Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Polymers 55 (2004) 367–377

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Effect of polymeric cosolutes on calcium pectinate gelation. Part 3. Gum arabic and overview

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

Addition of gum arabic (average $M_r \approx 450$ kDa; 0.5–2.0 wt%) to solutions of low methoxy pectin (DE 31; 2.0 wt%; pH $\approx$ 2.9–3.0) with stoichiometric Ca^{2+} caused massive increases in G' and G'' in the pre-gel state at 90 °C (attributed to segregative interactions promoting formation of calcium-mediated 'egg-box' junctions between pectin chains) but had little effect on the gels formed on cooling to 5 °C. This is in marked contrast to the behaviour of other polymeric cosolutes studied in the investigations reported in the two preceding papers, which caused large reductions in gel moduli (attributed to excessive association of calcium pectinate into large aggregated bundles); the difference is tentatively ascribed to strengthening of the calcium pectinate network by divalent counterions to the uronate residues in gum arabic. When the complication of cation exchange was eliminated by extensive dialysis of gum arabic against 100 mM Na^+ and use of the final dialysate in preparation of mixtures with calcium pectinate, massive increases in G' and G'' at high temperature were again observed, but with accompanying reductions in moduli at low temperature, which, at gum arabic concentrations above ~1.0 wt%, arose from collapse of the developing calcium pectinate network during cooling. The tentative conclusion from this work, and from the two preceding papers, is that enthalpically unfavourable (segregative) interactions between low methoxy pectin and polymeric cosolutes can be relieved in two ways: (i) Ca^{2+}-mediated self-association of pectin into compact ordered assemblies which occupy less of the total volume, and (ii) conformational rearrangement of the cosolute molecules to minimise segmental interactions with pectin; conformational rearrangement is inhibited by chain stiffness and by branching; thus polymeric cosolute molecules of limited flexibility are more effective in promoting self-association of pectin than more flexible molecules of comparable size, and branched molecules are more effective than linear chains of comparable stiffness.

Keywords: Biopolymer mixtures; Pectin; Gum arabic; Gelation; Rheology

---

1. Introduction

This is the last in a series of three related publications on Ca^{2+}-induced gelation of low methoxy pectin in the presence of other hydrocolloids. In the first paper (Giannouli, Richardson, & Morris, 2004a) it was shown that galactomannans (locust bean gum, and guar gum samples of different molecular weights) caused rheological changes similar to those observed previously on incorporation of oxidised starch (Picout, Richardson, Rolin, Abeysekera, & Morris, 2000b) or potato maltodextrin (Picout, Richardson, & Morris, 2000a). Increasing concentrations of each of these materials caused a progressive increase in moduli in the pre-gel solution state at high temperature, attributed (Picout et al., 2000b) to an increase in the extent of Ca^{2+}-mediated association of pectin chains into 'egg-box' junctions (Grant, Morris, Rees, Smith, & Thom, 1973; Morris, Powell, Gidley, & Rees, 1982; Powell, Morris, Gidley, & Rees, 1982) in response to segregative interactions with the polymeric cosolute. On cooling, however, plots of (log) modulus versus temperature for the mixed systems crossed the corresponding plot for calcium pectinate alone (at ~55 °C), giving weaker final gels (at 5 °C). The reduction in gel strength is attributed (Picout et al., 2000b) to segregative interactions promoting excessive self-association of calcium pectinate into large aggregated bundles that make little contribution to network crosslinking.

---

* 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.2003.09.015

The net effect of these two phenomena is that the increase in log G' on cooling from high temperature (85 °C) to low temperature (5 °C), $\Delta \log G'$, becomes progressively smaller as the concentration of polymeric cosolute is increased. Taking the percentage reduction in $\Delta \log G'$ as an overall index of the effectiveness of a given concentration of any specific polymeric cosolute in promoting self-association of low methoxy pectin (2.0 wt%) in the presence of Ca^{2+} (at stoichiometric equivalence to the unesterified carboxyl groups of the pectin), oxidised starch has a substantially greater effect than potato maltodextrin, but was found to have roughly the same effect as most of the galactomannan samples studied by Giannouli et al. (2004a). However, the effectiveness of guar gum samples of different molecular weights showed an unexpected inverse correlation with hydrodynamic volume, as characterised by intrinsic viscosity, $[\eta]$. Indeed, the effectiveness of the guar gum sample of lowest molecular weight (guar gum M7; $M_r \approx 60$ kDa) in driving formation of egg-box junctions at high temperature was so great that the highest concentration of this material at which fluid mixtures with calcium pectinate could be prepared was 1.0 wt% (in comparison with concentrations of up to 5 wt% for guar gums of higher molecular weight).

Because of this surprising finding, subsequent studies were focussed on other polymeric cosolutes of low hydrodynamic volume. As reported in the preceding paper (Giannouli, Richardson, & Morris, 2004b), branched dextrans of M_r 2000, 464 and 67 kDa and (linear) inulin of $M_r \approx 4.5$ kDa were found to have only limited effectiveness in promoting self-association of calcium pectinate, with the changes induced by the dextran samples being roughly comparable to those observed with potato maltodextrin, the least effective of the materials studied previously, and inulin having even less effect. In the present work, we have explored the changes in calcium pectinate gelation induced by incorporation of gum arabic.

Gum arabic (Whistler, 1993) is an exudate gum from trees of various *Acacia* species, and is also known as acacia gum. The primary structure is based on a core of β-D-galactose residues, comprising a (1 → 3)-linked backbone with (1 → 6)-linked branches, bearing L-arabinose, L-rhamnose, β-D-glucuronate and 4-*O*-methyl-β-D-glucuronate as single-sugar or oligosaccharide sidechains. The principal counterions to the uronate residues, which constitute ~20% of the total sugars, are Ca^{2+}, Mg^{2+} and K^{+}.

In addition to the carbohydrate constituents, gum arabic contains ~2.4% protein. As well as having a crucial role in emulsification, the protein component is central to the overall primary structure (Randall, Phillips, & Williams, 1989). Gum arabic can be separated into two principal fractions. The main fraction (Fraction 1), whose contribution to the total is around 86%, has a lower protein content than the unfractionated gum (<0.5%) and an average molecular mass of ~280 kDa, which does not change significantly on extensive proteolysis. The smaller fraction (Fraction 2) has a much higher protein content (~12%) and an average molecular mass about 5 times that of the major fraction (~1450 kDa).

On proteolysis, the molecular mass of Fraction 2 drops to that of Fraction 1, suggesting a 'wattle blossom' structure with, on average, five branched carbohydrate assemblies similar, or identical, to those in Fraction 1, linked together by a polypeptide chain. There is also a minor (~1.5%) 'glycoprotein' component, which contains about 25% of the total protein (Randall et al., 1989). Because of its densely branched structure, gum arabic has very low solution viscosity in comparison with linear polysaccharides (Goycoolea, Morris, Richardson, & Bell, 1995), and can be dissolved at concentrations up to ~50 wt%.

2. Materials and methods

Gum arabic was purchased from Sigma. It was supplied in powdered form, and described as having been harvested in the Sudan from trees of the *Acacia* species, but of unidentified mixed subspecies. Low methoxy pectin (DE 31%; free acid form) was a research sample kindly provided by the Copenhagen Pectin Division of Hercules, and was identical to the material used in the work reported in the two preceding papers (Giannouli et al., 2004a,b). All reagents were Analar grade from BDH. Distilled deionised water was used throughout.

