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Ca^{2+}-induced gelation of low methoxy pectin in the presence of oxidised starch. Part 1. Collapse of network structure

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Abstract

The effect of oxidised starch on the networks formed by low-methoxy pectin (2.0 wt%), on cooling (from 90 to 5°C) in the presence of Ca^{2+} (10–100% stoichiometric) has been explored by rheological measurements under low-amplitude oscillatory shear, and by light microscopy. At low concentrations of Ca^{2+}, incorporation of increasing concentrations of starch (across the range 0–30 wt%) causes a progressive increase in modulus (G' at 5°C), attributed to segregative interactions between the two polymers promoting conversion of pectin from the expanded coil conformation to a more compact associated form. Incorporation of starch at higher Ca^{2+} concentrations, however, causes large reductions in gel strength, which arise from sharp drops in G' during cooling. The Ca^{2+} concentration at the transition from enhancement to depletion of network strength is independent of starch concentration, and increases with increasing degree of esterification of the pectin component (across the DE range 31.1–55.8%). Reduction in gel strength is attributed to incipient precipitation of pectin into large aggregates within a supporting calcium pectinate network. This interpretation is supported by microscopy, which shows conversion from a homogeneous distribution of the two polymers to a grossly heterogeneous structure (length-scale ~10–50 μm) with increasing concentration of Ca^{2+}, and by quantitative analysis of gel moduli reported in the following paper.

Keywords: Ca^{2+}-induced gelation; Low methoxy pectin; Oxidised starch; Co-gel networks

1. Introduction

The work reported here, and in the three following papers (Picout, Richardson & Morris, 2000a,b,c), formed part of an industrial–academic LINK project "Behaviour of biopolymer mixtures in structuring food products", with public-sector funding from the UK Ministry of Agriculture, Fisheries and Food. The project consortium comprised two research groups in the University of York (Institute for Applied Biology and Department of Chemistry), one in Cranfield University (Food Research & Technology), two of the largest users of industrial biopolymers (Unilever and Nestlé), and three major producers (Cerestar, Hercules and SKW Biosystems). The research was focused on binary mixtures of three different types of biopolymer: partially depolymerised starch (oxidised starch and maltodextrins), pectin (high-methoxy and low-methoxy), and gelatin (type A and type B).

As anticipated from previous studies (e.g. Antonov, Lashko, Glotova, Malovikova & Markovich, 1996; Chilvers & Morris, 1987; Imeson, Ledward & Mitchell, 1977; Muchin, Wajnermann & Tolstogusow, 1976; Stainsby, 1980; Tolstoguzov, 1996; Tolstoguzow & Wajnermann, 1975; Tschumak, Wajnermann & Tolstogusow, 1976), mixtures of gelatin with the anionic polysaccharides (pectin and oxidised starch) under acidic conditions (i.e. where the gelatin component has substantial net positive charge) showed clear evidence of electrostatic association as the dominant mode of interaction between the two polymers (Gilsenan, Richardson & Morris, 2000b; Hember et al., 2000). The central aim of the project, however, was to gain a better understanding of the effect of segregative interactions ("thermodynamic incompatibility") in gelling mixtures.

Segregative effects in mixed systems are more common than associative interactions, and arise from the enthalpic advantage of individual molecules being surrounded by others of the same type. For small molecules, the enthalpic

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Table 1
Composition of pectin samples

Hercules batch number	4907	5006	4943	4942	4941
Pectin content of sample (%)	96.2	98.2	98.4	98.7	98.2
Galacturonate content of pectin (%)	85.2	82.1	82.7	86.4	89.0
Degree of esterification (%)	31.1	34.2	38.9	47.2	55.8
Effective formula weights:					
per galacturonate residue	220.0	224.2	223.0	214.1	210.3
per carboxyl group	319.4	340.8	364.9	405.6	475.8
Carboxyl concentration in 2.0 wt% solution (mM)	62.6	58.7	54.8	49.3	42.0
Stoichiometric equivalence of Ca^{2+} (mM)	31.3	29.3	27.4	24.7	21.0

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, and mixed systems often resolve spontaneously into two separate phases, each enriched in one polymer and depleted in the other (see, for example, Grinberg & Tolstoguzov, 1972; Grinberg & Tolstoguzov, 1997; Morawetz, 1965; Morris, 1990; Morris, 1986; Piculell, Nilsson, Falck & Tjerneld, 1991; Piculell et al., 1994; Piculell, Bergfeldt & Nilsson, 1995; Suchkov, Grinberg & Tolstoguzov, 1981; Tolstoguzov, 1986; Tolstoguzov, 1988; Tolstoguzov, 1991; Zasypkin, Braudo & Tolstoguzov, 1997). Phase separation in the solution state can usually be detected by immediate development of turbidity on mixing, due to formation of a "water-in-water" emulsion in which one phase exists as a continuous matrix with the other dispersed through it as small liquid droplets. The composition of the co-existing phases obtained from different starting compositions can be defined by a "binodal" or "cloud-point curve" which represents the boundary between monophasic and biphasic states of the system (where the enthalpic advantage of segregation and entropic advantage of mixing are in exact balance).

After initial phase separation, mixtures of non-gelling polymers normally show gradual resolution into two clear layers (in response to differences in density between the phases), but for gelling biopolymers the "water-in-water emulsion" structure can be trapped by network formation, giving a biphasic co-gel with one phase continuous and the other dispersed. Considerable progress has been made in relating the overall physical properties of biopolymer co-gels to those of the constituent polymers (Abdulmola, Hember, Richardson & Morris, 1996; Chronakis, Kaspis & Richardson, 1996; Clark, Richardson, Robinson, Ross-Murphy & Weaver, 1982; Clark, Richardson, Ross-Murphy & Stubbs, 1983; Ipsen, 1995; Kasapis, Morris, Norton & Clark, 1993a; Matser & Steeneken, 1997; McEvoy, Ross-Murphy & Clark, 1985; Mohammed, Hember, Richardson & Morris, 1998; Morris, 1992) by application of blending laws developed initially for composites of synthetic polymers in the condensed state (Takayanagi, Harima & Iwata, 1963).

