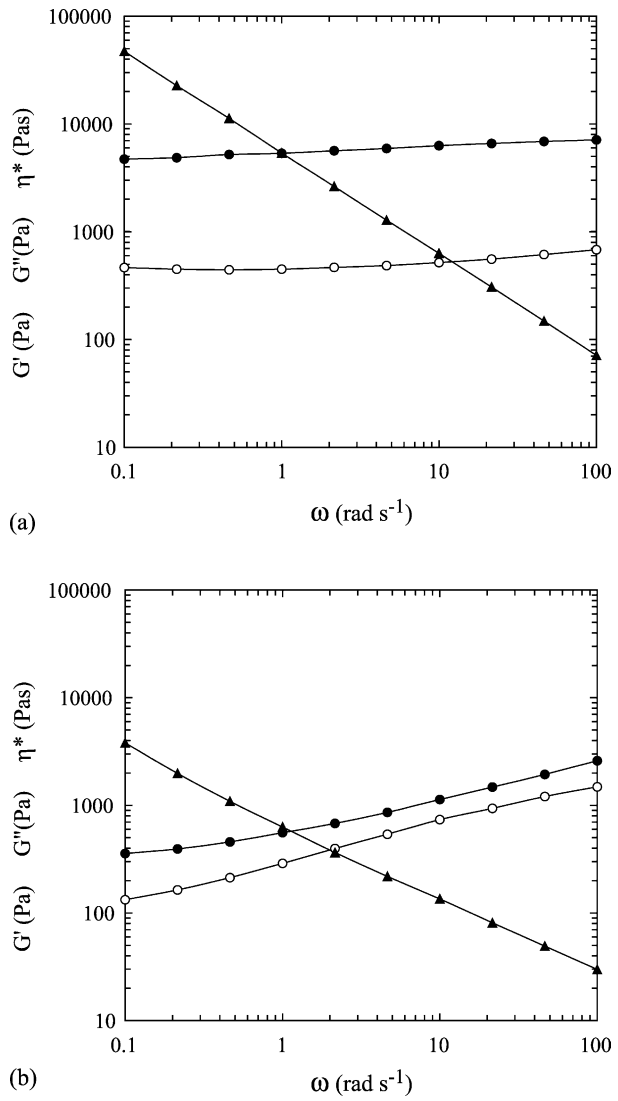


Fig. 5. Mechanical spectra (0.5% strain) showing the variation of G' (●), G'' (○) and η^* (▲) with frequency (ω) for 2.0 wt% calcium pectinate on completion of cooling from 90 to 5 °C (a) alone and (b) in the presence of 5.0 wt% guar gum M60.

solution was then added, with continuous stirring at 90 °C throughout. Pectin blanks were prepared in the same way, but omitting the maltodextrin. Method 2 gave stronger calcium pectinate gels, and is the procedure adopted in the present work. In the earlier investigation of calcium pectinate in combination with oxidised starch (Picout et al., 2000a) only Method 1 was used, so the results are not directly comparable. In both studies, however, preparations of low methoxy pectin with stoichiometric Ca^{2+} were found to have pourable 'weak gel' character at high temperature (90 °C), with conversion to a stronger 'true' gel structure then occurring as a separate process on cooling, as observed in the present work (Fig. 4).

The interpretation proposed by Picout et al. (2000a) was that the initial, pourable, network is crosslinked by thermally stable dimeric junctions (Morris, Powell, Gidley, & Rees, 1982) formed by 'egg-box' binding (Grant, Morris, Rees, Smith, & Thom, 1973) of Ca^{2+} between runs of unesterified carboxyl groups along the inner face of each of the participating chains, and that the true gel structure which develops at lower temperature involves weaker (thermally labile) association of dimers into larger aggregates which include some esterified residues (Powell, Morris, Gidley, & Rees, 1982), with perhaps some additional crosslinking through acid-induced association of 3-fold helices (Gilsenan, Richardson, & Morris, 2000) at the pH value used (~2.9–3.0, as in the present work).

3.3. Effect of partially depolymerised starches

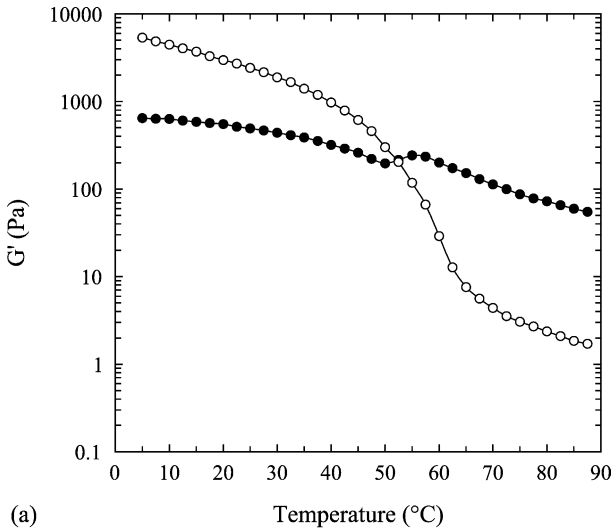
The purpose of including oxidised starch and potato maltodextrin in this investigation was not to duplicate the extensive studies by, respectively, Picout et al. (2000a) and Picout et al. (2000c), but simply to confirm that consistent results could be obtained by a different experimentalist (P.G.) and to provide a direct comparison with results reported here, and in the two following papers (Giannouli et al., 2004a,b), for other polymeric cosolutes. The partially depolymerised starches were therefore used at only one concentration (5.0 wt%).

Fig. 6 shows the changes in G' (Fig. 6(a)) and G'' (Fig. 6(b)) observed for 2.0 wt% pectin with stoichiometric Ca^{2+} on cooling from 90 to 5 °C in the presence of 5.0 wt% oxidised starch, in comparison with the averaged curves from Fig. 4 for pectin in the absence of starch. The initial moduli at 90 °C are much higher for the mixed system than for calcium pectinate alone, indicating that association of pectin chains is promoted by segregative interactions with starch. On cooling, there is an initial increase in both moduli, but this is followed (at ~55 °C) by a detectable decrease, as was also observed by Picout et al. (2000a). After this drop, which, as discussed in Section 1, can be attributed to partial collapse of the calcium pectinate network into large aggregated bundles in further response to segregative interactions with the starch component, the increase in moduli at lower temperatures is much smaller for the pectin–starch mixture than for calcium pectinate alone, and the final moduli at 5 °C are substantially smaller (by about an order of magnitude for G').