Solutions for determination of the ionic strength dependence of intrinsic viscosity for gum arabic were prepared by dissolving the gum in salt solution and dialysing against four changes of the same salt solution over a period of 2 days, with the final dialysate then being used for all subsequent dilutions. The salt solutions all incorporated 3 mM sodium azide (to inhibit bacterial growth), together with the amount of sodium chloride required to give overall Na^{+} concentrations of 10, 25, 100 and 1000 mM. In calculating concentrations of gum arabic, correction was made for changes in volume during dialysis, by weighing the sample at the beginning and end of the dialysis procedure. Measurements of dilute solution viscosity (at 20 °C) were made at relative viscosities in the approximate range $\eta_{rel} = 1.2–2.0$, using cup-and-bob geometry on a Contraves Low Shear 30 rotational viscometer.

The effect of gum arabic on calcium pectinate gelation was investigated in two series of experiments. The concentration of pectin was held fixed at 2.0 wt% in both, and the concentration of Ca^{2+} was also held fixed at stoichiometric equivalence to the unesterified carboxyl groups of the pectin.

In the first series of experiments, the procedure used was identical to that described in the two preceding papers (Giannouli et al., 2004a,b). Solutions of gum arabic, pectin and calcium chloride were prepared in water, and mixed at 90 °C in the proportions necessary to give the required final concentrations of each. Samples were loaded (at 90 °C) onto an oscillatory rheometer (with 50 mm cone-and-plate geometry), coated around their periphery with light silicone oil, and cooled to 5 °C at 1 °C/min, with measurement of G' and G'' at 10 rad/s and 0.5% strain.

In the second series of experiments, all samples incorporated 100 mM Na^+ (97 mM NaCl plus 3 mM NaN_3). A stock solution of gum arabic under these ionic conditions was prepared at a concentration of ~15 wt%, using the dialysis procedure described above for the more dilute solutions used in determination of intrinsic viscosity. The final dialysate was then used to dissolve the pectin and calcium chloride, and for all necessary dilutions. Mixtures were again prepared at 90 °C, and the subsequent rheological studies were identical to those described for the samples prepared without incorporation of Na^+. For brevity, the preparations used in the two series of experiments will be referred to as samples 'in water' and 'in 100 mM NaCl', respectively (i.e. omitting repeated mention of the calcium chloride and sodium azide constituents).

For comparative studies of the effect of Na^+ on gelation of calcium pectinate in the absence of gum arabic, the pectin was dissolved in water and mixed with concentrated solutions of NaCl and $CaCl_2$ in the proportions necessary to give the required final concentrations of pectin, Ca^{2+} and Na^+.

3. Results

3.1. Effect of gum arabic in water

Fig. 1 shows the changes in G' (Fig. 1a) and G'' (Fig. 1b) observed for 2.0 wt% low methoxy pectin with stoichiometric Ca^{2+} on cooling (at 1 °C/min) from 90 to 5 °C, alone, and in the presence of gum arabic (as received) at concentrations of 0.5, 1.0, 1.5 and 2.0 wt%. As found for other polymeric cosolutes (Giannouli et al., 2004a,b; Picout et al., 2000a,b), increasing concentrations of gum arabic cause a progressive increase in both G' and G'' in the pre-gel state at high temperature. In all cases, G' at high temperature is substantially greater than G'', so that the fluid preparations loaded onto the rheometer are more appropriately described as 'weak gels' (Ross-Murphy, 1984) rather than solutions. As discussed in Section 1, this initial gel-like character can be attributed to association of pectin chains into dimeric egg-box junctions (Morris et al., 1982; Powell et al., 1982), with the extent of association being increased by segregative interactions between pectin and gum arabic.

Consolidation of the initial 'weak gel' structure into a 'true' gel network can be seen as a large sigmoidal increase in moduli on cooling. As observed for oxidised starch and potato maltodextrin (Giannouli et al., 2004a; Picout et al., 2000a,b), locust bean gum and guar gum (Giannouli et al., 2004a), dextrans and inulin (Giannouli et al., 2004b), the magnitude of this increase diminishes with increasing concentration of gum arabic, but, in marked contrast to these other systems, the cooling curves for the mixtures do not cross the corresponding curves for calcium pectinate alone, and the final moduli on completion of cooling also increase with increasing concentration of gum arabic.

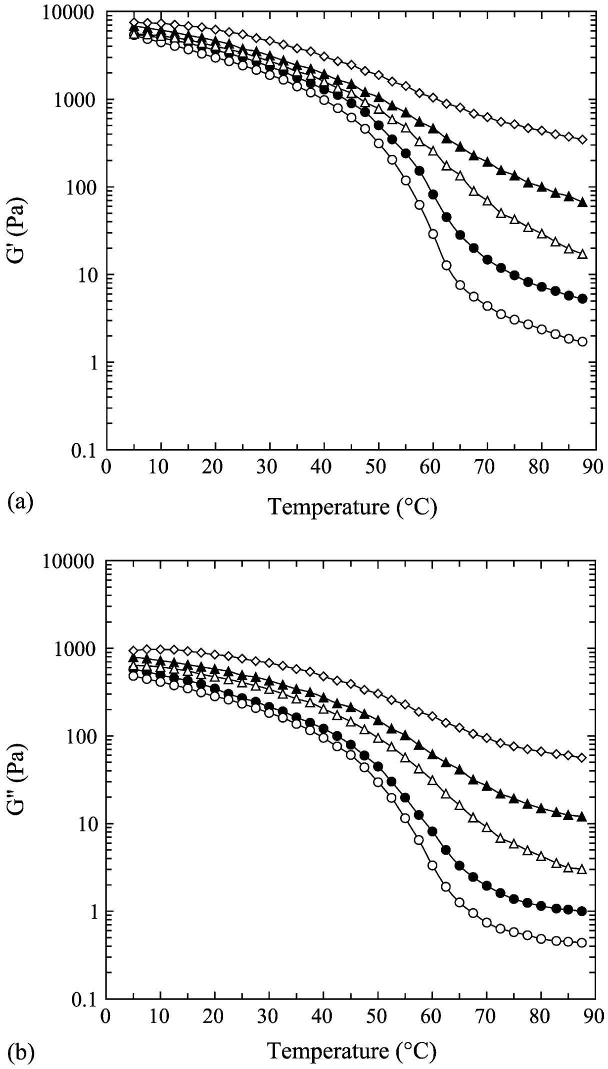

Fig. 1. Changes in (a) G' and (b) G'' (10 rad/s; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of gum arabic (in water) at concentrations (wt%) of 0.5 (●), 1.0 (△), 1.5 (▲) and 2.0 (◇).

The effect of gum arabic concentration on the values of G' and G'' recorded at 85 °C (5 min after the start of cooling, once readings had stabilised from loading) and at 5 °C, after completion of cooling, is shown directly in Fig. 2. At both temperatures, the separation of G' and G'' remains roughly constant. Raising the concentration of gum arabic from 0 to 2.0 wt% increases the moduli at 85 °C by more than two orders of magnitude. The increase at 5 °C is much smaller, but still significant (approximate doubling of both moduli).

