Carbohydrate Polymers 75 (2009) 269–281

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Gelation of high methoxy pectin by acidification with D-glucono-δ-lactone (GDL) at room temperature

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ARTICLE INFO

Article history:
Received 15 March 2007
Received in revised form 30 April 2008
Accepted 9 July 2008
Available online 18 July 2008

Keywords:
Pectin
GDL
Rheology
Gelation mechanism
Hydrophobic interactions

ABSTRACT

Rheological comparisons have been made between preparations of high methoxy pectin (DE ≈ 70%) gelled by acidification with D-glucono-δ-lactone (GDL) on holding for 16 h at 25 °C in the presence of 60 wt% sucrose, and otherwise identical preparations gelled by acidification with citric acid at high temperature and cooling from 90 to 25 °C at 1 °C/min. Two series of experiments were carried out for both methods of acidification. In the first series, the concentration of pectin (c) was held constant at 1.0 wt% and the final pH attained after holding (with GDL) or cooling (with citric acid) was varied from 3.75 to 2.25. In the second series, the final pH was held constant at 3.0 and c was varied from 0.25 to 2.00 wt%. All samples were then heated (1 °C/min) from 25 to 90 °C. Rheological changes on cooling/holding and heating were characterised by low-amplitude oscillatory measurements of storage modulus (G') and loss modulus (G'') at 1 rad s^{-1} and 0.5% strain, and mechanical spectra were recorded at 25 °C. Selected samples, gelled with GDL, were also characterised by compression testing (at 25 °C), and a direct linear relationship was found between the logarithm of yield stress and log G'.

The concentration-dependence of moduli for the samples acidified to pH 3.0 with GDL had the form typical of biopolymer gels, with log G' versus log c approaching a limiting slope of 2 as c was raised above the minimum critical gelling concentration ($c_o \approx 0.3$ wt%). Under all conditions of pH and pectin concentration studied, the values of G'' (at 25 °C) for the samples acidified with citric acid were higher than those of the corresponding GDL-induced networks. The values of G' were also higher, except at very low pH (below ~2.7 at $c = 1.0$ wt%) or very high concentrations of pectin. At pectin concentrations above ~1.5 wt%, the moduli of the samples gelled with citric acid (at pH 3.0) levelled out, or decreased slightly, with the values of G' dropping below those of the GDL-induced networks towards the end of the concentration range studied (at $c \approx 2$ wt%). All samples acidified with citric acid showed gel-like response ($G' > G''$) at 90 °C, attributed to hydrophobic association. The downturn in moduli at 25 °C for high concentrations of pectin is attributed to formation and disruption of strong networks during mixing with citric acid at high temperature ("pregelation"). It is suggested, however, that "weak gels" formed at lower concentrations or at pH values above ~2.7 may enhance gel properties by preserving a continuous network as hydrophobic junctions dissociate on cooling and are replaced by hydrogen-bonded junctions, in contrast to random percolation during gelation with GDL at 25 °C. On re-heating from 25 to 90 °C, the reverse processes (dissociation of hydrogen-bonded structures and formation of hydrophobic associations) were evident in an initial reduction and subsequent increase in moduli, as observed in previous studies. Similar heating traces were obtained for samples acidified with GDL to pH values above ~3.0 (at $c = 1.0$ wt%) or with pectin concentrations below ~1.0 wt% (at pH 3.0). However, at higher concentrations or lower values of pH (i.e. conditions favourable to extensive intermolecular association) an abrupt decrease in G', with an accompanying maximum in G'', was observed on heating through the temperature range ~60–80 °C. This is attributed to excessive hydrophobic association, causing collapse of network structure. It is further suggested that, for samples acidified with citric acid, there is preferential association of chain sequences of high ester content into hydrophobic junctions at 90 °C, leaving sequences with a high content of unesterified carboxyl groups available to form long hydrogen-bonded junctions during cooling, and thus giving gels that are stronger and more resistant to network collapse.

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doi:10.1016/j.carbpol.2008.07.016

1. Introduction

Pectin is used extensively in the food industry, mainly as a gelling agent in fruit-based preserves such as jams, jellies and marmalade (Christensen, 1986; May, 1990; Rolin, 1993). In nature, it occurs as one of the principal constituents of the plant cell wall. The primary structure of pectin (Ridley, O'Neill, & Mohnen, 2001; Visser & Voragen, 1996; Voragen, Schols, & Visser, 2003) is complex, containing as many as 17 different monosaccharides. The most abundant of these is α-D-galacturonate, which is present mainly in (1 → 4)-linked linear homogalacturonan sequences.

Other structural sequences present in pectin include xylogalacturonan, in which some of the galacturonate residues in a homogalacturonan sequence carry single-sugar side chains of β-D-xylose at O(3); rhamnogalacturonan I (RG-I), which has a regular disaccharide repeating sequence of: → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →; arabinan, which consists entirely of α-L-Ara*f* residues, present both in a (1 → 5)-linked backbone and in monomeric or oligomeric side chains attached at O(2) or O(3); arabinogalactan I (AG-I), which has a linear backbone of (1 → 4)-linked β-D-Gal*p* residues, some of which have single-sugar side chains of α-L-Ara*f* at O(3); and arabinogalactan II (AG-II), in which the side chains are short, usually branched, oligomers which contain both α-L-Ara*f* and β-D-Gal*p* residues and are attached at O(6) of a (1 → 3)-linked β-D-galactan backbone. AG-II is closely associated with protein, and it is not yet established whether protein–AG-II forms part of the pectin molecule or is present as a contaminant. The most complex constituent of pectin is rhamnogalacturonan II (RG-II). The backbone of RG-II, unlike that of RG-I, does not contain rhamnose, but consists of at least eight (1 → 4)-linked α-D-Gal*p*A residues to which assemblies of various sugars are attached. These include glucose, galactose, xylose and rhamnose, as well as several uncommon sugars that do not normally occur in polysaccharides. The full structure of RG-II has not yet been determined.

The way in which the various structural sequences are arranged in the pectin molecule has also not yet been fully elucidated. The most widely accepted proposal (Visser & Voragen, 1996) is that the polymer backbone consists of long homogalacturonate regions interspersed by occasional isolated residues of α-L-rhamnose and by long stretches of RG-I, carrying neutral-sugar side chains which attach the pectin to other constituents of the plant cell wall (cellulose and hemicelluloses). Recently, however, an alternative, and still highly speculative, model was proposed (Vincken et al., 2003), in which the backbone consists entirely of RG-I, and the homogalacturonate sequences are attached to it as side chains (along with other side chains, as in the conventional model).