The enhancement in moduli at 90 °C with 5 wt% maltodextrin as cosolute (Fig. 7) is less pronounced, and there is no detectable drop during cooling, indicating that the segregative interactions between pectin and maltodextin are weaker than those that occur between pectin and oxidised starch, but the moduli at 5 °C are again appreciably lower than for calcium pectinate alone. The curves shown in Fig. 7 are in good agreement with those obtained by Picout et al. (2000c) for the same concentrations of pectin and maltodextrin.

3.4. Effect of galactomannans

As a representative example of the effect of galactomannans on gelation of calcium pectinate, Fig. 8 shows



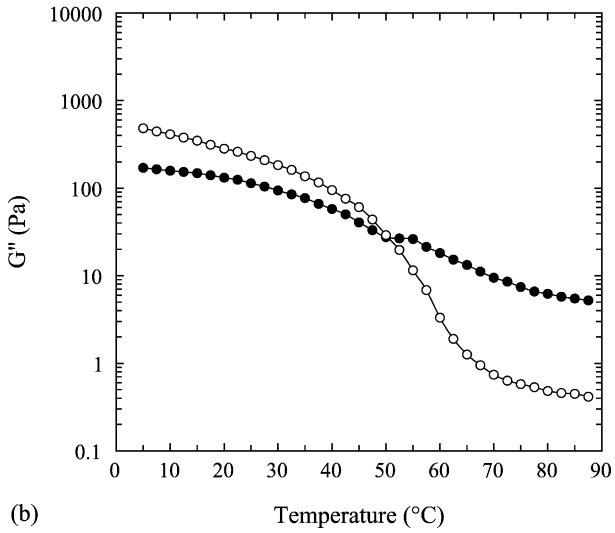
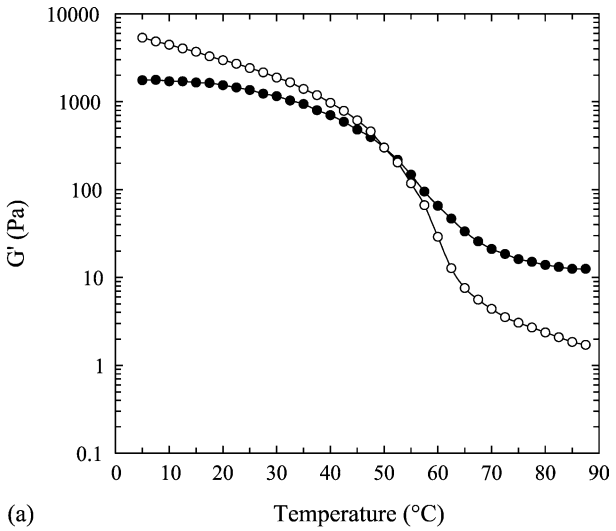


Fig. 6. Changes in (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of 5.0 wt% oxidised starch (●).



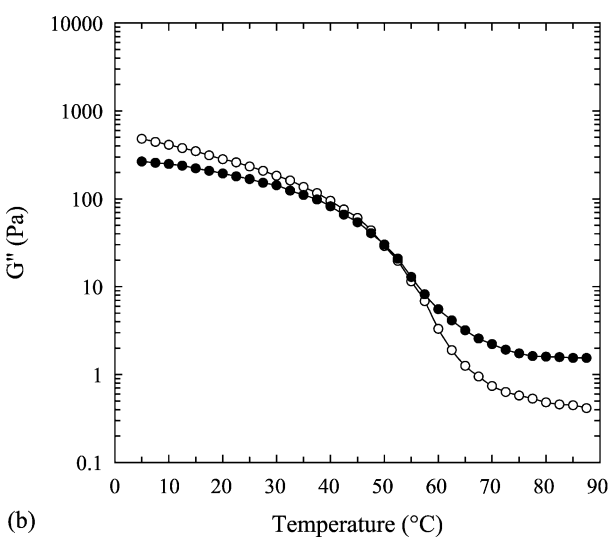


Fig. 7. Changes in (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of 5.0 wt% potato maltodextrin (●).

the changes in G' (Fig. 8(a)) and G'' (Fig. 8(b)) observed for low methoxy pectin (2.0 wt%; stoichiometric Ca^{2+}) on cooling from 90 to 5 °C in the presence of guar gum M60 at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 wt% (with the upper limit being imposed by the solubility of the galactomannan). The averaged curves from Fig. 4 for calcium pectinate alone are again included for direct comparison. Even the lowest concentration of M60 (0.5 wt%) caused a substantial increase in moduli at high temperature (90 °C) with, as seen for oxidised starch (Fig. 6) and potato maltodextrin (Fig. 7), an accompanying reduction in final moduli on completion of cooling to 5 °C. Both effects increased in magnitude with increasing concentration of galactomannan.

Fig. 9(a) shows the observed values of G' and G'' at high and low temperature plotted against concentration of M60. The low temperature values were taken at the end of the cooling process (at 5 °C); the high temperature readings were taken 5 min after the start of cooling (i.e. at 85 °C), to give more stable values than those recorded immediately after loading at 90 °C. The moduli at 85 °C increase monotonically as the concentration of galactomannan is raised, but with a progressive decrease in slope. At 5 °C, there is smaller monotonic reduction in G', with again a progressive decrease in slope with increasing concentration. The values of G'' at 5 °C also show an initial reduction with increasing concentration of M60 (up to ~2 wt%), but this is followed by a progressive increase, which may be caused, in part, by the direct contribution of the disordered galactomannan to overall viscous response, in addition to loss of cohesion in the pectin network. Curves broadly similar to those in Fig. 9 were obtained for the other samples of guar gum, and for the single sample of LBG.