The effect of segregative interactions can, however, also be seen at polymer concentrations below the binodal, where the mixture remains single phase. In systems where one component can undergo a transition from an expanded coil conformation to a more compact ordered form, the presence of low concentrations of a second polymer within the same phase may cause a large increase in the rate and/or extent of conformational ordering (Tolstoguzov, Belkina, Gulov, Grinberg, Titova & Belavzeva, 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, 1993b), with the fraction precipitated increasing in direct proportion to the protein concentration. In mixtures of maltodextrin with whey proteins the converse effect was observed (Manoj, Kaspis, Hember, Richardson & Morris, 2000), with precipitation of protein in direct proportion to maltodextrin concentration.

In the present work, we show evidence of an analogous precipitation process during formation of calcium pectinate gels in the presence of oxidised starch, leading to progressive collapse of the calcium pectinate network structure with increasing starch concentration. This qualitative interpretation is supported by quantitative analysis of gel moduli, presented in the next paper (Picout et al., 2000a). The two following papers (Picout et al., 2000b,c) report a related investigation and analysis of calcium pectinate gelation in the presence of potato maltodextrin, with essentially the same qualitative and quantitative conclusions.

2. Materials and methods

The oxidised starch used was C*Set 06598 (batch SH 1338) from Cerestar. This material is prepared by treatment of waxy maize starch with sodium hypochlorite under alkaline conditions, and contains approximately one carboxyl group per 35 glucose residues. It was developed primarily as an extender for gum arabic in confectionery gums and pastilles, and can be dissolved at concentrations up to ~50 wt%. Most of the experiments were carried out using citrus pectin with a degree of esterification (DE) of 34.2%, which is towards the lower end of the commercial range (Christensen, 1986; Rolin, 1993). Comparative studies were also made using samples with different degrees of esterification, which were prepared from a single batch of pectin (from lemon peel) by progressive de-esterification with pectin esterase (from *Aspergillus niger*). The pectin samples were analysed using standard procedures specified in detail elsewhere (Anon., 1996). Their composition (Table 1) is characterised by: (i) the pectin content of each sample,

expressed as a percentage of the total weight; (ii) the proportion of galacturonate residues (esterified and un-esterified), expressed as a percentage of the pectin content; and (iii) the percentage of galacturonate residues that are methyl esterified (i.e. the DE). These values then give the effective formula weight per carboxyl group, and hence the concentration of Ca^{2+} required for stoichiometric equivalence. The calcium source used for gel formation was calcium chloride dihydrate (AnalaR grade from BDH; formula weight 147).

Mixed solutions with oxidised starch were prepared by first dispersing the starch in cold water and stirring in a water bath at 90°C until a clear solution was obtained. The calcium salt was then dissolved in the starch solution and the pectin was slowly added, with continuous stirring until fully dissolved. Oxidised starch was used at concentrations across the range 0–35 wt%. Pectin concentration was held fixed at 2.0 wt% throughout. No adjustment was made to the natural pH of the pectin samples at this concentration (pH ≈ 2.9–3.0). Distilled deionised water was used throughout.

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 in rad s^{-1}) were made using highly truncated cone-and-plate geometry (50 mm diameter; 0.05 rad cone angle; 0.5 mm gap) on a sensitive prototype rheometer designed and constructed by one of us (R. K. R.). Temperature was controlled by a Haake circulating water bath and measured with 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, with measurement of G' and G'' at 10 rad s^{-1} and 0.5% strain, and held for a further 15 min; final values were recorded at the end of the holding period (i.e. after 100 min from the start of the cooling scan). A mechanical spectrum (frequency-dependence of G', G'' and η^*) was then recorded (at 0.5% strain). Some illustrative mechanical spectra were also measured at 80°C.

Selected samples were stained with iodine vapour and examined directly by light microscopy, using bright-field optics on a Nikon inverted microscope.

3. Results

3.1. Gelation of pectin in the absence of starch

In the main series of experiments, the pectin used was the sample of DE 34.2, at a fixed concentration of 2.0 wt%, and the Ca^{2+} concentration was varied between 10 and 100% of stoichiometric equivalence to the carboxyl groups of the polymer. Fig. 1 shows the changes in G' and G'' observed during cooling (1°C/min) for pectin in the absence of starch, with Ca^{2+} concentrations at the two extremes of the experimental range. In both cases there is an obvious sigmoidal transition between ~60 and ~30°C.

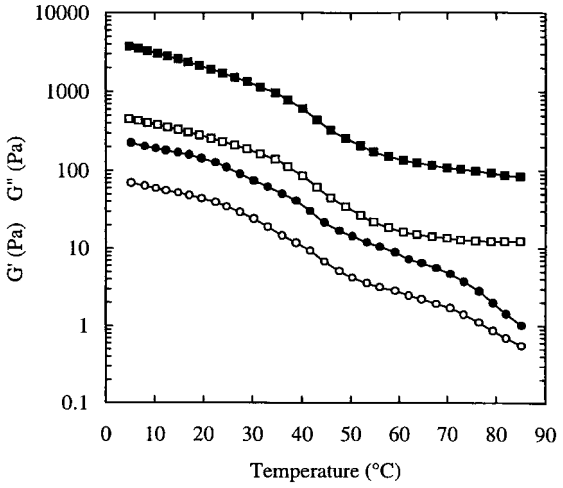

Fig. 1. Changes in G' (filled symbols) and G'' (open symbols), measured at 10 rad s^{-1} and 0.5% strain, for 2.0 wt% pectin (DE 34.2) on cooling (1°C/min) in the presence of Ca^{2+} at 10% (circles) and 100% (squares) of stoichiometric equivalence to the carboxyl groups of the polymer.