One difference between gum arabic and the other polymeric cosolutes studied so far is that gum arabic is

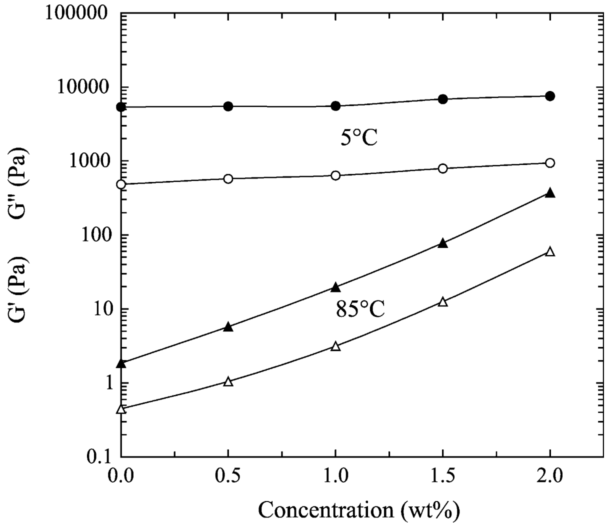

Fig. 2. Measured values (10 rad/s; 0.5% strain) of G' (filled symbols) and G'' (open symbols) at 85 (triangles) and 5 °C (circles) for 2.0 wt% calcium pectinate in the presence of gum arabic (in water) at all concentrations studied.

a charged polymer, whereas the others are either entirely uncharged (guar gum, locust bean gum, maltodextran, dextran and inulin) or have only a very low content of charged substituents (1 carboxyl group per 30 or 40 glucose residues in oxidised starch). It seemed possible, therefore, that the small increase in calcium pectinate gel strength at low temperature on incorporation of gum arabic, in contrast to the reduction seen with uncharged, or virtually uncharged, polymeric cosolutes, might be caused by direct interaction of pectin with divalent counterions to the carboxyl groups of gum arabic.

The purpose of the second series of experiments, made using preparations of gum arabic that had been dialysed extensively against monovalent (Na^+) salt solution, was to eliminate this complication. As a prelude to these experiments, two other studies were carried out. The first was to determine the effect of increasing concentrations of (monovalent) salt on the hydrodynamic volume (intrinsic viscosity) of gum arabic. The second was to determine the effect of Na^+ cations on gelation of calcium pectinate in the absence of gum arabic.

3.2. Salt-dependence of intrinsic viscosity for gum arabic

As described in Section 2, stock solutions of gum arabic were dialysed to Na^+ concentrations of 10, 25, 100 and 1000 mM, and were diluted with the final dialysate to give a range of relative viscosities between ~1.2 and ~2.0. As described previously (Giannouli et al., 2004a,b), Huggins and Kraemer plots of, respectively, η_{sp}/c and $\ln(\eta_{rel})/c$ versus polymer concentration (c) were fitted to the linear relationships

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \tag{1}$$

and

$$(\ln \eta_{rel})/c = [\eta] + (k' - 0.5)[\eta]^2 c \tag{2}$$

by varying $[\eta]$ and k' to minimise the combined root-mean-square deviation between observed and fitted values for both plots. The resulting values of $[\eta]$ for gum arabic at each of the four salt concentrations studied are listed in Table 1 and the standard of fit obtained is indicated in Fig. 3, using the sample incorporating 100 mM Na^+ as an illustrative example.

Table 1
Variation of intrinsic viscosity, $[\eta]$, with ionic strength (I) for gum arabic dialysed against Na^+ salt solutions

$[Na^+]$ (mM)	I	$I^{-1/2}$	$[\eta]$ (dl/g)	
			Observed	Fitted[a]
10	0.010	10.0	0.390	0.390
25	0.025	6.32	0.329	0.323
100	0.100	3.16	0.249	0.266
1000	1.000	1.00	0.237	0.227

[a] By the linear relationship between $[\eta]$ and $I^{-1/2}$ shown in Fig. 4 and Eq. (5).

The hydrodynamic volume of polyelectrolyte molecules is expanded by intramolecular electrostatic repulsion. Addition of increasing amounts of salt causes progressive shielding of these repulsions, and allows the molecules to contact towards the volume of an uncharged polymer with the same number of residues linked together in the same way. The extent of contraction depends on the inherent stiffness of the polymer, with the reduction in hydrodynamic

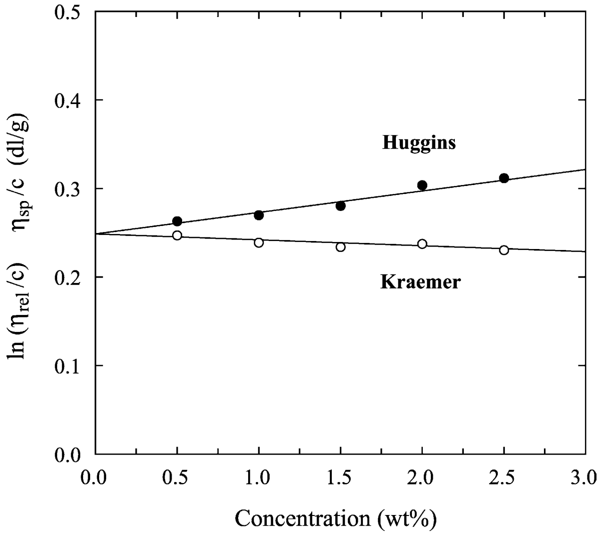

Fig. 3. Determination of intrinsic viscosity (20 °C) from Huggins and Kraemer plots of, respectively, η_{sp}/c (●) and $(\ln \eta_{rel})/c$ (○) against concentration (c), illustrated for gum arabic in 100 mM NaCl. Only two variable parameters were used to fit both plots, as described by Giannouli et al. (2004a).

volume on addition of salt being greater for flexible polyelectrolytes than for stiffer molecules. This is the basis of the semi-empirical ‘*B*-value’ method of Smidsrød and Haug (1971) for estimation of chain flexibility from reduction in polyelectrolyte intrinsic viscosity with increasing ionic strength (I).

In development of this method, a linear relationship between intrinsic viscosity and the reciprocal of the square root of ionic strength ($I^{-1/2}$) was demonstrated for a range of different polyelectrolytes. For any specific polyelectrolyte, the slope (S) of $[\eta]$ versus $I^{-1/2}$ was found to increase systematically with increasing molecular weight (M_r). To quantify this effect, intrinsic viscosity at a fixed ionic strength of 0.1 (denoted as $[\eta]_{0.1}$) was chosen as a convenient index of M_r, and a linear relationship between $\log S$ and $\log[\eta]_{0.1}$ was demonstrated:

$$\log S = \log B + m \log[\eta]_{0.1} \tag{3}$$

Plots of this type for different polyelectrolytes were found to have essentially the same gradient ($m = 1.3 \pm 0.1$), but with a systematic increase in the intercept ($\log B$) with increasing flexibility. The value of B for any specific polyelectrolyte therefore gives an indication of the flexibility of the molecule, and can be expressed as

$$B = S/([\eta]_{0.1})^{1.3} \tag{4}$$

Experimentally, the value of B for a single polyelectrolyte sample of unknown molecular weight can be determined by measuring intrinsic viscosity at a few different ionic strengths, constructing a (linear) plot of $[\eta]$ against $I^{-1/2}$, and inserting the slope (S) and intrinsic viscosity at $I = 0.1$ into Eq. (4). The plot obtained for gum arabic (from the experimental data in Table 1) is shown in Fig. 4. The linearity is reasonable, and the line of best (root-mean-square) fit is given by:

$$[\eta] = 0.21 + 0.018 I^{-1/2} \tag{5}$$

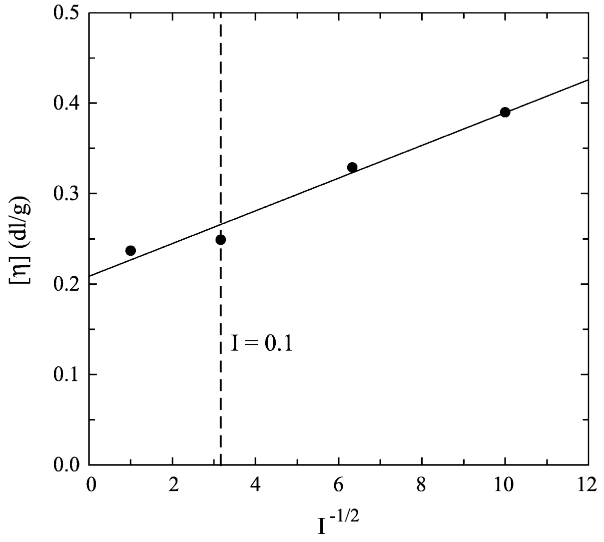

Fig. 4. Effect of ionic strength (I) on intrinsic viscosity, $[\eta]$, for gum arabic in the presence of NaCl at concentrations of 10, 25, 100 and 1000 mM. The vertical dashed line indicates the ionic strength ($I = 0.1$) used in determination of the flexibility parameter B by the method of Smidsrød and Haug (1971).

The values of $[\eta]$ derived from Eq. (5) for gum arabic at the four ionic strengths studied ($I = 0.010$, 0.025, 0.10 and 1.00) are listed in Table 1, along with the experimental values. The value of $[\eta]_{0.1}$ from Eq. (5) is 0.266 dl/g, giving $([\eta]_{0.1})^{1.3} = 0.179$, and the slope ($S$) is 0.018. Substituting these values in Eq. (4) gives $B = 0.10$ for gum arabic, which may be compared with literature values for other polysaccharides or their charged derivatives. The B values reported (Smidsrød & Haug, 1971) for alginates and pectins are around 0.04; carboxymethylcellulose has a somewhat higher B-value (0.065); the difference can be attributed (Morris & Ross-Murphy, 1981; Rees & Scott, 1971) to the difference in linkage geometry (1 ⟶ 4 diaxial in alginate and pectin; 1 ⟶ 4 diequatorial in cellulose). The B-value for carboxymethylamylose is substantially higher ($B = 0.20$), indicating significantly greater flexibility than in cellulose derivatives, and dextran sulphate is even more flexible ($B = 0.23$).

With the exception of dextran sulphate, the polymers used in developing the B-value method were linear, so comparison with (densely branched) gum arabic in terms of inherent chain stiffness is probably inappropriate. It does, however, seem reasonable to conclude from the relative magnitude of changes in intrinsic viscosity in response to changes in ionic strength that gum arabic ($B = 0.10$) is more resistant to expansion/contraction of hydrodynamic volume than dextran ($B = 0.23$) or starch polysaccharides ($B = 0.20$), but less resistant than cellulose ($B = 0.065$). Since the polymer backbone of the galactomannans has the same 1 ⟶ 4 diequatorial linkage geometry as in cellulose, giving similar persistence lengths (Picout, Ross-Murphy, Errington, & Harding, 2001), it is likely that they too will be somewhat less easily deformed than gum arabic. Similarly, it seems probable that inulin, like dextran, will be more readily deformable than gum arabic since, as discussed in the preceding paper (Giannouli et al., 2004b), the two materials are alike in having three, rather than two, bonds in the glycosidic linkage. Thus, deformability for gum arabic and the polymeric cosolutes studied by Giannouli et al. (2004a,b) can be ranked roughly in the following order: inulin ≈ dextran > partially depolymerised starches > gum arabic > galactomannans, although the balance of various contributing factors such as molecular weight, charge and solvent quality may be complex.

3.3. Effect of NaCl on calcium pectinate gelation

Fig. 5 shows the changes in G' (Fig. 5a) and G'' (Fig. 5b) observed for 2.0 wt% low methoxy pectin with stoichiometric Ca^{2+} on cooling from 90 to 5 °C, alone, and in the presence of NaCl at concentrations of 10, 100 and

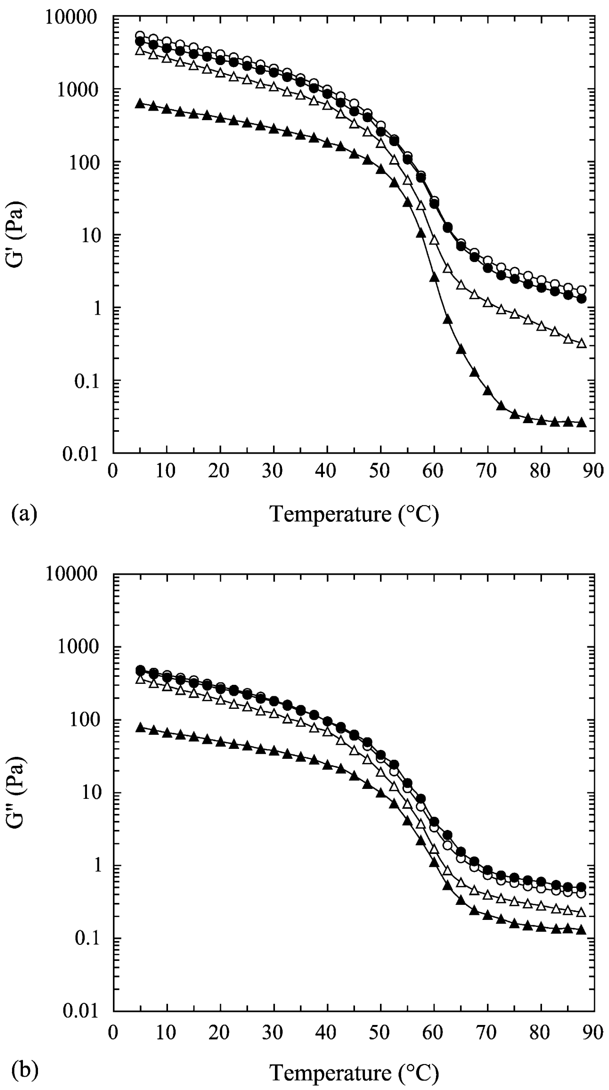

Fig. 5. Changes in (a) G' and (b) G'' (10 rad/s; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate alone (○) and in the presence of NaCl at concentrations of 10 mM (●), 100 mM (△), and 1000 mM (▲).

1000 mM. Previous studies by equilibrium dialysis and circular dichroism (Morris et al., 1982; Powell et al., 1982) have shown that monovalent cations can inhibit Ca^{2+}-induced association of low methoxy pectin (and of fully de-esterified poly-D-galacturonate). At very high concentrations (~2 M), monovalent salts appear to limit association in two ways: (i) egg-box binding of Ca^{2+} between pectin chains is restricted to formation of dimeric junctions, with no further association of these dimers into larger aggregates, and (ii) formation of stable dimers requires a minimum of eight consecutive unesterified galacturonate residues along the inner face of each of the participating chains. As might therefore be expected, incorporation of increasing amounts of NaCl causes a progressive reduction in both G' (Fig. 5a) and G'' (Fig. 5b) at all temperatures.