The mechanical spectrum recorded after completion of cooling to 5 °C for 2.0 wt% calcium pectinate in the presence

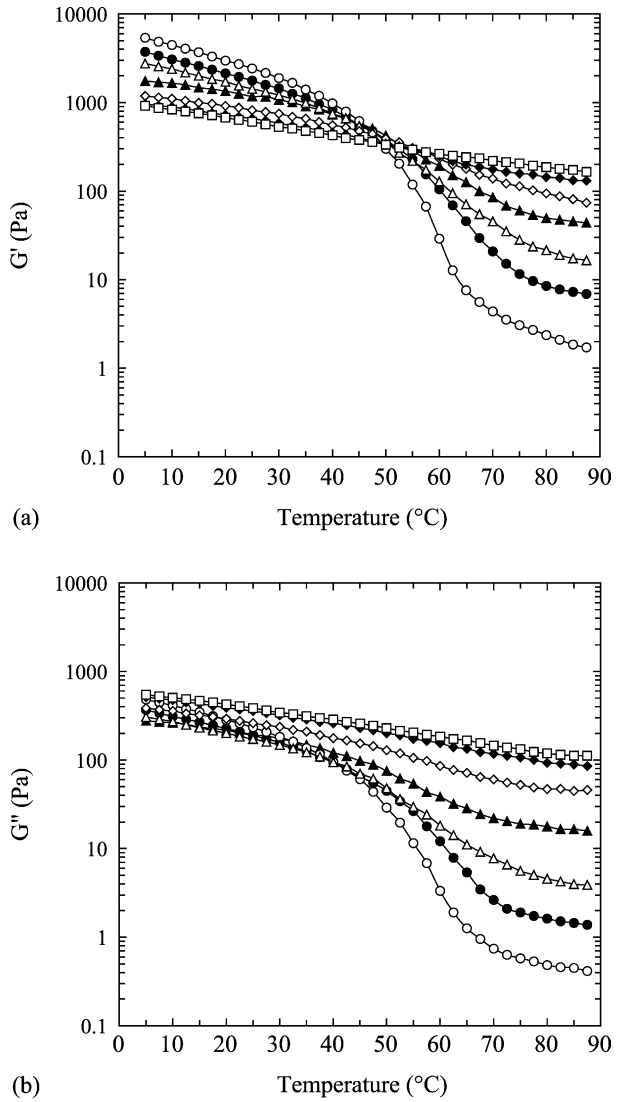


Fig. 8. Changes in (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of guar gum M60 at concentrations (wt%) of 0.5 (●), 1.0 (△), 2.0 (▲), 3.0 (◇), 4.0 (◆) and 5.0 (□).

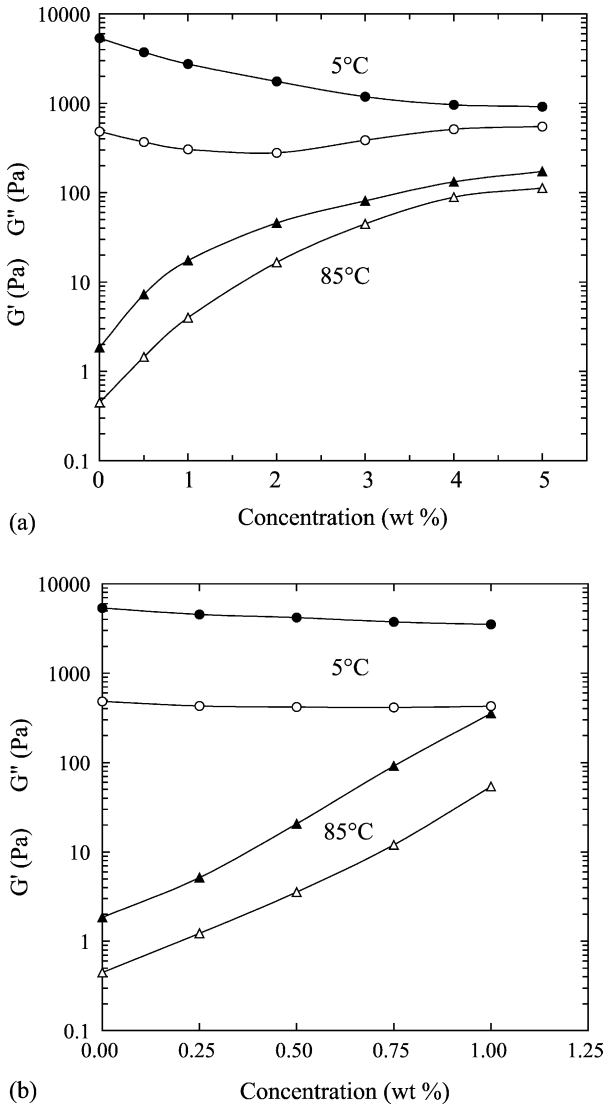


Fig. 9. Measured values (10 rad s^{-1}; 0.5% strain) of G' (filled symbols) and G'' (open symbols) at 85 °C (triangles) and 5 °C (circles) for 2.0 wt% calcium pectinate in the presence of (a) guar gum M60 and (b) guar gum M7 over a range of concentrations of each.

of M60 at the highest concentration studied (5.0 wt%) is shown in Fig. 5(b). The reduction in gel-like character in comparison with the spectrum shown in Fig. 5(a) for 2.0 wt% calcium pectinate alone is obvious. In addition to the much greater frequency-dependence and much smaller separation of G' and G'', which, as mentioned above, may arise in part from the direct contribution of entangled galactomannan chains to the overall spectrum, the values of G' at low frequency are more than an order of magnitude smaller for the pectin–guar gum mixture (Fig. 5(b)) than for calcium pectinate alone (Fig. 5(a)), indicating a less extensively crosslinked network.

As discussed at the beginning of Section 3.1, the reason for determining the intrinsic viscosities of the polymeric cosolutes was the expectation that their effectiveness in promoting self-association of pectin might be related to their molecular size, and in particular to their degree of space-occupancy, as characterised (Ross-Murphy, 1984) by the (dimensionless) product of concentration, c, and intrinsic viscosity, $[\eta]$. Fig. 10 shows the observed values of G' (Fig. 10(a)) and G'' (Fig. 10(b)) at 85 and 5 °C plotted against this 'space-occupancy parameter' $c[\eta]$ for 2.0 wt% pectin (with stoichiometric Ca^{2+}) in mixtures with each of the galactomannan samples studied, over the full range of experimentally tractable galactomannan concentrations. It is clear that the changes in moduli do not depend on the total volume occupied by the cosolute, and cannot therefore arise from a simple increase in the local concentration of pectin due to a 'filler' effect. The increase in G' and G'' at 85 °C and the decrease in G' at 5 °C occur at progressively higher values of $c[\eta]$ as the intrinsic viscosity of the cosolute (Table 1) increases. The values of G'' at 5 °C (Fig. 10(b)) appear to show reasonable superposition, but that is because