However, although its detailed molecular architecture is obviously central to understanding the structure of the plant cell wall, much of the structural complexity of pectin is lost during commercial extraction. The main sources of commercial pectin (Christensen, 1986; May, 1990; Rolin, 1993) are citrus peel and apple pomace, which are by-products of the fruit juice and cider industries. The normal method of extraction is by acid hydrolysis, which breaks down the polymeric neutral-sugar side chains and releases pectin from the plant tissue. The resulting extracts have relatively low molecular weight (~50–150 kDa) and a high content of galacturonate (typically ~65–85% of the polymeric material present), indicating that they consist predominantly of homogalacturonan sequences. Most of the galacturonate residues (up to ~80%) occur as the methyl ester. The percentage of GalA residues in the ester form is known as the degree of esterification (DE), and can be decreased enzymically using pectin methylesterase enzymes or chemically by hydrolysis with alkali or strong acid. Preparations with DE < 50% are known as low-methoxy (LM) pectins, and those with DE > 50% as high methoxy (HM) pectins.

Gelation of LM pectins is normally induced by incorporation of calcium ions. Intermolecular association appears to occur by formation of "egg box" junctions (Grant, Morris, Rees, Smith, & Thom, 1973), analogous to those in calcium alginate gels, with long arrays of site-bound Ca^{2+} ions sandwiched between (1 → 4)-diaxially linked polyuronate chains in a highly buckled twofold conformation (Jarvis & Apperley, 1995; Morris, Powell, Gidley, & Rees, 1982; Morris, Rees, Thom, & Boyd, 1978). Gelation of HM pectins (Rolin, 1993) occurs in the presence of high concentrations (typically ~60–65 wt%) of sucrose, or other cosolutes, at acidic pH. Reduction in pH promotes intermolecular association by converting a proportion of the GalA residues from the charged (COO^-) to the uncharged (COOH) form, thus suppressing electrostatic repulsion between the pectin chains. One obvious effect of incorporating large amounts of cosolute is to decrease the amount of water available to maintain the polymer in the solvated (solution) state. However, different cosolutes differ widely in their ability to promote formation of HM pectin gels (May, 1990; May & Stainsby, 1986; Tsoga, Richardson, & Morris, 2004a, 2004b).

The six possible pairwise interactions that can occur in a polymer–water–cosolute system are tabulated below.

Polymer–water	Water–water
Polymer–polymer	Cosolute–water
Polymer–cosolute	Cosolute–cosolute

The polymer–polymer interactions required to form a gel network occur in competition with polymer–water interactions in the solution state, and these in turn compete with water–water interactions. Most discussions of the role of cosolute molecules have focussed on cosolute–water interactions and their effect on water–water interactions (e.g. Nishinari & Watase, 1992; Oakenfull & Scott, 1986; Watase, Kohyama, & Nishinari, 1992). For sugars, particular attention has been paid to equatorial hydroxyl groups, whose spacing is compatible with the lattice structure of liquid water (Tait, Suggett, Franks, Ablett, & Quickenden, 1972). However, in an elegant theoretical and experimental study in which all pairwise interactions were considered explicitly in a lattice model, Nilsson, Piculell, and Malmsten (1990) concluded that cosolute–water interactions have only an indirect effect, by competing with polymer–cosolute interactions which control directly the association of polymer molecules into intermolecular junctions: the greater the extent to which the polymer chains are surrounded by cosolute molecules, the more difficult it is for them to associate with one another.

Finally, cosolute–cosolute interactions may also have a role in determining the effectiveness of different cosolutes in promoting gelation of HM pectin. In a recent series of experiments, Tsoga et al. (2004a, 2004b) studied a range of sugars and polyols, and found that liquid cosolutes gave weaker HM pectin gels than solid cosolutes. The proposed interpretation was that cosolute–cosolute interactions are stronger in solids than in liquids (as is evident from their higher melting points), and occur in competition with the polymer–cosolute interactions that inhibit association of pectin chains. A notable exception was fructose, which gave gels comparable in strength to those formed with liquid cosolutes such as glycerol. Calorimetric studies (Tsoga et al., 2004b), however, suggested that fructose inhibits gelation by binding directly to the pectin molecules, rather than by "condensing" around them.

X-ray fibre diffraction studies (Walkinshaw & Arnott, 1981a, 1981b) have shown that, under both neutral and acidic conditions, pectin exists in a threefold (3_1) helical conformation in the solid state. Interpretation of the diffraction patterns by molecular mod-

elling indicated stabilisation of the helical structure by extensive hydrogen bonding within and between the polymer chains.

Adoption of the threefold conformation under hydrated conditions can be induced by reduction in pH at fixed temperature (Cesàro, Ciana, Delben, Manzini, & Paoletti, 1982; Ravanat & Rinaudo, 1980) or by reduction in temperature (Gilsenan, Richardson, & Morris, 2000) at fixed values of pH (below ~4.0). Gels formed by cooling solutions of LM pectin (DE ≈ 31%) at acidic pH (in the absence of cosolute) were found to dissociate fully on re-heating (Gilsenan et al., 2000). HM pectin gels (formed by cooling under acidic conditions in the presence of sucrose or other cosolutes) also show loss of network structure (as characterised by dynamic moduli, G' and G'') on initial heating, but at higher temperatures the moduli remain constant, or increase (Evageliou, Richardson, & Morris, 2000; Tsoga et al., 2004a). The most likely interpretation is that (i) the junctions formed on cooling consist of aggregated threefold helices stabilised predominantly by hydrogen bonding; (ii) these junctions are thermally reversible (as seen for acid-induced gels of LM pectin); and (iii) new junctions are formed on heating by hydrophobic association (Oakenfull, 1991; Oakenfull & Scott, 1984) of methyl ester groups. For brevity, the hydrogen-bonded associations that form on cooling and dissociate again on heating will be referred to as "hydrophilic junctions", in contrast to hydrophobic junctions, which form on heating and dissociate on cooling (Tanford, 1980).

A practical problem that can occur in conventional gelation of HM pectin by cooling at acidic pH in the presence of high concentrations of sugar (in manufacture of jams, jellies and marmalade) is that the onset of gel formation may occur at high temperature, as the product is boiled or filled into containers, resulting in a broken network (May, 1990). The initial aim of this investigation was to explore the effect of such "pregelation" on final gel strength by making comparisons with gels prepared at room temperature by acidification with D-glucono-δ-lactone (GDL). However, when the networks formed by these two methods were then heated, some surprising differences were observed, which may give new insights into the mechanism of HM pectin gelation by the conventional procedure.

2. Materials and methods

The HM pectin used was a commercial sample from Danisco, with DE ≈ 70%. Sucrose was normal food grade, purchased locally. GDL was from ADM, Ringaskiddy, Co. Cork, Ireland, and citric acid from Merck.