The mechanical spectra recorded at 5°C (100 min after the start of the cooling scan) are shown in Fig. 2. The spectrum obtained for the sample with stoichiometric Ca^{2+} (Fig. 2a) is typical of a strong gel (see, for example, Morris, 1984; Ross-Murphy, 1984), with $G' \gg G''$, little frequency-dependence in either modulus, and a linear decrease in log η^* with increasing log ω. The spectrum for the sample of lower Ca^{2+} content (10% stoichiometric) also has gel-like character (Fig. 2b), but with greater frequency-dependence of both moduli and smaller separation of G' and G''.

Fig. 3 shows mechanical spectra obtained for replicate loadings of the same preparations after cooling to 80°C. Although both samples remained fluid at temperatures above the gelling transition shown in Fig. 1, they show predominantly solid-like response ($G' > G''$) to low-amplitude deformation, and can therefore be classified as "weak gels" (Morris, Gothard, Hember, Manning & Robinson, 1996; Ross-Murphy, 1984).

It should be noted that the moduli at 10 rad s^{-1} in the spectrum for the sample with 10% stoichiometric Ca^{2+} (Fig. 3b) are substantially (~3×) higher than the corresponding (80°C) values in the cooling traces shown in Fig. 1. This is a kinetic effect, which also gives rise to the initial sharp increase in G' and G'' in the early stages of cooling. Before the mechanical spectra were recorded, the samples were held at 80°C until stable readings were obtained. At high Ca^{2+} concentration, there was virtually no time-dependent change. For samples of low Ca^{2+} content, however, there was a progressive increase over a period of ~10–15 min (from ~2 to ~6 Pa for G' and from ~0.8 to ~2 Pa for G'' in the preparation with 10% stoichiometric Ca^{2+}). The changes in moduli below the range of these initial time-dependent effects (i.e. below ~70°C) are fully

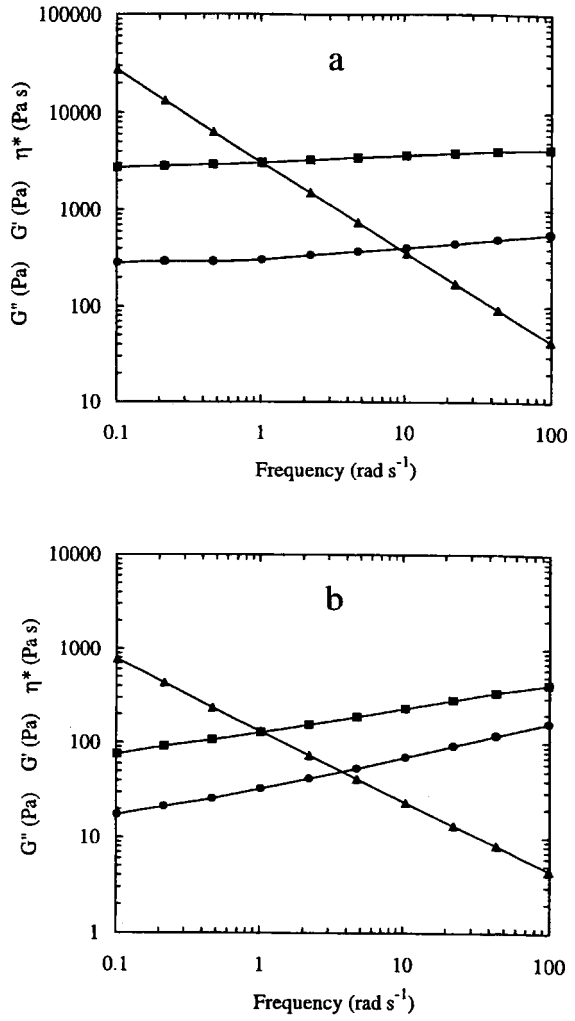

Fig. 2. Mechanical spectra (0.5% strain), showing the frequency-dependence of G' (■), G'' (●) and η^* (▲) for 2.0 wt% pectin (DE 34.2) after cooling from 90 to 5°C at 1°C/min and holding for 15 min, in the presence of Ca^{2+} at (a) 100% and (b) 10% of stoichiometric equivalence to the carboxyl groups of the polymer.

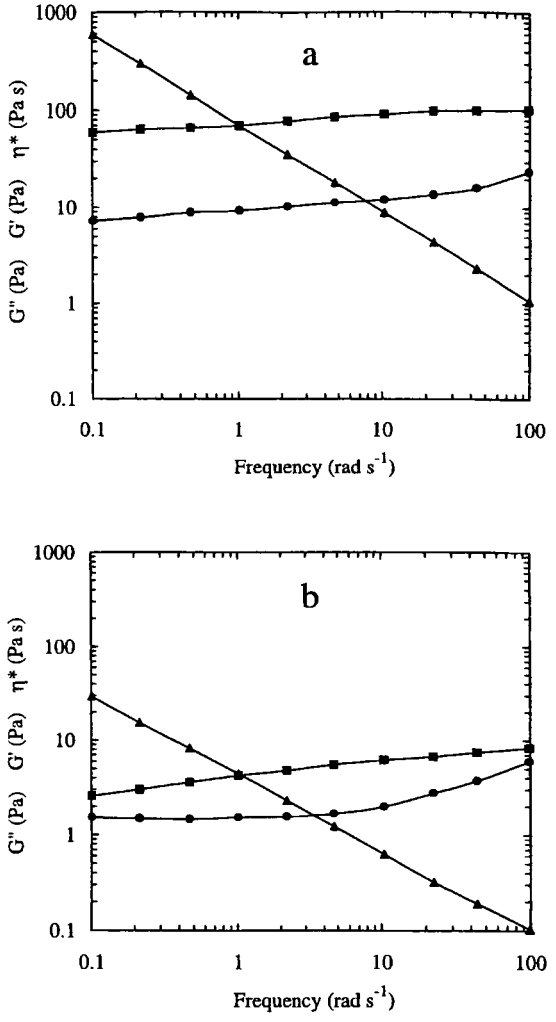

Fig. 3. Mechanical spectra (0.5% strain), showing the frequency-dependence of G' (■), G'' (●) and η^* (▲) for 2.0 wt% pectin (DE 34.2) in the presence of Ca^{2+} at (a) 100% and (b) 10% stoichiometric equivalence, after holding at 80°C until stable moduli were attained.

reversible, with ~10°C hysteresis between cooling and heating (Gilsenan, Richardson & Morris, 2000c).