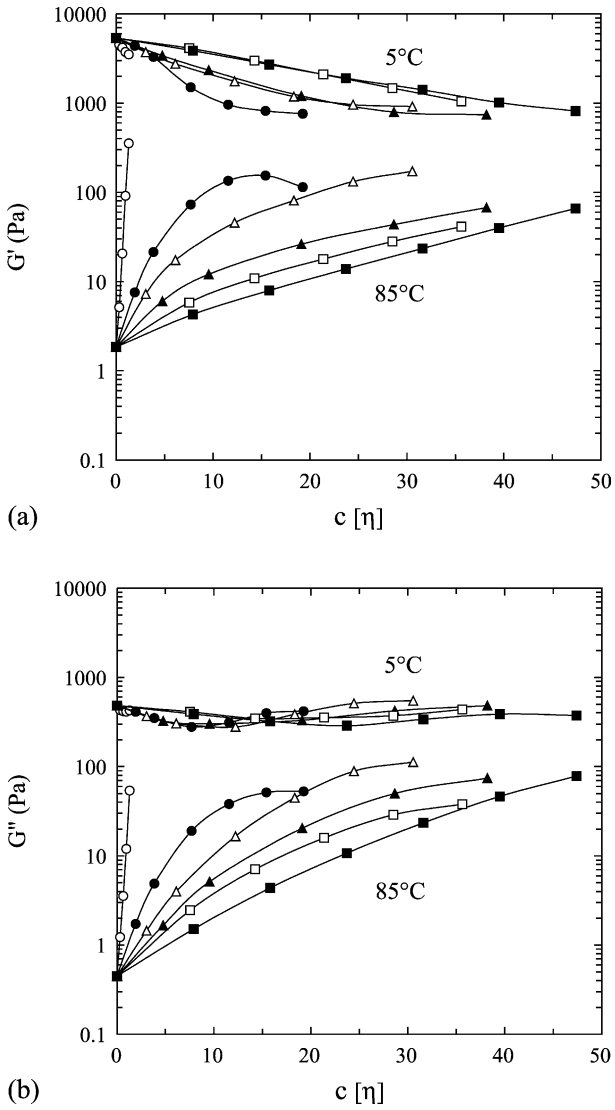


Fig. 10. Measured values of (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) at 85 °C (lower curves) and 5 °C (upper curves), plotted against the (dimensionless) product of concentration, c, and intrinsic viscosity, $[\eta]$, of the galactomannan component for mixtures of 2.0 wt% calcium pectinate with locust bean gum (■) and with guar gum samples M7 (○), M30 (●), M60 (△), M90 (▲) and M150 (□).

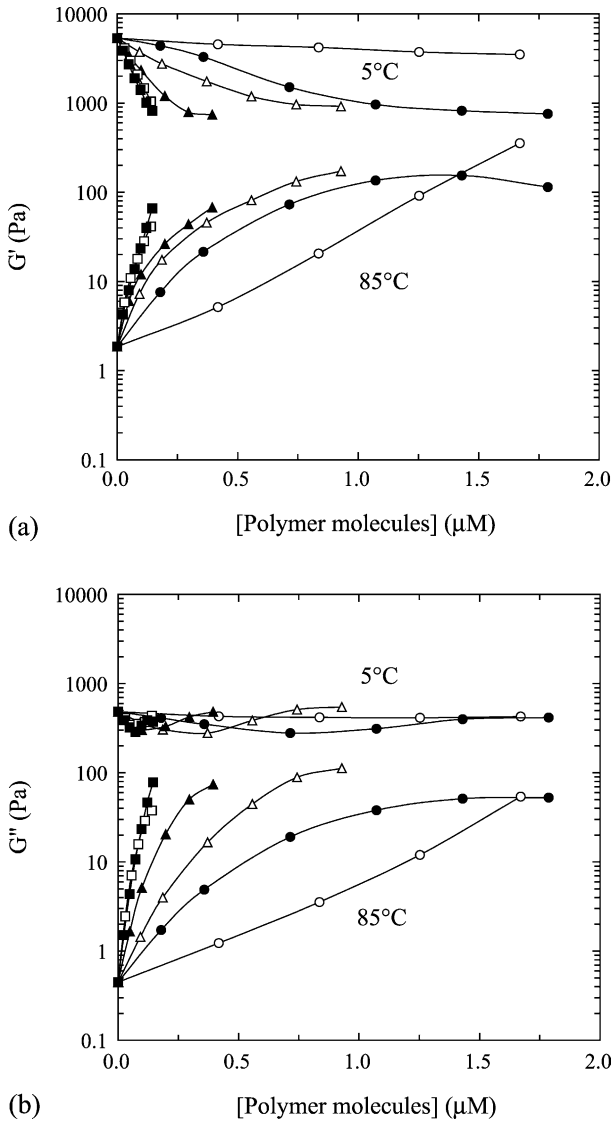


Fig. 11. Measured values of (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) at 85 °C (lower curves) and 5 °C (upper curves), plotted against the molar concentration of galactomannan chains for mixtures of 2.0 wt% calcium pectinate with locust bean gum (■) and with guar gum samples M7 (○), M30 (●), M60 (△), M90 (▲) and M150 (□).

they remain virtually constant (Fig. 9(a)) on varying the concentration of each cosolute.

Another possibility tested was that the drive to segregation might depend on the number of galactomannan molecules present, irrespective of their size (i.e. be related to osmotic pressure). However, when the experimental moduli shown in Fig. 10 are plotted against molar concentration of cosolute (Fig. 11), derived using the values of M_r from Table 1 for the guar gum samples and the value of $M_r = 2067$ kDa for LBG, the pattern shown in Fig. 10 is reversed, with the changes in moduli occurring at progressively lower values of molar concentration as the molecular weight of the cosolute increases.

Closer superposition than in either Fig. 10 or Fig. 11 was obtained (Fig. 12) by simply plotting the experimental moduli against weight concentration of cosolute. For most of the galactomannans studied, the results are grouped fairly tightly, although there is a systematic trend to lower values of G' at 85 °C (Fig. 12(a)) with increasing molecular weight of cosolute. The increase in moduli induced by the guar gum sample of lowest molecular weight (M7), however, is far steeper than for any of the other galactomannans. That is why this material was used (Fig. 12) only at concentrations up to 1.0 wt%; at slightly higher concentration (1.25 wt%) it caused formation of a cohesive, non-pourable calcium pectinate network at the mixing temperature of 90 °C.