All samples for rheological measurements were prepared using distilled deionised water, and incorporated a fixed sucrose concentration of 60 wt%. The required amount of pectin was added (at room temperature) as a 5 wt% stock solution, and mixtures were stirred mechanically at ~95 °C until the sucrose was fully dissolved, with subsequent addition of water to compensate for evaporation. For conventional gelation by cooling, the required amount of citric acid was added as a 10 wt% stock solution, preheated to ~95 °C. The acidified mixture was then stirred rapidly until homogeneous, and immediately loaded onto an oscillatory rheometer at 90 °C. For gelation with GDL, the pectin–sucrose solutions were allowed to cool to room temperature, and the required amount of GDL was added as solid powder. The mixture was then homogenised by brief (~3 min) mechanical stirring and loaded onto the oscillatory rheometer at 25 °C. Alternatively, selected samples were filled into cylindrical moulds for compression testing.

Oscillatory measurements of storage modulus (G'), loss modulus (G'') and complex dynamic viscosity (η^*) were made at a fixed strain of 0.5% using parallel plate geometry (4 cm diameter; 0.5 mm gap) on a CarriMed CSL-100 rheometer. After loading, all samples were coated around their periphery with light silicone oil, to minimise loss of water by evaporation. Samples acidified with citric acid were cooled from 90 to 25 °C at 1 °C/min; those acidified with GDL were held for 16 h at 25 °C. In both cases development of network structure was monitored by oscillatory measurements at 1 rad s^{-1}, a mechanical spectrum (variation of G', G'' and η^* with frequency, ω/rad s^{-1}) was recorded (at 25 °C) and the samples were heated from 25 to 90 °C at 1 °C/min, with measurements again being made at 1 rad s^{-1}.

Two series of experiments were carried out for both methods of acidification. In the first series, the pectin concentration was held constant at 1.0 wt%, and the pH of the gels formed by cooling from 90 to 25 °C (with citric acid) or by holding for 16 h at 25 °C (with GDL) was varied. The final values of pH used were 2.25–3.75 in increments of 0.25. In the second series of experiments, the pH reached after cooling (with citric acid) or holding (with GDL) was held constant at 3.0, and the concentration of pectin was varied from 0.25 to 2.00 wt%, in increments of 0.25. The amount of citric

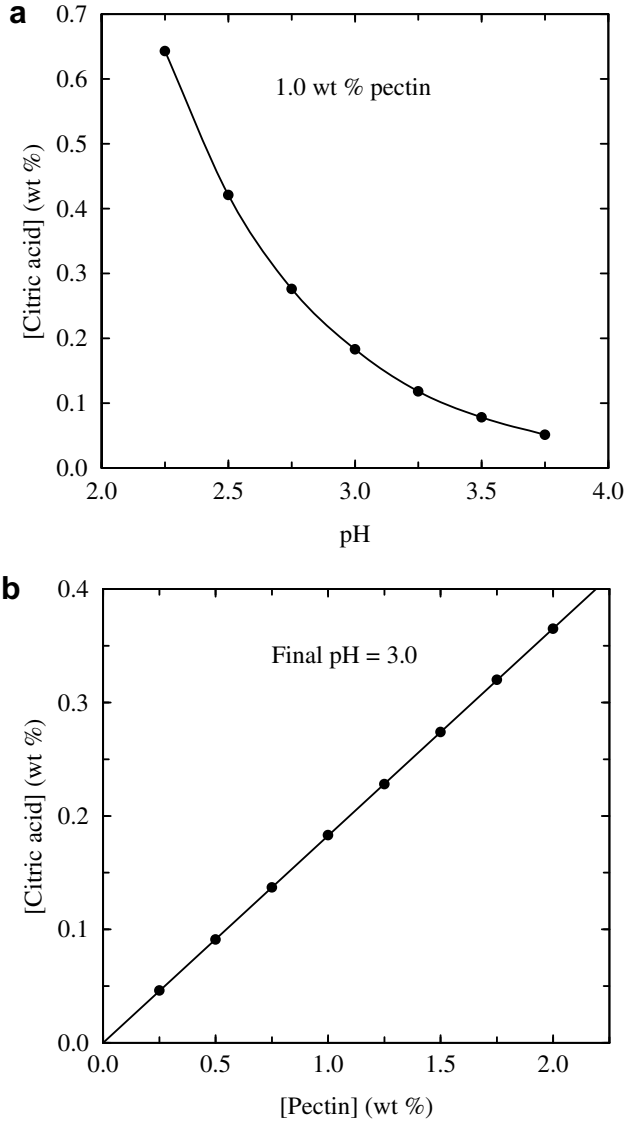

Fig. 1. Concentrations of citric acid required to give (a) target values of pH (2.25–3.75 in increments of 0.25) for gels of 1.0 wt% HM pectin (DE ≈ 70%), and (b) a fixed pH of 3.0 on varying pectin concentration from 0.25 to 2.00 wt% in increments of 0.25. All gels incorporated 60 wt% sucrose, and were formed by cooling from 90 to 25 °C at 1 °C/min.

acid or GDL needed to reach the target values of pH was determined using calibration curves constructed from preliminary experiments. In these experiments, the gels obtained after completion of cooling or holding were reduced to pastes by manual stirring, and left in contact with the pH electrode (at 25 °C) until stable readings were attained (typically after ~15 min). It was also established in preliminary experiments that a holding time of 16 h at 25 °C was sufficient to give stable final values of pH by hydrolysis of GDL.

Samples for compression testing (acidified with GDL) were filled (at 25 °C) into lubricated cylindrical moulds (diameter 12.6 mm; height 13.5 mm), sealed with lubricated cover slips, and held in a water bath for 16 h at 25 °C (as in the oscillatory studies). The resulting gels were then compressed (at 25 °C) on a TA-XT2 texture analyser (from Stable Microsystems), using a cylindrical probe of diameter 50 mm and a compression rate of 1 mm/s. Results are reported as the average of two replicate compression curves for each sample.

3. Results

HM pectin gels formed on cooling after acidification with citric acid have been characterised extensively in previous investigations, including particularly those by Evageliou et al. (2000) and Tsoga et al. (2004a, 2004b). The experiments in which the same method of gelation was used in the present work were not intended to duplicate, or supersede, these earlier studies, but were carried out to allow direct comparisons to be made with gels acidified with GDL, using the same sample of pectin and identical values of concentration and pH for both procedures.

The preparations acidified with citric acid were cooled to a final temperature of 25 °C, rather than to the lower temperature of 5 °C used in the previous investigations cited above; 25 °C was chosen to allow reduction in pH with GDL to occur over an experimentally tractable timescale, while still giving essentially complete development of network structure on cooling with citric acid.