Detailed discussion of the mechanism of calcium pectinate gelation, including the development of "weak gel" properties at high temperature, is outside the scope of the present paper. In the outline, however, we suggest, tentatively, 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 (Fig. 2) 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). At the pH value used in the present work (~2.9–3.0), however, there may also be some crosslinking through acid-induced association of three-fold helices (Gilsenan, Richardson & Morris, 2000a), particularly in preparations with a low content of Ca^{2+}.

For the purposes of the present study, however, the most important consideration is the effect of ionic environment of the mechanical properties of the networks formed on cooling. As shown in Fig. 4, increasing concentration of Ca^{2+} causes an initial sharp rise in the final values of G' at 5°C, with a more gradual increase at higher concentrations.

3.2. Gelation with oxidised starch

Fig. 5 shows the results of the main investigation, in which pectin concentration was held constant (2.0 wt%; DE 34.2), Ca^{2+} concentration was also held fixed (at 10, 20, 22.5, 25, 30, 50 or 100% stoichiometric), and the concentration of starch was varied across the range 0–30 wt%. It is immediately evident that progressive incorporation of oxidised starch causes massive changes

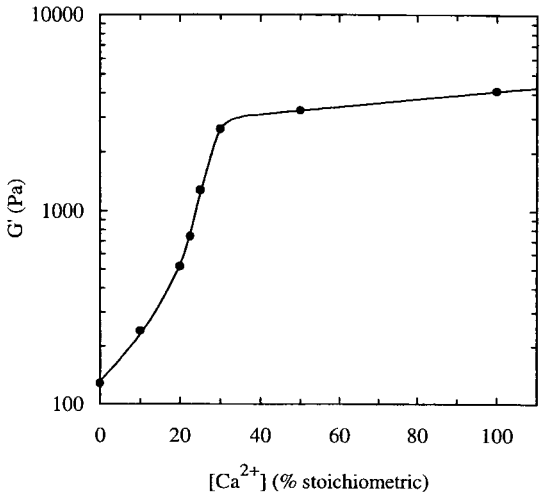

Fig. 4. Variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with Ca^{2+} concentration for 2.0 wt% pectin (DE 34.2).

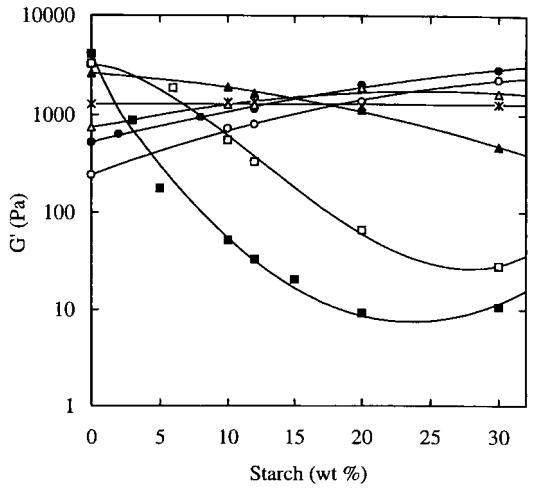

Fig. 5. Variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with concentration of oxidised starch in mixtures with pectin (2.0 wt%; DE 34.2) in the presence of Ca^{2+} at concentrations (% stoichiometric) of 10 (○), 20 (●), 22.5 (△), 25 (∗), 30 (▲), 50 (□) and 100 (■).

in gel moduli (G' at 5°C), and that the direction of change (i.e. to higher or lower values of G') depends on Ca^{2+} concentration. For clarity, the same data are shown in Fig. 6, scaled to the corresponding values of G' for 2.0 wt% pectin alone at the same concentrations of Ca^{2+} (Fig. 4). It is now apparent that there is a systematic progression from an increase in modulus with increasing starch concentrations in mixtures with a low content of Ca^{2+} to a (much larger) reduction at high Ca^{2+} concentrations.

As shown in Fig. 7, the transition from reinforcement to depletion of network strength occurs at essentially the same concentration of Ca^{2+} (~25% stoichiometric) for different concentrations of starch. The same effect can be seen directly in Figs. 5 and 6, with the moduli of calcium pectinate gels prepared at 25% stoichiometric Ca^{2+} remaining independent of starch concentration across the full range studied (0–30 wt%).

Figs. 8–10 illustrate the changes in G' observed during cooling for Ca^{2+} concentrations at the extremes of the range studied (10 and 100% stoichiometric) and at 25% stoichiometric (the "crossover" concentration from Fig. 7). At the lowest concentration of Ca^{2+} (Fig. 8), progressive incorporation of oxidised starch causes a monotonic increase in G' at all temperatures (i.e. reinforcing both the "weak gel" and "true gel" structures). At 25% stoichiometric Ca^{2+} (Fig. 9), the insensitivity of G' at 5°C to the presence of oxidised starch is also evident at higher temperatures, with mixtures prepared at starch concentrations across the full range studied giving closely similar cooling traces.

The most striking effects, however, are seen at higher Ca^{2+} concentration (100% stoichiometric; Fig. 10). At starch concentrations up to ~12 wt%, the initial moduli at high temperature remain virtually constant, and the cooling curves also begin by following the increase in G' seen for pectin alone at the same concentration of Ca^{2+}. There is then, however, a sharp drop in modulus, which increases in magnitude and moves to progressively higher temperature as the concentration of starch is raised. The obvious interpretation is that, as proposed in a preliminary study by Abdulmola, Richardson and Morris (2000), the calcium pectinate network begins to develop in the normal way, and then collapses in response to segregative interactions with oxidised starch. Ultimately, at starch concentrations of ~15 wt% and above, the collapse appears to start above the loading temperature of 90°C, with a systematic reduction in measured values of G' for the initial "weak gel" state.