Fig. 13 shows the changes in G' (Fig. 13(a)) and G'' (Fig. 13(b)) observed for low methoxy pectin (2.0 wt%; stoichiometric Ca^{2+}) on cooling in the presence of guar gum

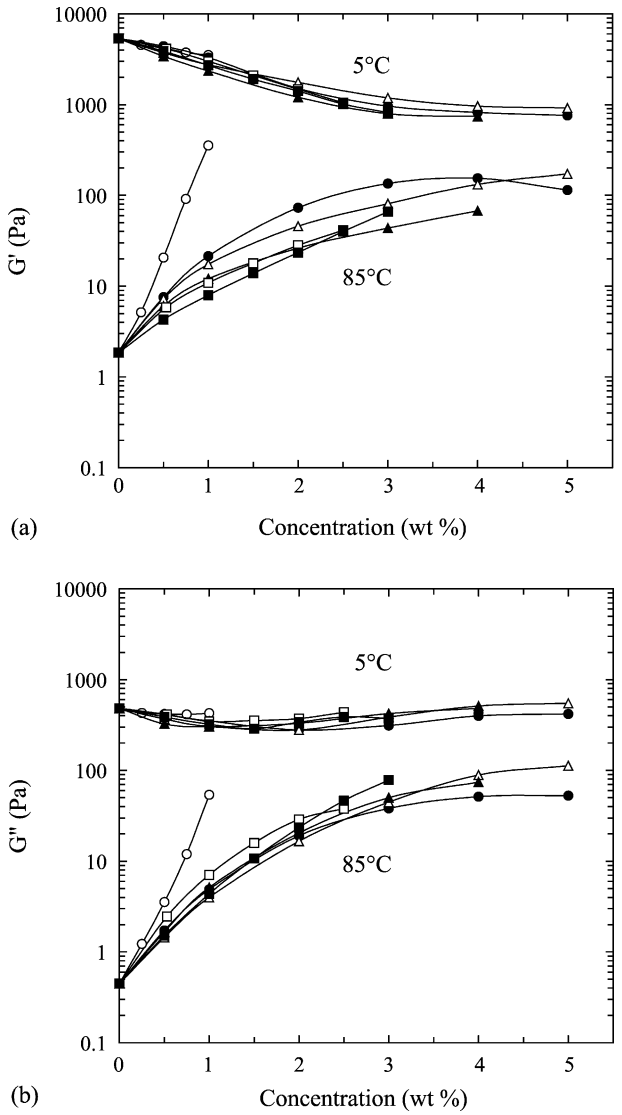


Fig. 12. Measured values of (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) at 85 °C (lower curves) and 5 °C (upper curves), plotted against the concentration (wt%) of the galactomannan component for mixtures of 2.0 wt% calcium pectinate with locust bean gum (■) and with guar gum samples M7 (○), M30 (●), M60 (△), M90 (▲) and M150 (□).

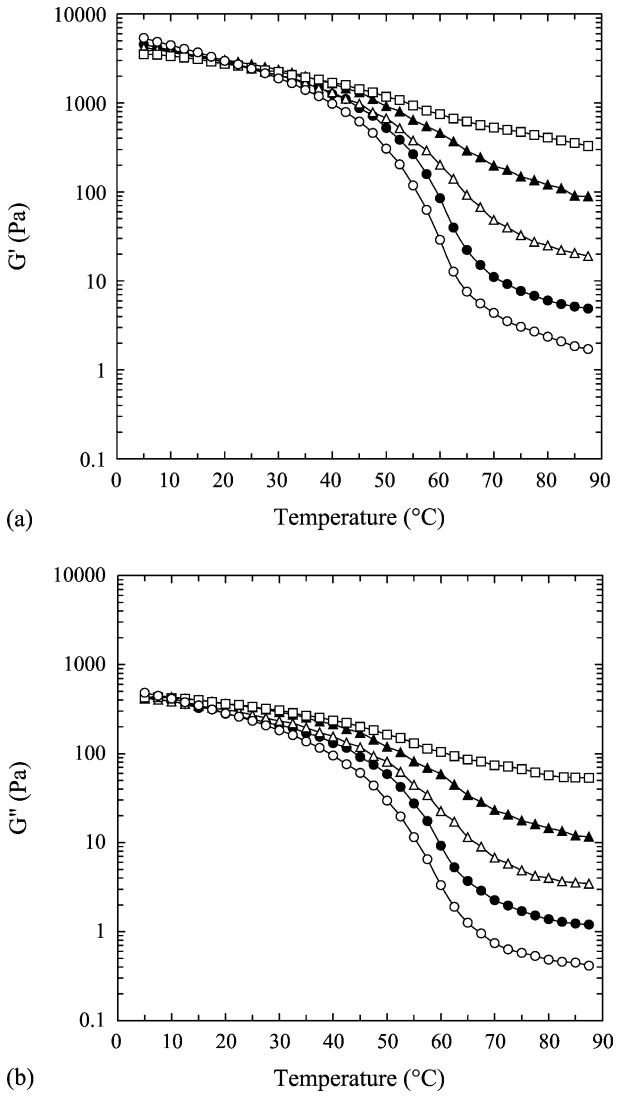


Fig. 13. Changes in (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of guar gum M7 at concentrations (wt%) of 0.25 (●), 0.50 (△), 0.75 (▲) and 1.0 (□).

M7 at concentrations of 0.25, 0.50, 0.75 and 1.0 wt%. Increasing concentration of M7 causes only a marginal reduction in final moduli on completion of cooling to 5 °C but, as shown in Fig. 12 and described above, the increases in moduli at high temperature are massive. The values of G' and G'' at 85 and 5 °C are shown in Fig. 9(b), for direct comparison with the corresponding values (Fig. 9(a)) for mixtures of calcium pectinate with guar gum of higher molecular weight (M60). Over the same range of galactomannan concentration (0.0–1.0 wt%), the results are qualitatively similar, with no significant change in the separation between G' and G'' at either high or low temperature. Quantitatively, however, the increase in moduli at 85 °C is about 15 times greater with M7 as cosolute than it is with M60.