At the highest salt concentration studied (1 M NaCl), G' is reduced by an order of magnitude in the final gel state at 5 °C, and by almost two orders of magnitude at the loading temperature of 90 °C. At 100 mM NaCl, the reductions in moduli are much smaller, and at 10 mM they are barely detectable.

In the experiments on Ca^{2+}-induced gelation of low methoxy pectin in mixtures with gum arabic that had been dialysed extensively against Na^+ salt solution, gum arabic was used at concentrations up to 8 wt%. As mentioned in Section 1, approximately 20% of the sugars in gum arabic are negatively charged (D-glucuronate and 4-*O*-methyl-D-glucuronate). Assuming a mean residue weight of ~160 Da, the molecular weight per charge is therefore ~800 Da, and thus the concentration of carboxyl groups in an 8 wt% solution is ~100 mM. An Na^+ concentration of 100 mM was therefore chosen as high enough to fully balance the negative charge of gum arabic at the highest concentration used, while not causing any serious inhibition of calcium pectinate gelation.

3.4. Gelation of calcium pectinate with gum arabic in 100 mM NaCl

Fig. 6 shows the observed changes in G' (Fig. 6a) and G'' (Fig. 6b) on cooling from 90 to 5 °C at 1 °C/min for 2.0 wt% calcium pectinate in 100 mM NaCl, in comparison with the corresponding curves for mixtures of the same concentration of calcium pectinate with 0.5, 1.0 and 1.5 wt% of gum arabic dialysed extensively against 100 mM Na^+ (3 mM NaN_3 plus 97 mM NaCl). As observed for other polymeric cosolutes (Giannouli et al., 2004a,b; Picout et al., 2000a,b), and for gum arabic in water (Fig. 1), there is a progressive increase in high-temperature values of both G' and G'' as the concentration of gum arabic is increased. At the lowest concentration of gum arabic studied (0.5 wt%), both moduli show a smooth, sigmoidal increase on cooling to 5 °C. With 1.0 wt% gum arabic present, G' passes through a shallow maximum towards the end of the cooling process (at ~25 °C). On further increase in gum arabic concentration to 1.5 wt%, there is a substantial drop in G' at ~ 50 °C (Fig. 6a), with an accompanying reduction in G'' (Fig. 6b). As discussed previously (Picout et al., 2000b), this phenomenon, which was also observed for moderate concentrations of oxidised starch (Giannouli et al., 2004a; Picout et al., 2000b) and for higher concentrations of potato maltodextrin (Picout et al., 2000a), can be attributed to collapse of the developing calcium pectinate network in response to segregative interactions with the polymeric cosolute. At higher concentrations of gum arabic (above 1.5 wt%) the reductions in moduli during cooling became sharper and more pronounced, as illustrated in Fig. 7 for 2.0 wt% calcium pectinate (with 100 mM Na^+) in mixtures with 2.5 and 4.0 wt% gum arabic.

The values of G' and G'' recorded at 85 °C (5 min after commencement of cooling) and at 5 °C (on completion of cooling) are shown in Fig. 8 for all concentrations of (dialysed) gum arabic studied (0.5, 1.0, 1.5, 2.0, 2.5, 3.0,

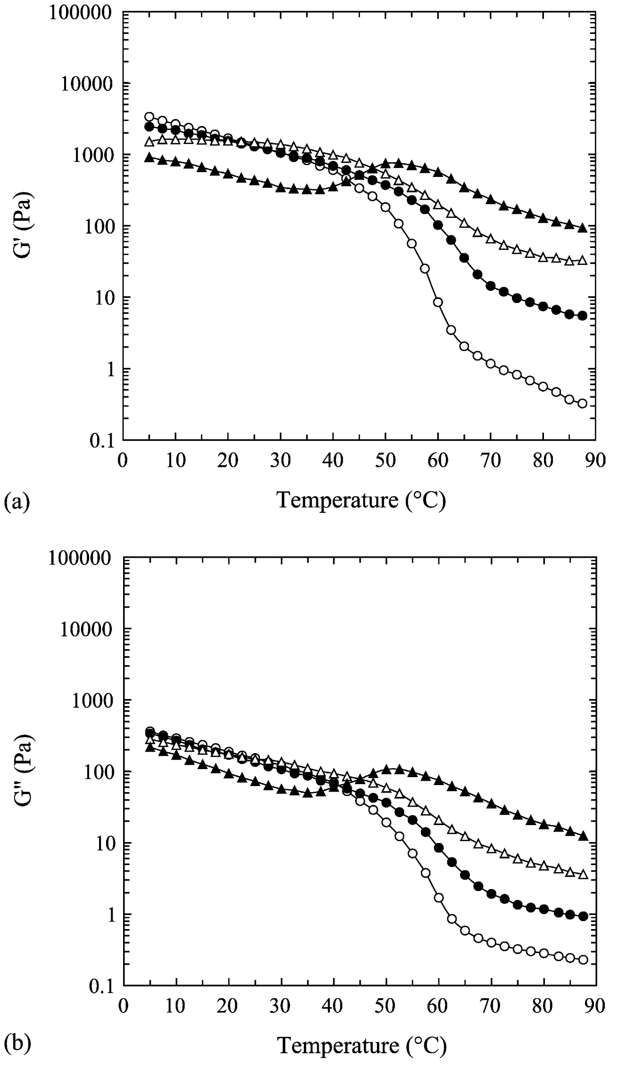

Fig. 6. Changes in (a) G' and (b) G'' (10 rad/s; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate in 100 mM NaCl, alone (○) and in the presence of gum arabic at concentrations (wt%) of 0.5 (●), 1.0 (△) and 1.5 (▲).

3.5, 4.0 and 8.0 wt%). Increasing concentrations of gum arabic cause an initial sharp increase in G' and G'' at high temperature (85 °C), but both moduli then level out towards constant values at the highest concentrations of cosolute used. The initial steep increase in moduli at high temperature is accompanied by a steep reduction in the values attained on completion of cooling to 5 °C, but at higher concentrations of gum arabic the final moduli again level out towards constant values. Indeed, G'' at 5 °C passes through a shallow minimum at ~3 wt% gum arabic, and then increases slightly at higher concentrations. As discussed previously (Giannouli et al., 2004a,b), the increase probably arises from the direct contribution of high concentrations of the (non-gelling) polymeric cosolute to overall viscous response (G'').

At the highest concentrations of gum arabic used (above ~3 wt%), the final values of G'' on completion of cooling to 5 °C are slightly lower than the initial values in the pre-gel solution state at high temperature. The same effect is even more evident for G', with the final values at 5 °C dropping below the initial values at high temperature when the concentration of gum arabic in the mixtures reaches ~2.5 wt%, and at the highest concentrations of gum arabic used (4.0 and 8.0 wt%) there is about a 5-fold reduction in G' between high and low temperature. As can be seen from the cooling curves shown in Fig. 7, the reduction arises from the sharp drop in modulus attributed to collapse of calcium pectinate network structure during cooling.