Fig. 10 includes the cooling trace for a sample incorporating 35 wt% oxidised starch (i.e. above the range shown in Figs. 5 and 6). After the initial sharp decrease, the moduli remain close to those observed at lower concentration of starch (20 wt%) across the temperature range ~70 to ~20°C, but then show a sharp up-turn on further cooling

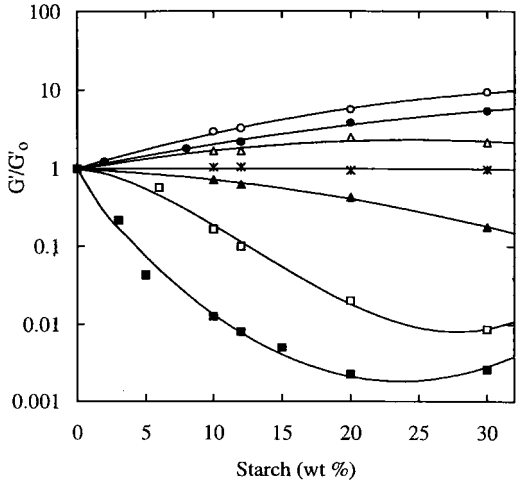

Fig. 6. Moduli from Fig. 5 divided by the corresponding values (G'_o) for 2.0 wt% pectin alone at the same Ca^{2+} concentrations (Fig. 4); symbols and conditions as in Fig. 5.

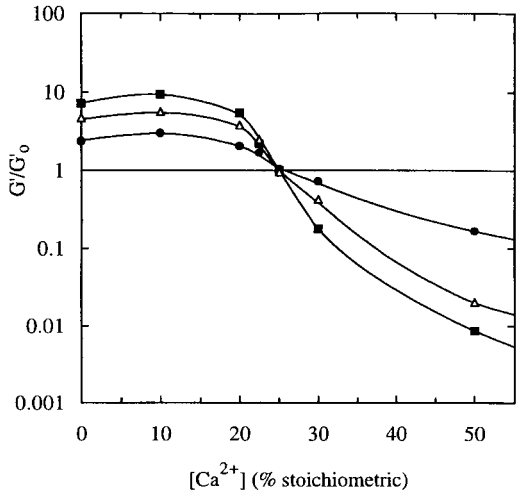

Fig. 7. Observed moduli (G'; 10 rad s^{-1}; 0.5% strain; 5°C) for 2.0 wt% pectin (DE 34.2) in combination with oxidised starch at concentrations (wt%) of 10 (●), 20 (△) and 30 (■), divided by the corresponding moduli for pectin alone at the same concentrations of Ca^{2+}.

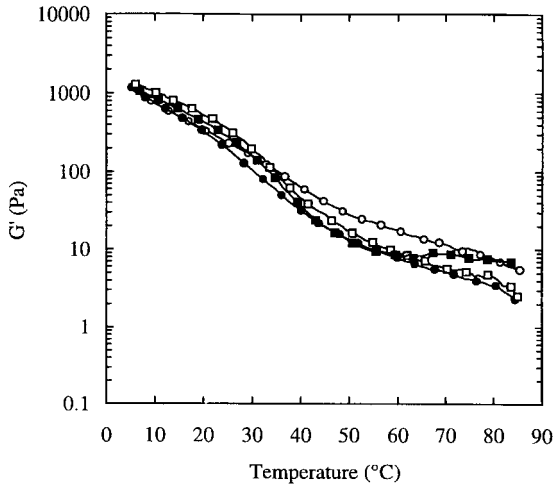

Fig. 9. Changes in G' (10 rad s^{-1}; 0.5% strain) for 2.0 wt% pectin (DE 34.2) with 25% stoichiometric Ca^{2+} during cooling (1°C/min) in the presence of oxidised starch at concentrations (wt%) of 0 (○), 10 (●), 20 (□) and 30 (■).

to 5°C. As discussed in greater detail in the following paper (Picout et al., 2000a), development of additional structure at low temperature comes from the onset of gelation of oxidised starch.

To test the proposal (Abdulmola et al., 2000) that the large reductions in modulus seen at high concentrations of Ca^{2+} are due to collapse of the calcium pectinate network, selected samples were visualised by light microscopy. Fig. 11 shows the micrographs obtained for mixtures of 2.0 wt% pectin (DE 34.2) and 20 wt% oxidised starch after cooling from 90 to 5°C in the presence of Ca^{2+} at concentrations of 20 and 50% stoichiometric (i.e. below and above the "cross-over" from reinforcement to weakening of the calcium pectinate network by incorporation of oxidised starch). At the lower Ca^{2+} concentration (Fig. 11a), both components are homogeneously dispersed at the resolution of light microscopy. At the higher concentration, however, the network is grossly heterogeneous (Fig. 11b), with regions of dense iodine staining (i.e. starch-rich) interspersed with other regions (white areas in the micrograph) which are essentially devoid of starch (i.e. pectin-rich), on a length-scale of ~10–50 μm. It seems evident, therefore, that at high concentrations of Ca^{2+}, the presence of oxidised starch causes at least partial segregation of the calcium pectinate network into pectin-rich and pectin-depleted regions.