The investigation was confined to a fixed concentration (60 wt%) of a single cosolute, sucrose, chosen for its industrial relevance. A potential complication is that some hydrolysis of sucrose to glucose and fructose occurs on heating under acidic conditions. However, it was shown by Evageliou et al. (2000) that, under a time–temperature regime virtually identical to that used in the present work, the extent of hydrolysis was insufficient to have any significant effect on gel moduli.

The results obtained for the samples acidified with citric acid are presented first (Sections 3.1 and 3.2), followed by those for samples acidified with GDL (Sections 3.3–3.5).

3.1. Gelation with citric acid – varying pH

The concentrations of citric acid needed to give the target values of pH (2.25, 2.50, 2.75, 3.00, 3.25, 3.50 and 3.75) in the gels formed by cooling 1.0 wt% pectin from 90 to 25 °C in the presence of 60 wt% sucrose are shown in Fig. 1a. As would be expected, the required concentration increases smoothly with decreasing pH.

The values of G' and G'' attained on completion of cooling are shown in Fig. 2. There is a progressive increase in both moduli with decreasing pH, which, as discussed previously (Section 1), can be attributed to progressive reduction in electrostatic repulsion be-

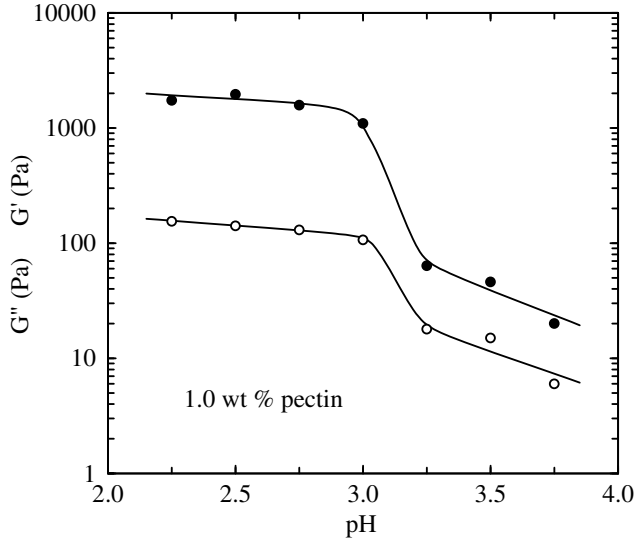

Fig. 2. Variation of G' (●) and G'' (○) with pH in gels of 1.0 wt% HM pectin acidified with citric acid and cooled from 90 to 25 °C in the presence of 60 wt% sucrose. Measurements (25 °C) were made at 1 rad s^{-1} and 0.5% strain.

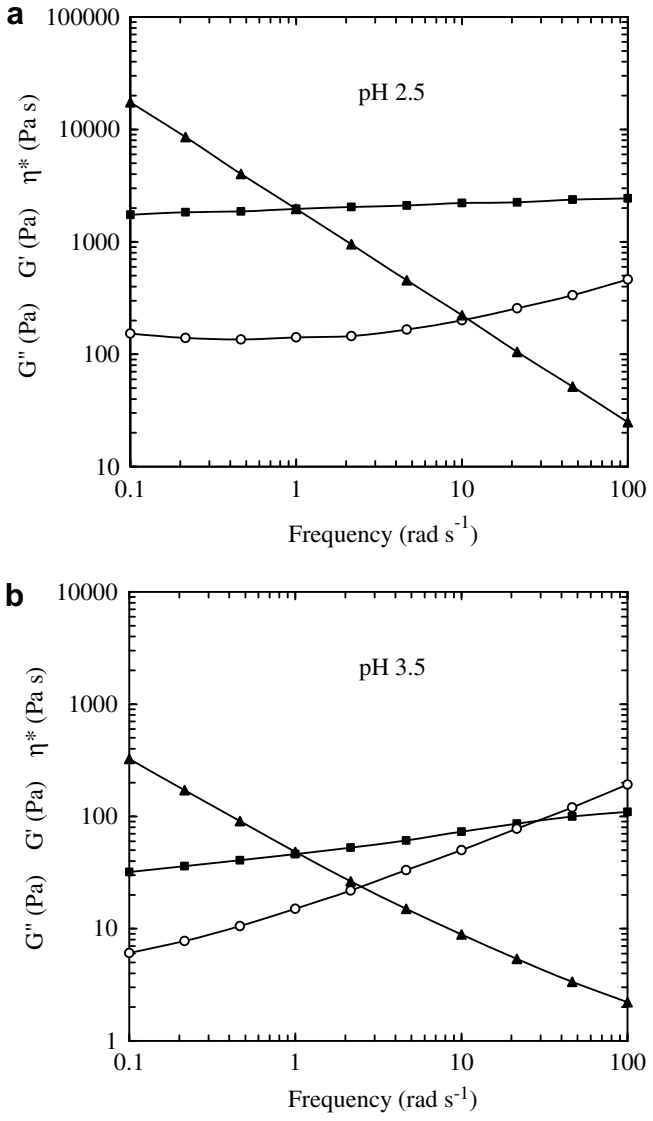

Fig. 3. Mechanical spectra (25 °C; 0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for gels incorporating 1.0 wt% HM pectin and 60 wt% sucrose and acidified with citric acid to pH values of (a) 2.5 and (b) 3.5.

tween the polymer chains on conversion of carboxyl groups from the charged (COO^-) form to the uncharged (COOH) form. The increase in moduli is particularly steep on reduction of pH from ~3.25 to ~3.0. The pH range over which individual carboxyl groups in pectin (or other polyuronates) convert from the charged form to the uncharged (protonated) form depends on the ionisation state of other carboxyl groups in the molecule (Milas & Rinaudo, 1997). Thus the apparent pK_a of pectin varies with pH, but it extrapolates to a minimum value (pK_o) of ~3.3 in the limit of complete protonation (Ravanat & Rinaudo, 1980; Rinaudo, 1996). The sharp increase in moduli shown in Fig. 2 is centred around this value. At pH values below ~2.8, where virtually all the carboxyl groups will have been converted to the uncharged form, continued reduction in pH has little effect on gel moduli, as would be expected.

Fig. 3 shows illustrative mechanical spectra recorded on completion of cooling (i.e. at 25 °C) for samples prepared at pH values of 3.5 and 2.5, which are, respectively, well above and well below the steep increase in moduli (Fig. 2) with decreasing pH. The spectrum for the sample prepared at pH 2.5 (Fig. 3a) has the form typical (Ross-Murphy, 1984) of a strong gel ($G' >> G''$; little frequency-dependence of either modulus; linear reduction in $\log\eta^*$ on increasing $\log\omega$, with a slope close to −1). In the spectrum recorded at pH 3.5 (Fig. 3b), the moduli, as well as being much lower, show much greater variation with frequency and much smaller separation between G' and G''. Indeed at high frequency G'' rises steeply above G', indicating a substantial "sol fraction" of chains, or chain segments, that have not been incorporated into the gel network.