The effect of molecular size is demonstrated more explicitly in Fig. 14, where the observed values of G' (Fig. 14(a)) and G'' (Fig. 14(b)) at 85 and 5 °C for low methoxy pectin (2.0 wt%; stoichiometric Ca^{2+}) in the presence of each of the galactomannans at a fixed concentration of 1.0 wt% (the upper end of the range accessible for M7) are plotted against intrinsic viscosity of the galactomannans. G' and G'' at 5 °C both appear to show a slight reduction as the intrinsic viscosity increases, but the magnitude of this effect is comparable to the experimental scatter of the individual moduli. At 85 °C, however, there is an obvious systematic reduction in G' (Fig. 14(a)) with increasing intrinsic viscosity of the cosolute. The change is steepest on going from M7 ($[\eta] = 1.30$) to M30 ($[\eta] = 3.85$), but appears to continue for the samples of higher intrinsic viscosity. The values of G'' at 85 °C (Fig. 14(b)) also show an initial sharp decrease, but this is followed by a slight increase at higher intrinsic viscosities,

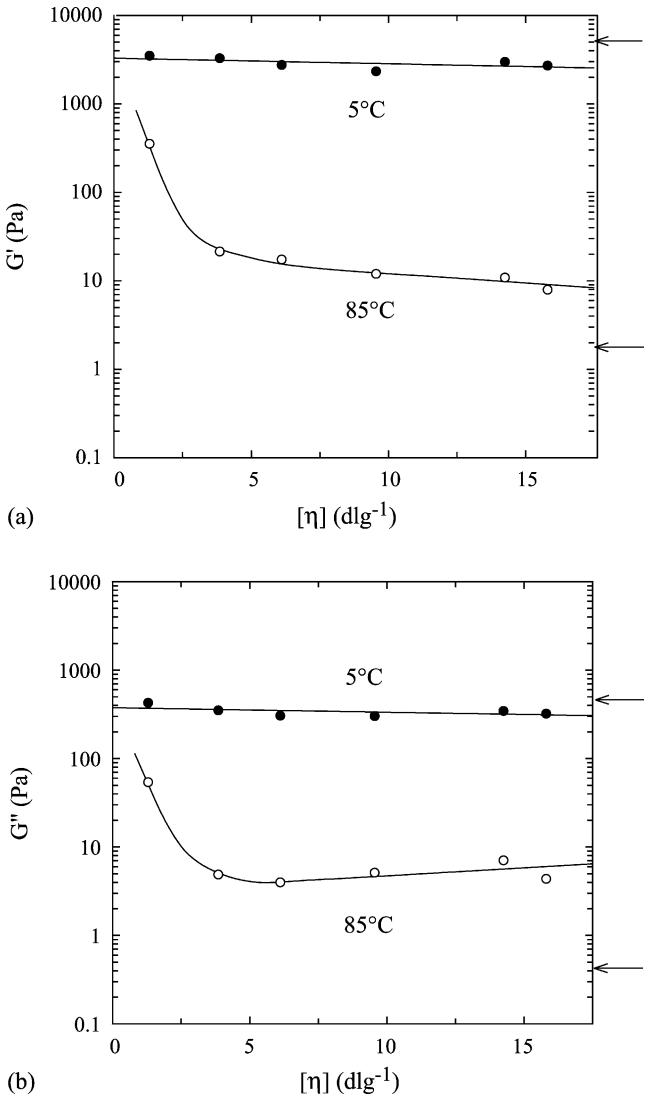


Fig. 14. Variation of (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) at 85 °C (○) and 5 °C (●) with intrinsic viscosity, [η], of the galactomannan component for mixtures of 2.0 wt% calcium pectinate with 1.0 wt% of each of the galactomannans studied. The arrows on the right-hand axis show the corresponding moduli for 2.0 wt% calcium pectinate alone.

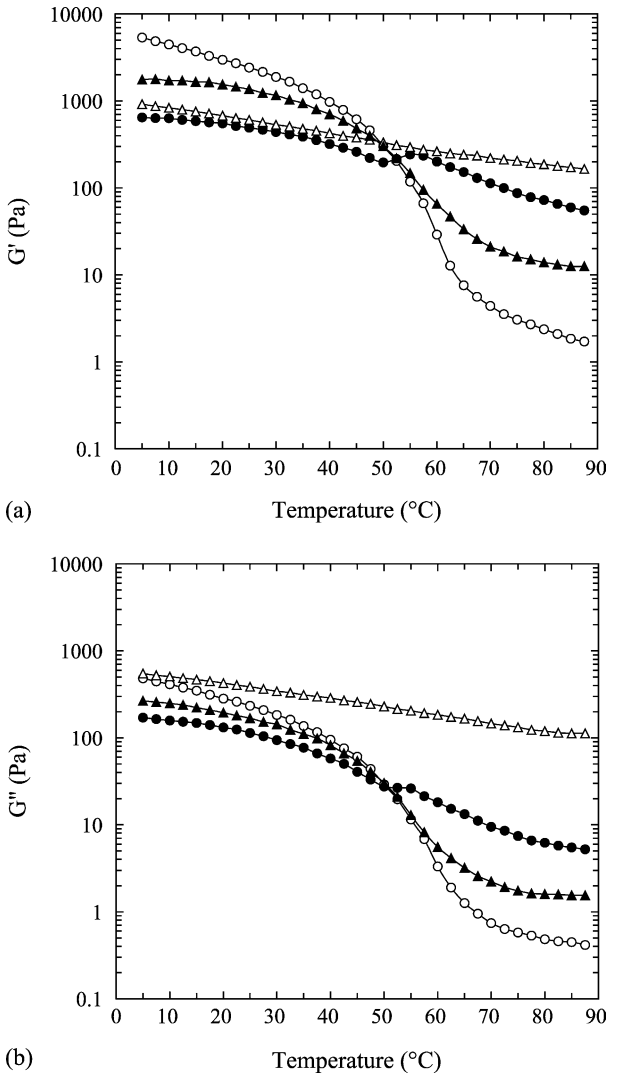


Fig. 15. Changes in (a) G' and (b) G'' (10 rad s^{-1}; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of 5.0 wt%, potato maltodextrin (▲) oxidised starch (●) and guar gum M60 (△).

which probably comes from the increasing solution viscosity of the galactomannan component in the mixtures.

4. Discussion and conclusions

One obvious conclusion is that the changes in calcium pectinate network structure induced by oxidised starch (Picout et al., 2000a) and potato maltodextrin (Picout et al., 2000c) are not unique to those materials, since the galactomannans used in the present investigation were found to cause similar changes. Although, strictly, galactomannans are branched, the branch points lead only to single-sugar stubs, and thus the molecules are essentially linear. It is also clear, therefore, that a densely branched structure such as that of amylopectin is not a necessary requirement for polymeric cosolutes to promote extensive self-association of pectin.