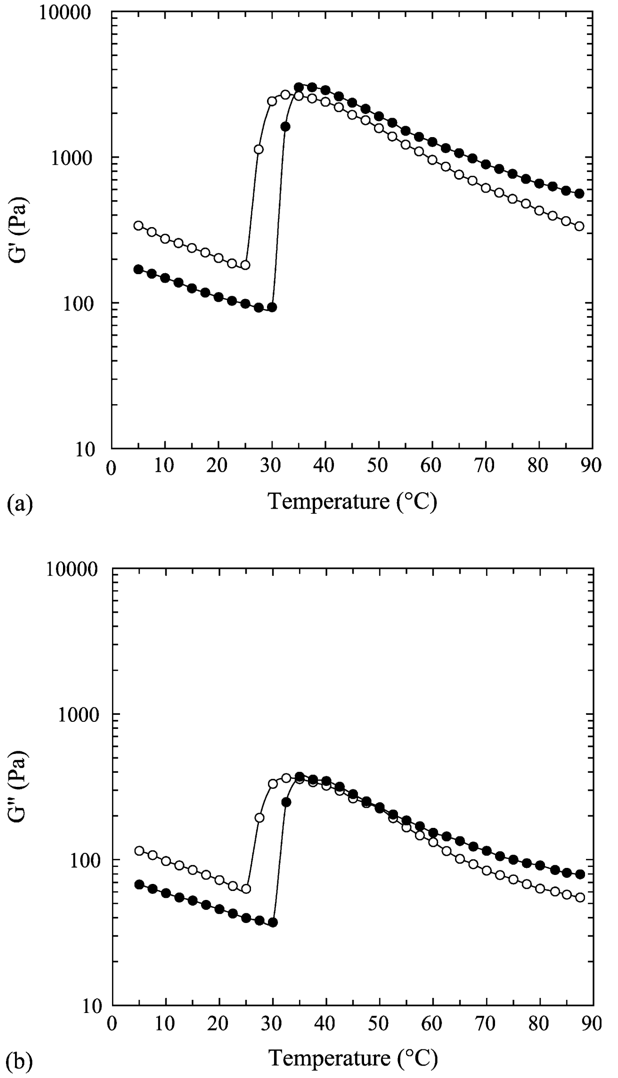

Fig. 7. Changes in (a) G' and (b) G'' (10 rad/s; 0.5% strain) during cooling from 90 to 5 °C for 2.0 wt% calcium pectinate in 100 mM NaCl, in the presence of gum arabic at concentrations (wt%) of 2.5 (○) and 4.0 (●).

4. Discussion

As shown in Figs. 1 and 2 and discussed in Section 3.1, low concentrations (2.0 wt%, or less) of gum arabic in water

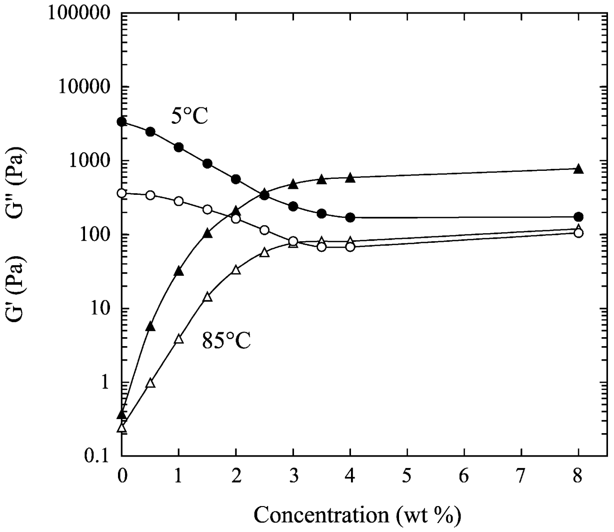

Fig. 8. Measured values (10 rad/s; 0.5% strain) of G' (filled symbols) and G'' (open symbols) at 85 (triangles) and 5 °C (circles) for 2.0 wt% calcium pectinate in 100 mM NaCl, in the presence of gum arabic at all concentrations studied.

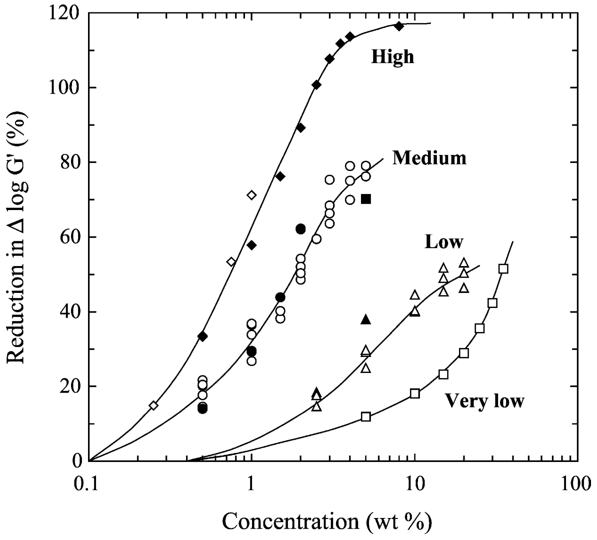

Fig. 9. Percentage reduction of the change in log G' on cooling from 85 to 5 °C for 2.0 wt% calcium pectinate on incorporation of gum arabic (◆) in 100 mM NaCl. The corresponding values from Giannouli et al. (2004a) for guar gum M7 (◇), other galactomannans (○), 5.0 wt% oxidised starch (●) and 5.0 wt% potato maltodextrin (▲) and from Giannouli et al. (2004b) for dextrans (△) and inulin (□) are also shown.

caused massive increases in G' and G'' of calcium pectinate in the pre-gel state at high temperature, but had little effect on the gels formed on cooling to 5 °C. Because of the likely complication of transfer of divalent counterions from gum arabic to pectin, these results are difficult to interpret, and will not be discussed further.

When the complication of cation exchange was eliminated by extensive dialysis of gum arabic against 100 mM Na$^+$ and use of the final dialysate in preparation of mixtures with calcium pectinate, gum arabic was again found to cause massive increases in G' and G'' at high temperature (Figs. 6 and 8), but with accompanying reductions in moduli at low temperature (5 °C), as observed for calcium pectinate in the presence of other polymeric cosolutes (Giannouli et al., 2004a,b; Picout et al., 2000a,b).

As discussed in Section 1, the percentage reduction in $\Delta \log G'$, the increase in $\log G'$ on cooling from high temperature (85 °C) to low temperature (5 °C), can be taken as an overall index of the relative effectiveness of different polymeric cosolutes in promoting Ca^{2+}-mediated self-association of low methoxy pectin. Fig. 9 shows a direct comparison, by this criterion, of the results from the studies reported in the two preceding papers (Giannouli et al., 2004a,b) and from the present investigation of (dialysed) gum arabic. Because of the very different ranges of concentration required to produce significant changes in the rheology of calcium pectinate, the concentrations of the various polymeric cosolutes studied are compared on a logarithmic scale (horizontal axis in Fig. 9).

The results fall into four separate groups. The reductions in $\Delta \log G'$ produced by incorporation of gum arabic (after dialysis against 100 mM Na$^+$) are closely similar to those observed for equivalent concentrations of guar gum M7, the smallest of the galactomannans studied by Giannouli et al. (2004a). These two materials, guar gum M7 and gum arabic (in 100 mM Na$^+$), can therefore be grouped together as having 'high' effectiveness in promoting association of calcium pectinate. The range of concentrations at which fluid mixtures with calcium pectinate could be prepared was, however, wider for gum arabic than for M7, and the reductions in $\Delta \log G'$ are correspondingly greater, extending to values above 100% (which means that, as shown in Figs. 7 and 8, the final values of G' at 5 °C for mixtures incorporating high concentrations of gum arabic were lower than the initial values at high temperature).