3.3. Effect of DE

The purpose of the remaining experiments was to determine the effect of degree of esterification on the concentration of Ca^{2+} needed to go from strengthening to weakening of the

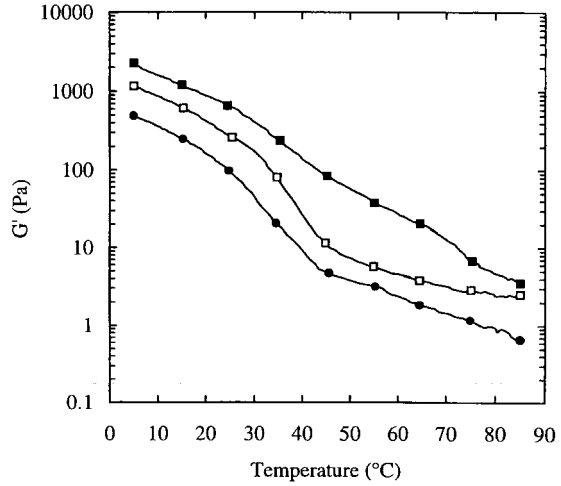

Fig. 8. Changes in G' (10 rad s^{-1}; 0.5% strain) for 2.0 wt% pectin (DE 34.2) with 10% stoichiometric Ca^{2+} during cooling (1°C/min) in the presence of oxidised starch at concentrations (wt%) of 10 (●), 20 (□) and 30 (■).

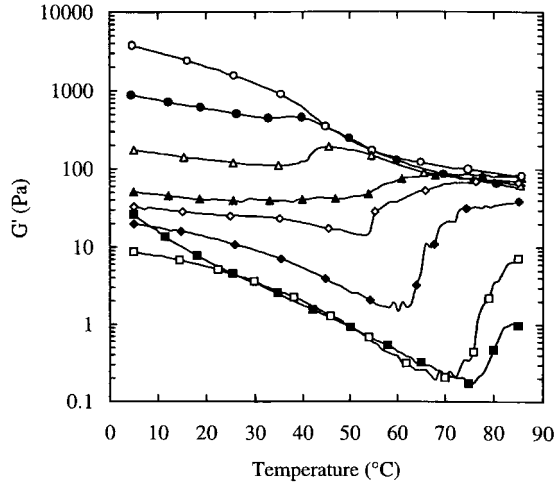

Fig. 10. Changes in G' (10 rad s^{-1}; 0.5% strain) for 2.0 wt% pectin (DE 34.2) with 100% stoichiometric Ca^{2+} during cooling (1°C/min) in the presence of oxidised starch at concentrations (wt%) of 0 (○), 3 (●), 5 (△), 10 (▲), 12 (◇), 15 (◆), 20 (□) and 35 (■).

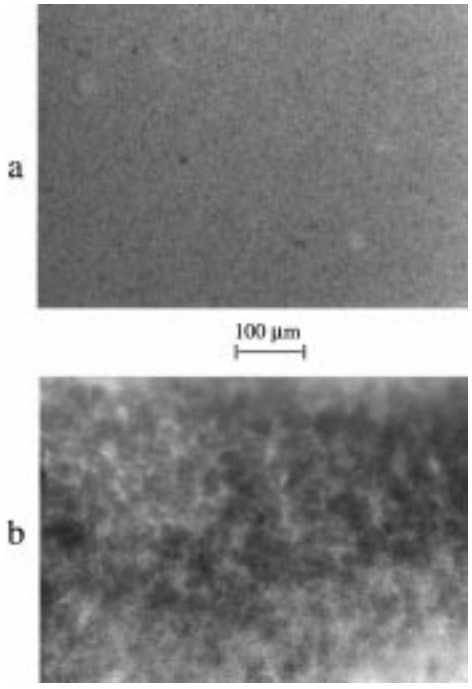

Fig. 11. Micrographs for mixtures of 2.0 wt% pectin (DE 34.2) and 20 wt% oxidised starch, cooled to 5°C at ~1°C/min in the presence of (a) 20% and (b) 50% stoichiometric Ca^{2+}; dark areas show the distribution of starch (stained with iodine vapour); white areas are starch-depleted (i.e. pectin-rich).

calcium pectinate network by incorporation of oxidised starch. As shown in Fig. 7, the transition from reinforcement to depletion occurs at the same Ca^{2+} concentration for different concentrations of starch. Comparison of different pectins (Table 1) was therefore made at a single concentration of oxidised starch. The value chosen was 12 wt%, which, as shown in Fig. 10, was the highest concentration at which collapse of network structure in the presence of stoichiometric Ca^{2+} (for the sample of DE 34.2) was confined entirely to temperatures below the loading temperature of 90°C used in the rheological studies.

Fig. 12a shows the effect of Ca^{2+} on the final modulus (G' at 5°C) at this concentration for mixtures with pectin of DE 34.2 (as in the studies described so far), in comparison with the Ca^{2+}-dependence of G' for the pectin sample alone. For ease of comparison with the behaviour of the other pectins, Ca^{2+} concentration is now expressed as molarity, rather than as % stoichiometric. Fig. 13 shows corresponding plots for the samples of DE 31.1, 38.9, 47.2 and 55.8 (Table 1). In all cases, the mixtures with 12 wt% oxidised starch give higher moduli than pectin alone at low concentrations of Ca^{2+}, and then drop to lower values as the Ca^{2+} concentration is increased. The effect of DE on the transition from enhancement to depletion can be seen directly in Fig. 14, which shows the moduli of the mixed systems divided by those of pectin alone at the same concentrations of Ca^{2+}. The corresponding plot for the sample of DE 34.2 is shown in Fig. 12b.