Fig. 4 shows the changes in G' (Fig. 4a) and G'' (Fig. 4b) observed for the same two samples (pH 3.5 and 2.5) during cooling from 90 to 25 °C and re-heating. In the previous studies by Evageliou et al. (2000), where cooling was continued to 5 °C, the initial reduction in moduli on heating occurred predominantly at temperatures below ~30 °C, and is therefore less evident in the present work. A slight reduction in G' and, particularly, G'' can, however, be observed (Fig. 4) in the early stages of heating. At higher temperatures, the moduli observed on heating are consistently higher than those obtained during cooling, which, as discussed above, can be attributed to association of methyl ester groups into hydrophobic junctions as the temperature is raised.

The values of G' and G'' attained on completion of heating to 90 °C are shown in Fig. 5, in direct comparison with the corresponding values (Fig. 2) at 25 °C. The pH-dependence of both moduli at 90 °C has the same general form as at 25 °C, with a steep increase on reduction in pH from ~3.25 to ~ 3.0. The absolute values of G'', however, are consistently lower at 90 °C than at 25 °C, indicating incorporation of chain sequences into the gel network by hydrophobic association during heating. At pH values above the steep increase in moduli (i.e. above pK_o), the values of G' at 90 °C are slightly higher than at 25 °C, which is again consistent with reinforcement of the gel network by formation of hydrophobic junctions as the temperature is raised. At lower pH, however, there is a net decrease in G' between 25 and 90 °C, suggesting that at pH values well below pK_o, where there is extensive formation of hydrophilic junctions during cooling, dissociation of these junc-

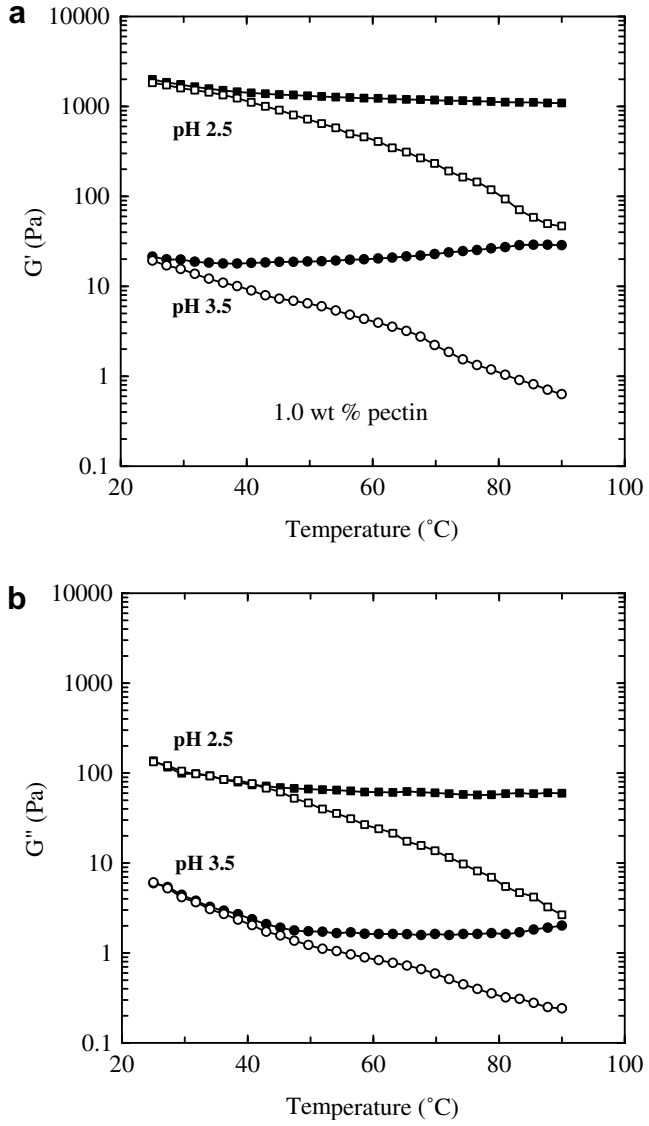

Fig. 4. Variation of (a) G' and (b) G'', measured at 1 rad s^{-1} and 0.5% strain, during cooling (open symbols) and heating (filled symbols) at 1 °C/min for preparations of 1.0 wt% HM pectin with 60 wt% sucrose acidified with citric acid to pH 3.5 (circles) or pH 2.5 (squares).

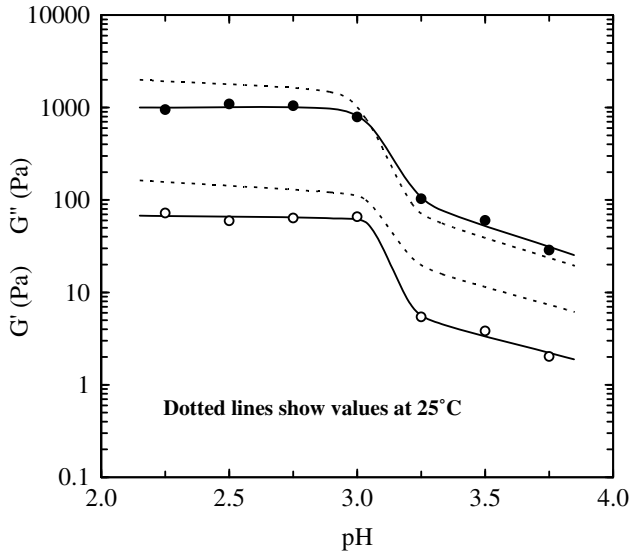

Fig. 5. pH-dependence of G' (●) and G'' (○) at 90 °C for gels of 1.0 wt% HM pectin in the presence of 60 wt% sucrose, after acidification with citric acid, cooling from 90 to 25 °C, and re-heating. The dotted lines show the corresponding moduli at 25 °C (Fig. 2).

tions on heating has a greater effect than formation of new junctions by hydrophobic association.

3.2. Gelation with citric acid – varying pectin concentration

In the studies reported here, the pH of the gels formed on cooling to 25 °C (with 60 wt% sucrose) was held constant at 3.0 and the concentration of pectin was varied. The pectin concentrations used were 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 wt%. The concentrations of citric acid needed to maintain the pH at 3.0 are shown in Fig. 1b. The values are directly proportional to pectin concentration, demonstrating that pH is determined by the ratio of citric acid to pectin, irrespective of the absolute concentration of either component.