The next group, with 'medium' effectiveness in driving self-association of pectin, consists of oxidised starch, locust bean gum, and the remaining (higher molecular weight) guar gum samples studied by Giannouli et al. (2004a). The third group, with 'low' effectiveness, contains the three branched dextrans used by Giannouli et al. (2004b). The reduction in $\Delta \log G'$ caused by the sample of potato maltodextrin used, at a single concentration of 5.0 wt%, by Giannouli et al. (2004a) is marginally higher, but it too can be regarded as having 'low' effectiveness in promoting initial association and subsequent aggregation of calcium pectinate. The fourth group, with 'very low' effectiveness, contains only the inulin sample used by Giannouli et al. (2004b).

As discussed in Section 1, the most striking finding from the work reported in the first paper of this series (Giannouli et al., 2004a) was an inverse relationship between the intrinsic viscosity of the various guar gum samples studied and their ability to promote self-association of pectin, with

Table 2
Intrinsic viscosity, $[\eta]$, and relative effectiveness (Fig. 9) in promoting self-association of pectin, for gum arabic and the polymeric cosolutes studied by Giannouli et al. (2004a,b)

Material	$[\eta]$ (dl/g)	Effectiveness
Locust bean gum	15.8	Medium
Guar gum M150	14.3	Medium
Guar gum M90	9.55	Medium
Guar gum M60	6.11	Medium
Guar gum M30	3.85	Medium
Guar gum M7	1.30	High
Dextran H	0.89	Low
Dextran M	0.59	Low
Oxidised starch	0.35	Medium
Dextran L	0.32	Low
Gum arabic in 0.1 M salt	0.25	High
Potato maltodextrin	0.20	Medium
Inulin	0.066	Very low

the sample of lowest molecular weight (guar gum M7; $[\eta] = 1.30$ dl/g) having a particular dramatic effect on calcium pectinate rheology. The present investigation shows that gum arabic, which has even lower hydrodynamic volume ($[\eta] = 0.25$ dl/g in 100 mM Na^+), causes equally dramatic changes in rheology. There does not, however, appear to be any simple or direct correlation between hydrodynamic volume and effectiveness in promoting Ca^{2+}-mediated association of low methoxy pectin.

In Table 2, the polymeric cosolutes used in the investigations reported here and in the two preceding papers (Giannouli et al., 2004a,b) are listed in descending order of intrinsic viscosity, and are classified as having ‘high’, ‘medium’, ‘low’ or ‘very low’ effectiveness in driving self-association of pectin, according to the groupings shown in Fig. 9. It is evident that their relative effectiveness does not depend solely on molecular size, and that other features of the different polymeric cosolutes must therefore be considered.

One possibility is chain stiffness. As shown in Fig. 9, the materials that are least effective in promoting self-association of pectin are the dextran and inulin samples studied by Giannouli et al. (2004b), which, as discussed in Section 3.2, have three bonds in their predominant glycosidic linkages, and are therefore much more flexible than most other polysaccharides. One way in which mixtures of pectin with polymeric cosolutes may respond to segregative interactions between chain segments of the two polymers is by pectin forming compact, ordered structures which occupy less of the total volume. Another way in which enthalpically unfavourable segmental interactions could be relieved, however, is by segments of the polymeric cosolute chains moving away from the pectin molecules, and forming more favourable enthalpic contacts with other segments of the same chain or with segments of neighbouring chains of the same type. The ability of chain segments to respond in this way (i.e. by ‘retreating’, rather than by forcing the pectin to retreat) would be expected to decrease as their freedom of movement decreases, which could explain why stiffer molecules, such as the galactomannans, are more effective than dextrans or inulin in driving self-association of pectin. Relief of enthalpically unfavourable interactions by the converse process of pectin segments moving away from the polymeric cosolute would similarly be inhibited by conformational constraints, since, as discussed in Section 3.2, pectin (like alginate) has very restricted chain flexibility.

The inverse correlation between molecular size and effectiveness in promoting self-association of pectin, which was displayed most clearly by galactomannans with widely different intrinsic viscosities (Giannouli et al., 2004a), could also be explained, at least qualitatively, by consideration of segmental interactions. For disordered polymeric cosolutes of high molecular weight, a substantial proportion of the constituent segments will be ‘buried’ within the interior of the polymer coil, and will not, therefore, be exposed to contacts with pectin. As the molecular weight is decreased (i.e. as the individual coils became smaller), there will be a progressive increase in surface-to-volume ratio, with a progressively higher proportion of the chain segments making enthalpically unfavourable contacts with pectin and hence promoting formation of calcium pectinate junctions.

It is obvious, however, that the inverse relationship between molecular size and segregative interactions with pectin cannot continue indefinitely, since incorporation of monosaccharides or small oligomers at concentrations equivalent to those used for the polymeric cosolutes does not have any significant effect on Ca^{2+}-mediated gelation of low methoxy pectin (Christensen, 1986; May, 1990). Thus, at some stage the trend to increasing effectiveness with decreasing molecular size must be reversed, which may explain why potato maltodextrin ($[\eta] = 0.20$ dl/g) is less effective (Fig. 9) in promoting self-association of pectin than oxidised starch ($[\eta] = 0.35$ dl/g), despite both materials consisting predominantly of partially depolymerised amylopectin. It may also explain why inulin (average degree of polymerisation ≈ 28) is much less effective (Fig. 9) than dextrans of comparable flexibility but much higher molecular weight (Giannouli et al., 2004b).

Another factor that may be relevant to the greater effectiveness of dextran in comparison with inulin is that the ability of polymeric cosolute molecules to respond to segregative interactions with pectin by conformational rearrangement (rather than by forcing self-association of pectin into a more compact structure) will be restricted by branching, which occurs extensively in dextran (about 1 branch point per 20 residues) but is essentially absent in inulin. The same consideration is likely to be much more significant for gum arabic. As described in Section 1, gum arabic consists of two main fractions, which occur in the approximate ratio 6:1 and have M_r values of ~ 280 and ~ 1450 kDa, respectively. The weighted average of these individual values gives an overall mean molecular weight of ~450 kDa. One of the dextran samples studied by Giannouli et al. (2004b) has a closely similar molecular weight (dextran M; $M_r = 464$ kDa), but the intrinsic viscosity is much higher ($[\eta] = 0.59$ dl/g for dextran M, in comparison with $[\eta] = 0.25$ dl/g for gum arabic in 100 mM NaCl), indicating that the structure of gum arabic is much denser and more closely packed, and would therefore be expected to show much greater resistance to conformational rearrangement in response to segregative interactions with pectin.

Restriction of segmental mobility by branching as well as by inherent chain stiffness may also explain why self-association of pectin is promoted to roughly the same extent by oxidised starch and galactomannans (Fig. 9) although, as discussed in Section 3.2, the predominant (1 → 4)-linked α-D-glucan sequences of starch are less rigid than the (1 → 4)-linked β-D-mannan backbone of the galactomannans.

The discussion so far has centred on the overall effectiveness of different polymeric cosolutes in promoting formation of calcium pectinate junctions, as assessed (Fig. 9) by reduction of the increase in log G' on cooling. There are, however, indications that the structural features that are most important in driving initial formation of egg-box junctions at high temperature are not necessarily the same as those that promote further excessive aggregation at lower temperatures.