To indicate the effectiveness of galactomannans in comparison with oxidised starch and potato maltodextrin, Fig. 15 shows the cooling curves for 2.0 wt% calcium pectinate in the presence of 5.0 wt% oxidised starch (Fig. 6) and 5.0 wt% maltodextrin (Fig. 7) plotted together with the corresponding curves for the mixture with 5.0 wt% guar gum M60 (Fig. 8) and for calcium pectinate alone (Fig. 4). All three cosolutes cause an increase in G' (Fig. 15(a)) at high temperature and a decrease at low temperature, with the cooling curves crossing the curve for calcium pectinate alone at ~55 °C. A similar pattern of intersection occurs (Fig. 15(b)) for G'', but with the trace for M60 shifted upwards by the direct contribution of the galactomannan to overall viscous response. Since, as discussed above, the increase in G' at high temperature and the accompanying

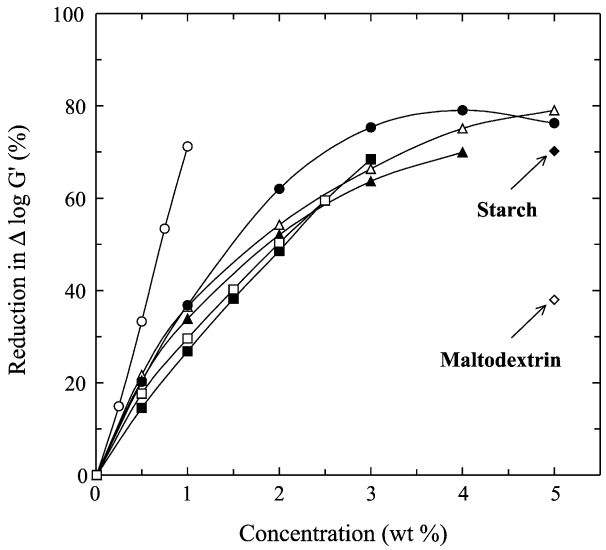


Fig. 16. Percentage reduction of the change in log G' on cooling from 85 to 5 °C for 2.0 wt% calcium pectinate on incorporation of locust bean gum (■) and guar gum samples M7 (○), M30 (●), M60 (△), M90 (▲) and M150 (□) at all galactomannan concentrations studied. The corresponding values for 5.0 wt% potato maltodextrin (◇) and oxidised starch (◆) are also shown (labelled points with arrows).

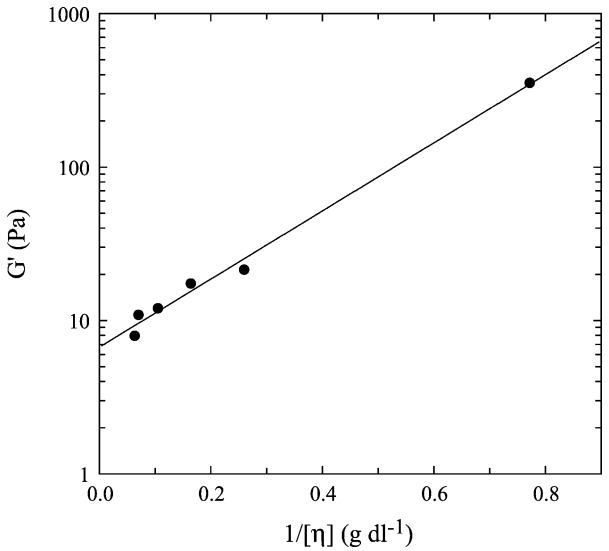


Fig. 17. Variation of G' at 85 °C (●) with the reciprocal of intrinsic viscosity, $1/[\eta]$, of the galactomannan component for mixtures of 2.0 wt% calcium pectinate with 1.0 wt% of each of the galactomannans studied.

decrease at low temperature can both be attributed to self-association of pectin, driven by segregative interactions with the cosolute, the reduction in the overall change in modulus on cooling may provide a useful single index of the extent of segregation induced by different concentrations of different polymeric cosolutes.

Fig. 16 shows the percentage reduction in the change in log G' between 85 and 5 °C (i.e. in the overall change between high and low temperature in cooling curves such as those shown in Fig. 15(a)), plotted against the concentration of cosolute in all the mixtures studied. It is evident that, by this criterion, oxidised starch and the galactomannans of high and intermediate molecular weight are roughly comparable in their ability to promote self-association of pectin, and that the potato maltodextrin is substantially less effective.

The most striking conclusion, however, is that the guar gum sample of lowest molecular weight (M7) causes much stronger segregative interactions than the samples of higher molecular weight. This cannot be attributed to decreased galactose content (Table 1), since LBG, which has a much lower content of galactose, behaves similarly (Figs. 12 and 16) to guar gum of comparable molecular weight (M150). Indeed, Fig. 14(a) indicates an inverse correlation between the hydrodynamic volume of the galactomannans (as characterised by intrinsic viscosity) and their effectiveness in promoting association of pectin chains. This inverse correlation is demonstrated more clearly in Fig. 17, which shows a direct linear relationship between log G' at 85 °C for 2.0 wt% pectin in mixtures with 1.0 wt% of each of the galactomannans (as in Fig. 14(a)) and the reciprocal of galactomannan intrinsic viscosity.

Subsequent studies were therefore focussed on other polymeric materials of low hydrodynamic volume. The materials used were gum arabic (Acacia gum), dextrans and inulin. The results obtained for dextrans and inulin are presented in the following paper (Giannouli et al., 2004a). The investigation of calcium pectinate gelation in the presence of gum arabic showed some features of particular interest, and is reported separately (Giannouli et al., 2004b).

References

Bohdanecký, M., & Kovár, J. (1982). Viscosity of polymer solutions. Amsterdam: Elsevier.

Buffington, L. A., Stevens, E. S., Morris, E. R., & Rees, D. A. (1980). Vacuum ultraviolet circular dichroism of galactomannans. *International Journal of Biological Macromolecules*, *2*, 199–203.

Chilvers, G. R., & Morris, V. J. (1987). Coacervation of gelatin–gellan gum mixtures and their use in microencapsulation. *Carbohydrate Polymers*, *7*, 111–120.

Giannouli, P., Richardson, R. K., & Morris, E. R. (2004a). Effect of polymeric cosolutes on calcium pectinate gelation. Part 2. Dextrans and inulin. *Carbohydrate Polymers*, *55* (4), 355–363.

Giannouli, P., Richardson, R. K., & Morris, E. R. (2004b). Effect of polymeric cosolutes on calcium pectinate gelation. Part 3. Gum arabic and overview. *Carbohydrate Polymers*, *55* (4), 365–375.

Gilsenan, P. M., Richardson, R. K., & Morris, E. R. (2000). Thermally-reversible acid-induced gelation of low-methoxy pectin. *Carbohydrate Polymers*, *41*, 339–349.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Letters*, *32*, 195–198.

Grinberg, V. Ya., & Tolstoguzov, V. B. (1972). Thermodynamic compatibility of gelatin with some D-glucans in aqueous media. *Carbohydrate Research*, *25*, 313–321.

Grinberg, V. Ya., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, *11*, 145–158.