As shown in Fig. 15, the Ca^{2+} concentration at the "cross-

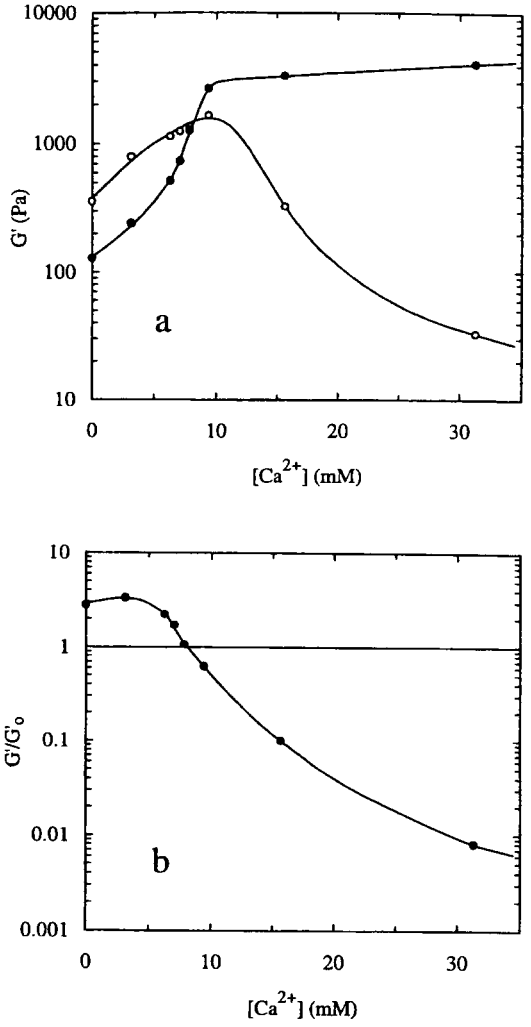

Fig. 12. (a) Variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with Ca^{2+} concentration for 2.0 wt% pectin (DE 34.2) in the presence (○) and absence (●) of 12.0 wt% oxidised starch. (b) Moduli for the pectin–starch mixtures in (a), divided by the corresponding values (G'_o) for pectin alone at the same concentrations of Ca^{2+}.

over" point (i.e. where the mixed systems have the same modulus as pectin alone) increases linearly as the DE is raised (Fig. 15a), with an accompanying linear decrease (Fig. 15b) in the common value of G'.

4. Discussion

The increase in modulus with increasing concentration of oxidised starch in mixtures with pectin at low concentrations of Ca^{2+} (Figs. 5 and 6) can be readily explained by segregative interactions between the two polymers promoting conversion of pectin from the expanded coil conformation to a more compact associated form. The decrease in gel strength at higher Ca^{2+} concentrations, however, is less straightforward.

One obvious interpretation of the sharp reductions in

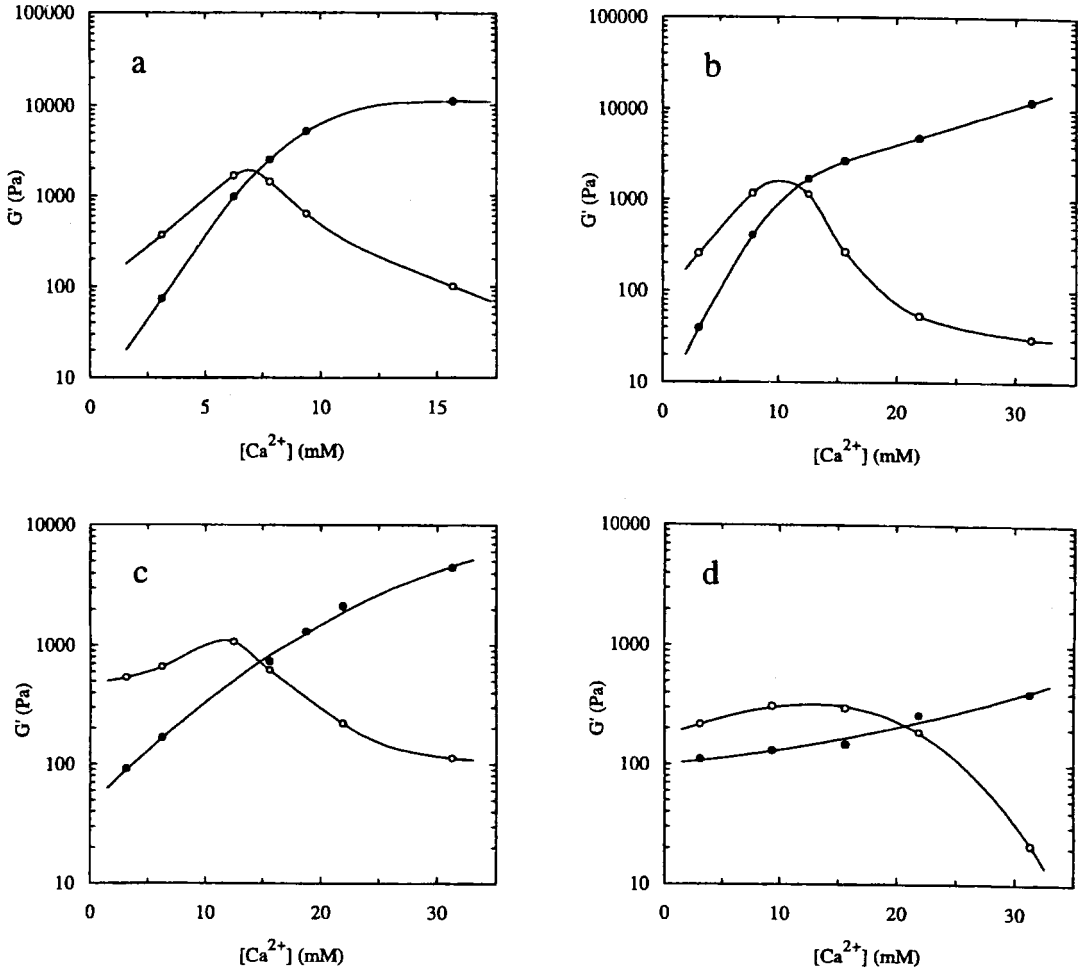

Fig. 13. Variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with Ca^{2+} concentration for 2.0 wt% pectin of DE (a) 31.1, (b) 38.9, (c) 47.2 and (d) 55.8, in the presence (○) and absence (●) of 12.0 wt% oxidised starch.

modulus seen during cooling at high concentrations of Ca^{2+} (Fig. 10), and of the heterogeneous structure observed by microscopy (Fig. 11b), is that the system undergoes phase separation, with the starch-rich phase forming a continuous matrix and the pectin component confined largely to dispersed particles. The observed moduli could then be attributed to gelation of the minor pectin component in the starch-rich continuous phase, and the progressive reduction in G' to increasingly complete segregation as the starch concentration is raised. Indeed, an interpretation along these general lines has been proposed (Kalichevsky, Orford & Ring, 1986) for mixtures of agarose and dextran, where similar effects were observed.