In particular, for the dextran samples studied by Giannouli et al. (2004b), it was found that the moduli of the calcium pectinate 'weak gel' networks formed at high temperature increased as the molecular weight of the dextran decreased, whereas the reductions in final moduli on completion of cooling to 5 °C became more pronounced as the molecular weight of the dextran was raised. As indicated in Fig. 9, however, these two opposing trends virtually balanced one another, with the overall change in log G' on cooling being essentially independent of molecular weight. A similar inverse relationship between high-temperature and low-temperature response was also observed (Giannouli et al., 2004a) for guar gum, although, because of the ability of low concentrations of the sample of lowest molecular weight (guar gum M7) to promote formation of cohesive calcium pectinate networks at high temperature, the maximum concentration of cosolute at which comparisons could be made was 1.0 wt% and the changes in gel moduli at 5 °C were smaller than those observed for dextran, which could be used at higher concentrations. In both cases, however, it appears that initial association of disordered pectin into Ca^{2+}-mediated junctions is promoted preferentially by small polymeric cosolute molecules, indicating that it is driven by surface contacts, but that larger molecules are more effective in promoting further excessive aggregation, with associated reduction in gel moduli, suggesting that space occupancy by the polymeric cosolute becomes more significant when the pectin chains are already associated into rigid, ordered structures.

The way in which the reductions in final moduli arise also seems to depend on the nature of the polymeric cosolute. For most of the cosolutes studied, there was a sigmoidal increase in moduli during cooling, as seen for calcium pectinate alone, but the magnitude of the increase became progressively smaller as the concentration of cosolute was increased, suggesting a smooth progression from a large number of small intermolecular junctions to a smaller number of large, aggregated assemblies. For low concentrations of oxidised starch (Picout et al., 2000b) and higher concentrations of potato maltodextrin (Picout et al., 2000a), however, sharp reductions in moduli during cooling were observed, and attributed (Picout et al., 2000b) to collapse of the developing calcium pectinate network in response to segregative interactions with the starch polysaccharides. As shown in Figs. 6 and 7, similar sharp reductions also occurred with (dialysed) gum arabic as cosolute. The common feature of these materials is that oxidised starch and potato maltodextrin both consist predominantly of partially depolymerised amylopectin, and therefore, like gum arabic, contain densely branched chains which might be expected to be particularly resistant to interpenetration with stiff, extended structures such as the egg-box junctions of calcium pectinate gel networks.

Finally, reduction in gel strength on incorporation of polymeric cosolutes is not unique to calcium pectinate. Similar effects have been observed for other gelling polysaccharides that associate readily into larger aggregates after initial conformational ordering. Examples include mixtures of amylose with dextran (Kalichevsky, Orford, & Ring, 1986) and of agar polysaccharides with gum arabic or alginate of low molecular weight (Smith, Richardson and Morris, unpublished).

5. Conclusions

The main conclusions from the present work, and from the investigations reported in the two preceding papers (Giannouli et al., 2004a,b) can be summarised as follows.

- Enthalpically unfavourable (segregative) interactions between low methoxy pectin (DE 31; 2.0 wt%; stoichiometric Ca^{2+}; pH ≈ 2.9–3.0) and polymeric cosolutes can be relieved in two ways: (i) by Ca^{2+}-mediated self-association of pectin into compact ordered assemblies which occupy less of the total volume, and (ii) by conformational rearrangement of the polymeric cosolute molecules to minimise segmental interactions with pectin.
- Conformational rearrangement is inhibited by chain stiffness and by branching; thus stiff cosolute molecules are more effective in promoting self-association of pectin than flexible molecules of comparable size, and branched

molecules are more effective than linear chains of comparable stiffness.

- Initial association of disordered pectin into egg-box junctions in the pre-gel solution state at high temperature is promoted preferentially by segmental contacts with small polymeric cosolute molecules of high surface-to-volume ratio; larger cosolute molecules are more effective in driving further, excessive association of ordered pectin into aggregated bundles.
- Densely branched (impenetrable) cosolute molecules (gum arabic; partially depolymerised amylopectin) cause an abrupt collapse of calcium pectinate network structure during cooling; linear chains (galactomannans; inulin) or more expanded branched structures (dextrans) promote more gradual aggregation, reflected in a progressive reduction in final gel moduli with increasing concentration of cosolute.

It must be emphasised, however, that these conclusions are highly speculative, and are, at best, an empirical rationalisation of the experimental evidence. The main purpose of the three papers in this series is to draw attention to an aspect of the behaviour of biopolymer mixtures that has received little attention, in the hope of prompting further investigations by other experimental approaches, and theoretical analysis of the interactions that can occur between gelling and non-gelling polymeric materials.

References

Christensen, S. H. (1986). Pectins In M. Glicksman (Ed.), (*Vol. III*) (pp. 205–230). *Food hydrocolloids*, Boca Raton, FL: CRC Press.

Giannouli, P., Richardson, R. K., Morris, E. R (2004a). Effect of polymeric cosolutes on calcium pectinate gelation. Part 1. Galactomannans in comparison with partially depolymerised starches. D.O.I.: 10.1016/j.carbol.2003.09.013, PII: S0144–8617(03) 00309–6.

Giannouli, P., Richardson, R. K., Morris, E. R (2004b). Effect of polymeric cosolutes on calcium pectinate gelation. Part 2. D.O.I.: 10.1016/j.carbol.2003.09.014, PII: S0144–8617(03) 00310–2.

Goycoolea, F. M., Morris, E. R., Richardson, R. K., & Bell, A. E. (1995). Solution rheology of mesquite gum in comparison with gum arabic. *Carbohydrate Polymers*, *27*, 37–45.

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.

Kalichevsky, M. T., Orford, P. D., & Ring, S. G. (1986). The incompatibility of concentrated aqueous solutions of dextran and amylose and its effect on amylose gelation. *Carbohydrate Polymers*, *6*, 145–154.

May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers*, *12*, 79–99.

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, E. R., & Ross-Murphy, S. B. (1981). Chain flexibility of polysaccharides and glycoproteins from viscosity measurements. *Techniques in carbohydrate metabolism, B310* (pp. 201–246). London: Elsevier.

Picout, D. R., Richardson, R. K., & Morris, E. R. (2000a). 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., Rolin, C., Abeysekera, R. M., & Morris, E. R. (2000b). 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., Ross-Murphy, S. B., Errington, N., & Harding, S. E. (2001). Pressure cell assisted solution characterisation of polysaccharides. 1. Guar gum. *Biomacromolecules*, *2*, 1301–1309.

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.

Randall, R. C., Phillips, G. O., & Williams, P. A. (1989). Fractionation and characterisation of gum from *Acacia Senegal*. *Food Hydrocolloids*, *3*, 65–75.

Rees, D. A., & Scott, W. E. (1971). Polysaccharide conformation. Part VI. Computer model building for linear and branched pyranoglycans. Correlation with biological function. Preliminary assessment of inter-residue forces in aqueous solution. Further interpretation of optical rotation in terms of chain conformation. *Journal of the Chemical Society B*, 469–479.

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.

Smidsrød, O., & Haug, A. (1971). Estimation of the relative stiffness of the molecular chain in polyelectrolytes from measurements of viscosity at different ionic strengths. *Biopolymers*, *10*, 1213–1227.

Whistler, R. L. (1993). Exudate gums. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial gums: Polysaccharides and their derivatives* (3rd ed.) (pp. 309–339). San Diego, CA: Academic Press.