Harding, S. E., Hill, S. E., & Mitchell, J. R. (1995). *Biopolymer mixtures*, Nottingham, UK: Nottingham University Press.

Imeson, A. P., Ledward, D. A., & Mitchell, J. R. (1977). On the nature of the interaction between some anionic polysaccharides and proteins. *Journal of the Science of Food and Agriculture*, *28*, 661–668.

Kasapis, S., & Morris, E. R. (1994). Conformation and physical properties of two unusual microbial polysaccharides: *Rhizobium trifolii* CPS and levan. In K. Nishinari, & E. Doi (Eds.), *Food hydrocolloids—structures, properties, and functions* (pp. 97–103). New York: Plenum Press.

Kasapis, S., Morris, E. R., Norton, I. T., & Gidley, M. J. (1993). Phase equilibria and gelation in gelatin/maltodextrin systems—Part II: Polymer incompatibility in solution. *Carbohydrate Polymers*, *21*, 249–259.

Michon, C., Cuvelier, G., Launay, B., Parker, A., & Takerkart, G. (1995). Study of the compatibility/incompatibility of gelatin/iota-carrageenan/water mixtures. *Carbohydrate Polymers*, *28*, 333–336.

Morawetz, H. (1965). Macromolecules in solution. New York: Wiley.

Morris, E. R. (1990). Mixed polymer gels. In P. Harris (Ed.), *Food gels* (pp. 291–359). London: Elsevier.

Morris, E. R., Powell, D. A., Gidley, M. J., & Rees, D. A. (1982). Conformations and interactions of pectins. I. Polymorphism between gel and solid states of calcium polygalacturonate. *Journal of Molecular Biology*, *155*, 507–516.

Morris, V. J. (1986). Multicomponent gels. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.), (pp. 87–99). *Gums and stabilisers for the food industry 3*, London, UK: Elsevier.

Muchin, M. A., Wajnermann, E. S., & Tolstogusow, W. B. (1976). Complex gels of proteins and acidic polysaccharides. *Die Nahrung*, *20*, 313–319.

Picout, D. R., Richardson, R. K., Rolin, C., Abeysekera, R. M., & Morris, E. R. (2000a). Ca^{2+}-induced gelation of low methoxy pectin in the presence of oxidised starch: Part 1—Collapse of network structure. *Carbohydrate Polymers*, *43*, 113–122.

Picout, D. R., Richardson, R. K., & Morris, E. R. (2000b). Ca^{2+}-induced gelation of low methoxy pectin in the presence of oxidised starch: Part 2—Quantitative analysis of moduli. *Carbohydrate Polymers*, *43*, 123–131.

Picout, D. R., Richardson, R. K., & Morris, E. R. (2000c). Co-gelation of calcium pectinate with potato maltodextrin: Part 1—Network formation on cooling. *Carbohydrate Polymers*, *43*, 133–141.

Picout, D. R., Richardson, R. K., & Morris, E. R. (2000d). Co-gelation of calcium pectinate with potato maltodextrin: Part 2—Analysis of co-gel moduli. *Carbohydrate Polymers*, *43*, 143–153.

Picout, D. R., & Ross-Murphy, S. B. (2002). On the chain flexibility of arabinoxylans and other β-(1 → 4) polysaccharides. *Carbohydrate Research*, *337*, 1781–1784.

Picullel, L., Bergfeldt, K., & Nilsson, S. (1995). Factors determining phase behaviour of multicomponent biopolymer systems. In S. E. Harding, S. E. Hill, & J. R. Mitchell (Eds.), *Biopolymer mixtures* (pp. 13–35). Nottingham, UK: Nottingham University Press.

Piculell, L., Iliopoulos, I., Linse, P., Nilsson, S., Turquois, T., Viebke, C., & Zhang, W. (1994). Association and segregation in ternary polymer solutions and gels. In G. O. Phillips, P. A. Williams, & D. J. Wedlock (Eds.), (pp. 309–322). *Gums and stabilisers for the food industry 7*, Oxford, UK: IRL Press.

Piculell, L., Nilsson, S., Falck, L., & Tjerneld, F. (1991). Phase separation in aqueous mixtures of similarly charged polyelectrolytes. *Polymer Communications*, *32*, 158–160.

Powell, D. A., Morris, E. R., Gidley, M. J., & Rees, D. A. (1982). Conformations and interactions of pectins. II. Influence of residue sequence on chain association in calcium pectate gels. *Journal of Molecular Biology*, *155*, 517–531.

Ross-Murphy, S. B. (1984). Rheological methods. In H. W.-S. Chan (Ed.), *Biophysical methods in food research* (pp. 195–290). *Critical reports on applied chemistry*, London, UK: SCI.

Stainsby, G. (1980). Proteinaceous gelling systems and their complexes with polysaccharides. *Food Chemistry*, *6*, 3–14.

Suchkov, V. V., Grinberg, V. Ya., & Tolstoguzov, V. B. (1981). Steady-state viscosity of the liquid two-phase disperse system water–casein–sodium alginate. *Carbohydrate Polymers*, *1*, 39–53.

Tolstoguzov, V. B. (1986). Functional properties of protein–polysaccharide mixtures. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional properties of food macromolecules* (pp. 385–415). London, UK: Elsevier.

Tolstoguzov, V. B. (1988). Concentration and purification of proteins by means of two-phase systems: Membraneless osmosis process. *Food Hydrocolloids*, *2*, 195–207.

Tolstoguzov, V. B. (1991). Functional properties of food proteins and role of protein–polysaccharide interaction. *Food Hydrocolloids*, *4*, 429–468.

Tolstoguzov, V. B., Belkina, V. P., Gulov, V. Ja., Grinberg, V. Ja., Titova, E. F., Belavzeva, E. M., Titova, E. F., & Belavzeva, E. M. (1974). State of phase, structure and mechanical properties of the gelatinous system water–gelatin–dextran. *Die Stärke*, *26*, 130–137.

Tolstoguzow, W. B., & Wajnermann, E. S. (1975). Investigation of the interaction between certain proteins and acidic polysaccharides in aqueous medium. *Die Nahrung*, *19*, 45–60.

Tschumak, G. Ja., Wajnermann, E. S., & Tolstogusow, W. B. (1976). Structure and properties of complex gels of gelatin and pectin. *Die Nahrung*, *20*, 321–328.

Zasypkin, D. V., Braudo, E. E., & Tolstoguzov, V. B. (1997). Multicomponent biopolymer gels. *Food Hydrocolloids*, *11*, 159–170.