Fig. 6 shows the variation of G' (Fig. 6a) and G'' (Fig. 6b) during cooling and heating for illustrative values of pectin concentration. In all cases, the moduli recorded on heating rise above the corresponding values during cooling, as observed (Fig. 4) in the experiments where pectin concentration was held constant at 1.0 wt% and pH was varied.

The concentration-dependence of G' and G'' on completion of cooling to 25 °C (at pH 3.0) is shown in Fig. 7a. Both moduli increase monotonically as the concentration is increased from 0.25 to 1.50 wt%, but then level out, or decrease slightly, at higher concentrations. The possible origin of this behaviour is discussed later (Section 4). As shown in Fig. 7b, there is little difference between the moduli measured on completion of cooling to 25 °C and on completion of subsequent heating to 90 °C, suggesting that, at pH 3.0, loss of network structure by dissociation of hydrophilic junctions on heating is almost exactly balanced by hydrophobic association.

3.3. Gelation with GDL – varying pH

The concentrations of GDL needed to give the target values of final pH (2.25–3.75 in increments of 0.25) in gels of 1.0 wt% pectin

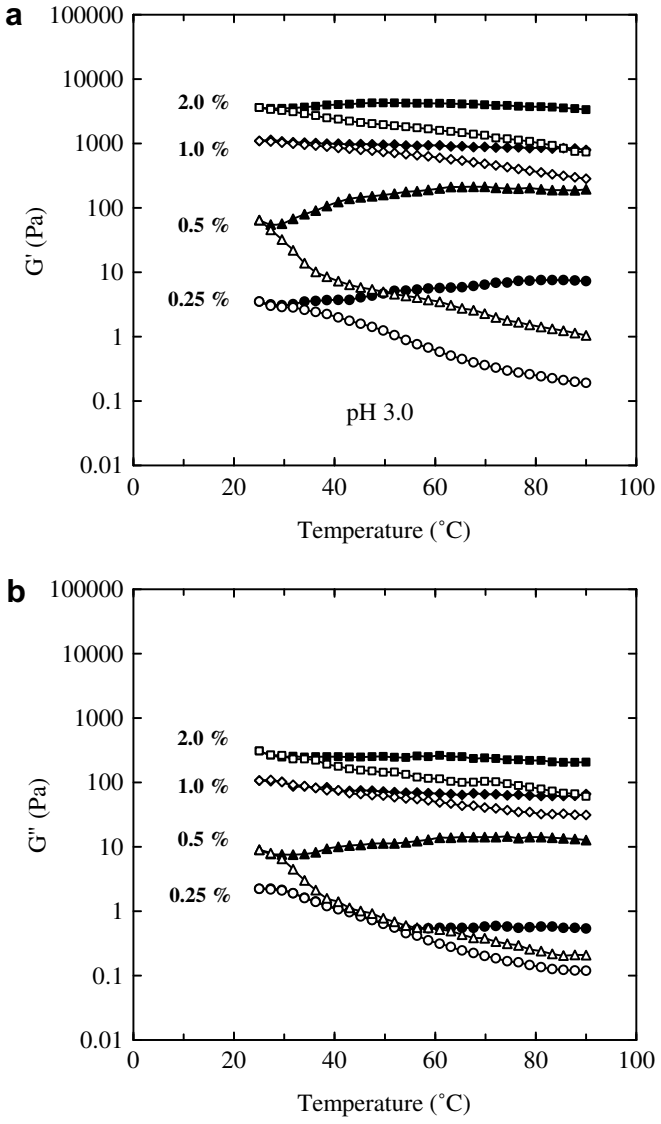

Fig. 6. Variation of (a) G' and (b) G'', measured at 1 rad s^{-1} and 0.5% strain, during cooling (open symbols) and heating (filled symbols) at 1 °C/min for samples with pectin concentrations (wt%) of 0.25 (circles), 0.5 (triangles), 1.0 (diamonds) and 2.0 (squares) acidified with citric acid to a fixed pH of 3.0 and incorporating a fixed sucrose concentration of 60 wt%.

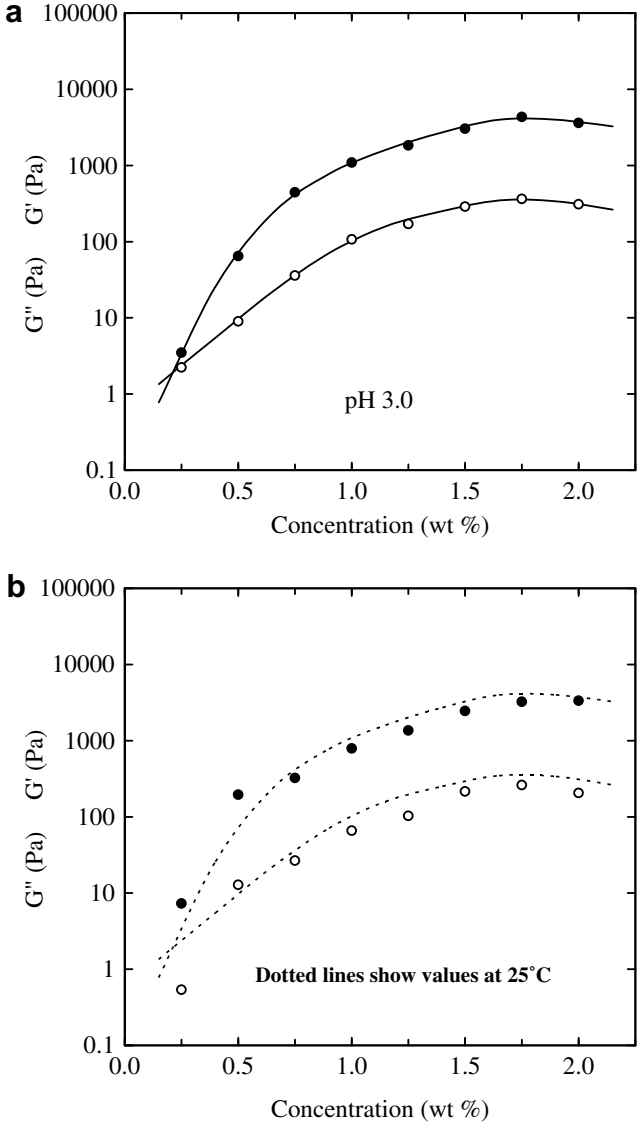

Fig. 7. Concentration-dependence of G' (●) and G'' (○), measured at 1 rad s^{-1} and 0.5% strain, for gels of HM pectin in the presence of 60 wt% sucrose, acidified with citric acid to pH 3.0, (a) at 25 °C after initial cooling from 90 °C, and (b) at 90 °C, after re-heating from 25 °C. The dotted lines in (b) show the moduli at 25 °C (a).