The effect of Ca^{2+} concentration on the behaviour of mixtures of pectin with oxidised starch, however, is difficult to explain in this way. Qualitatively, addition of salt (calcium chloride) would be expected to promote phase separation (Piculell et al., 1991, 1994): preservation of electrical neutrality requires segregation of sufficient counterions to balance the charge on the polymer chains, thus introducing a substantial entropic barrier to separation;

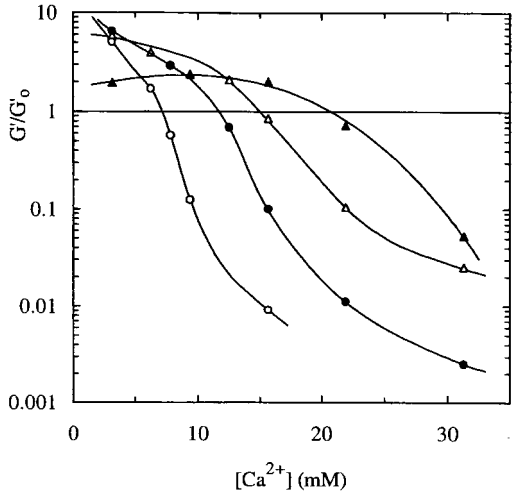

Fig. 14. Ca^{2+}-dependence of G' values from Fig. 13 for mixtures of 12.0 wt% oxidised starch with 2.0 wt% pectin of DE 31.1 (○), 38.9 (●), 47.2 (△) and 55.8 (▲), divided by the corresponding moduli (G'_o) for the same pectin preparations in the absence of starch.

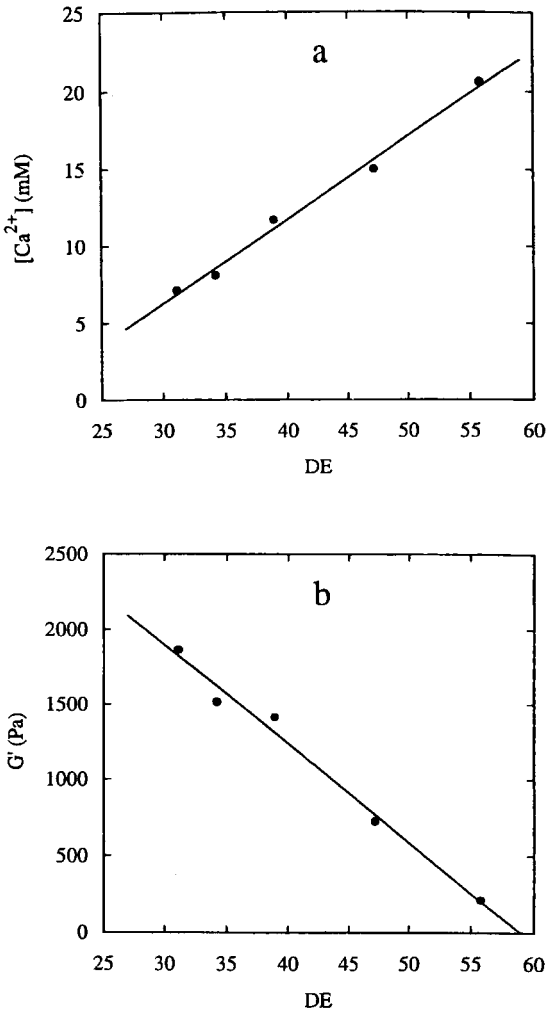

Fig. 15. Effect of DE on (a) the concentration of Ca^{2+} at which oxidised starch does not change G' (5°C) of pectin, and (b) the common value of G' at this Ca^{2+} concentration.

addition of extraneous salt can allow segregation to occur, by reducing the imbalance of ionic concentrations between the two phases. However, since the charge density (and hence the counterion requirement) of pectin decreases with increasing DE, it might be reasonable to expect that the salt concentration needed to trigger phase separation would decrease as the DE is raised, whereas in practice we have observed the opposite effect (Fig. 14), with loss of network strength coming at progressively higher Ca^{2+} concentrations with increasing DE.

An alternative possibility, however, is that the collapse of network structure (Fig. 10) is, in effect, a precipitation process, analogous to those seen for other systems (Kasapis et al., 1993b; Manoj et al., 2000), but that, because of the "weak-gel" structure already present at high temperature (Fig. 3), the result is formation of dense bundles of pectin chains supported within a continuous network, rather than macroscopic precipitation. As discussed previously, Ca^{2+}-induced gelation of pectin under acidic conditions is complex. It seems reasonable to suggest, however, that ester substituents will hinder association of pectin chains into large Ca^{2+}-mediated assemblies, by (i) introducing an entropic barrier to close-packing (loss of rotational freedom of the substituent groups) and (ii) reducing the electrostatic drive to interchain chelation of calcium ions. Thus the observed displacement of network collapse to progressively higher concentrations of Ca^{2+} with increasing DE seems more consistent with a precipitation mechanism than with classic phase separation, although both would, of course, arise from segregative interactions (thermodynamic incompatibility) between pectin and oxidised starch.

In the following paper (Picout et al., 2000a), the concept of precipitation of pectin chains within a supporting calcium pectinate network is explored further, by quantitative analysis of the effect of starch concentration on the moduli of the gels formed in the presence of stoichiometric Ca^{2+}, where, as shown in Figs. 5 and 6, the reductions in strength are greatest.

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