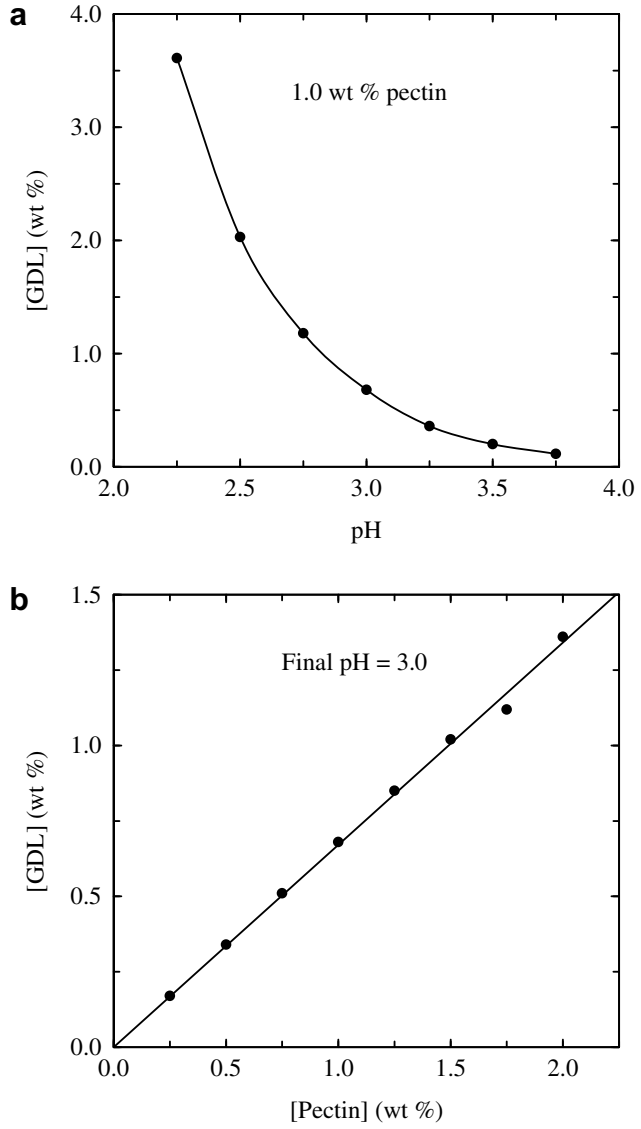

Fig. 8. Concentrations of GDL required to give (a) target values of pH (2.25–3.75 in increments of 0.25) for gels of 1.0 wt% HM pectin (DE ≈ 70%), and (b) a fixed pH of 3.0 on varying pectin concentration from 0.25 to 2.00 wt% in increments of 0.25. All gels incorporated 60 wt% sucrose, and were formed by holding for 16 h at 25 °C.

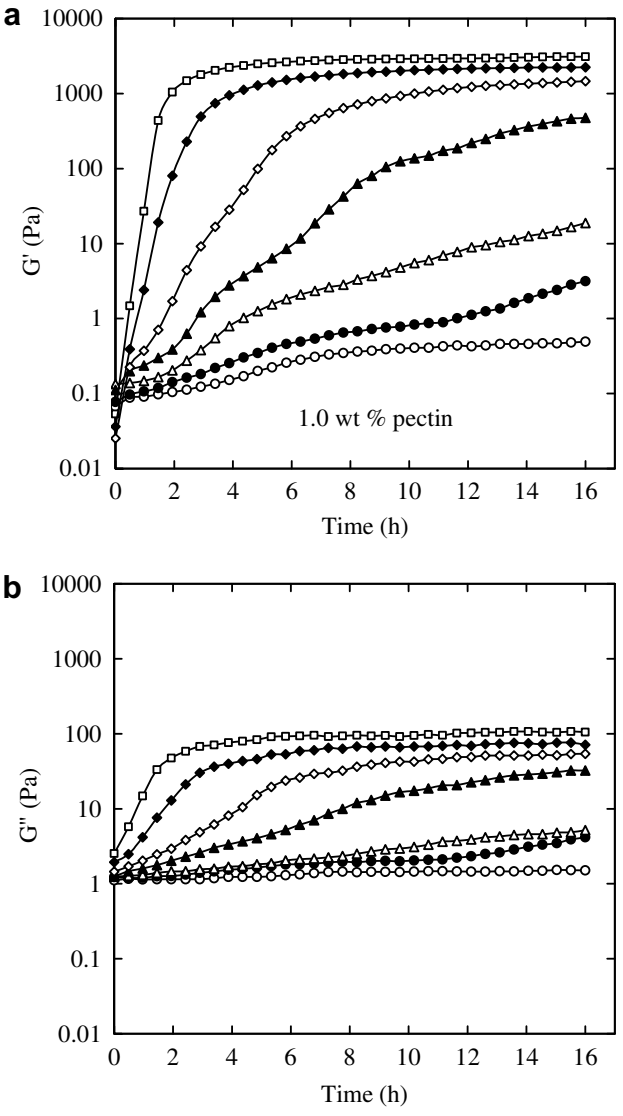

Fig. 9. Variation of (a) G' and (b) G'', measured at 1 rad s^{-1} and 0.5% strain during holding at 25 °C, for mixtures of 1.0 wt% HM pectin and 60 wt% sucrose, acidified with GDL to give final pH values (after 16 h at 25 °C) of: 3.75 (○), 3.50 (●), 3.25 (△), 3.00 (▲), 2.75 (◇), 2.50 (♦) and 2.25 (□).

with 60 wt% sucrose after holding for 16 h at 25 °C are shown in Fig. 8a. The curve has the same general form as that observed for citric acid (Fig. 1a), although the absolute concentrations required are, of course, different.

Fig. 9 shows the changes in G' (Fig. 9a) and G'' (Fig. 9b) observed during the holding period at 25 °C for each of the pH values studied. As would be expected, the sharp increase in moduli marking the onset of network formation moves to progressively shorter times with decreasing values of final pH (i.e. increasing concentration of GDL; Fig. 8a). The samples with final pH values of 3.00 and 2.75 show two discernable "waves" of increase in G'. A possible interpretation is that the second steep increase corresponds to the sharp reduction in charge density, and hence in electrostatic repulsion between the polysaccharide chains, that occurs as the pH drops below pK_o.

The moduli attained on completion of the 16 h holding period at 25 °C are shown in Fig. 10, in comparison with the corresponding values (Fig. 2) observed on completion of cooling to 25 °C for samples acidified with citric acid. The values of G'' for the samples acidified with GDL are consistently lower than for those acidified with citric acid, but the general form of pH-dependence is similar, with again a steep increase on reduction in pH from ~3.25 to ~3.0. However, in contrast to the gels formed with citric acid, where G' is higher than G'' at all pH values studied, G' for the gels formed with GDL is below G'' at the highest value of pH (3.75), but then rises steeply as the pH is decreased and at pH values below ~2.6 it exceeds G' for the samples gelled with citric acid.

Fig. 11 shows mechanical spectra recorded at pH values of 2.25 (Fig. 11a) and 3.0 (Fig. 11b), which are, respectively, below and above the point at which the curves of G' versus pH for the two methods of acidification cross one another (Fig. 10). At pH 2.25, the values of G', G'' and η^* for the sample gelled with GDL are consistently higher than those for the sample gelled with citric acid, but the spectra (Fig. 11a) are otherwise virtually identical, with the form typical (Ross-Murphy, 1984) of a strong gel. At pH 3.0 (Fig. 11b), the values of G', G'' and η^* are lower for

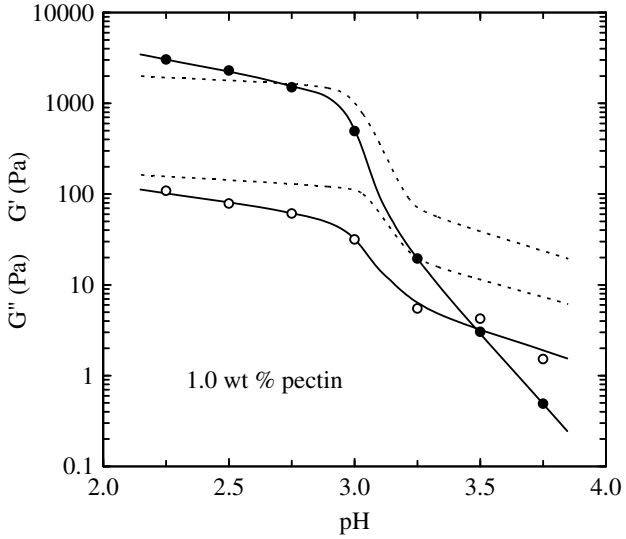

Fig. 10. Variation of G' (●) and G'' (○) with pH in gels of 1.0 wt% HM pectin acidified with GDL and held for 16 h at 25 °C in the presence of 60 wt% sucrose. Measurements were made at 1 rad s^{-1} and 0.5% strain. The dotted lines show the corresponding values (at 25 °C) for samples acidified with citric acid and cooled from 90 °C (Fig. 2).

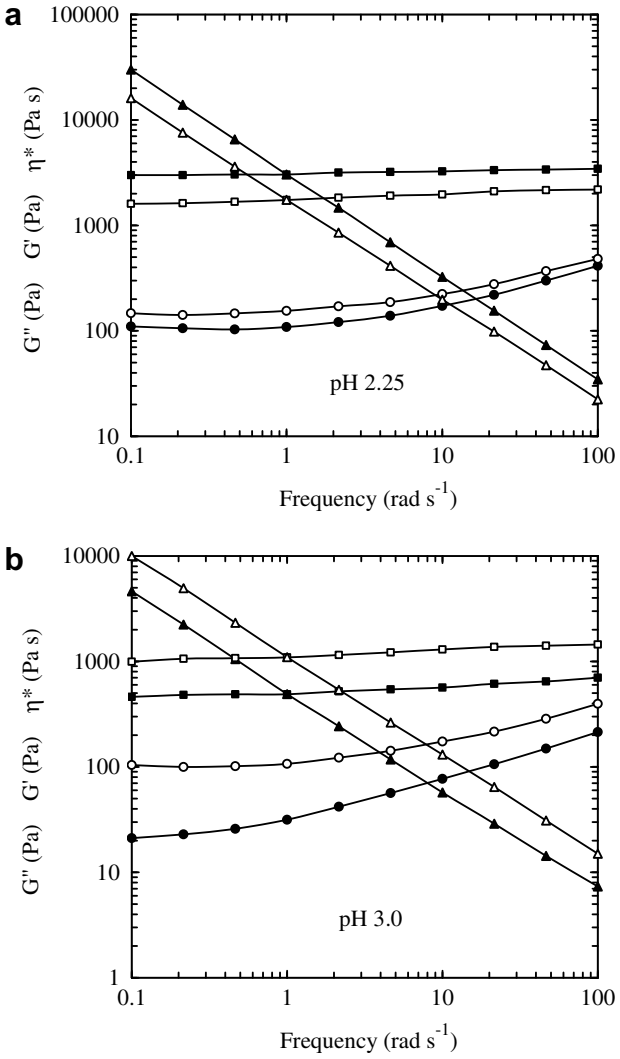

Fig. 11. Mechanical spectra (25 °C; 0.5% strain) showing the frequency-dependence of G' (squares), G'' (circles) and η^* (triangles) for gels incorporating 1.0 wt% HM pectin and 60 wt% sucrose and acidified to pH values of (a) 2.25 and (b) 3.0 with citric acid (open symbols) or with GDL (filled symbols).

the sample gelled with GDL, but both methods of acidification again give similar mechanical spectra, with somewhat greater frequency-dependence of moduli than at pH 2.25 (Fig. 11a), consistent with a less extensively crosslinked network.

Fig. 12 shows compression curves for mixtures of 1.0 wt% HM pectin and 60 wt% sucrose, acidified with GDL to final pH values of 3.0, 2.75 and 2.5 (after holding for 16 h at 25 °C). All three curves show an initial increase in resistance (stress) with increasing extent of compression, followed by a sharp decrease as network structure breaks. Fracture occurs at progressively higher deformation (i.e. higher strain) as the pH is decreased, and the stress at the point of fracture ("yield stress", σ_b) also increases. As shown in Fig. 13, there is a direct linear correlation between $\log\sigma_b$ from compression testing and $\log G'$ from low-amplitude oscillatory measurement (Fig. 10), demonstrating that the large-deformation (failure) properties of the pectin networks are affected by pH in a similar way to their small-deformation response.

3.4. Gelation with GDL – varying pectin concentration

The concentrations of GDL needed to give a final pH of 3.0 after holding for 16 h at 25 °C for pectin concentrations ranging from 0.25 to 2.00 wt% are shown in Fig. 8b. As was found for citric acid (Fig. 1b), the concentrations required are directly proportional to pectin concentration, again demonstrating that pH is determined by the ratio of acidifying agent to pectin, rather than the absolute concentration of either component.

Fig. 14 shows the changes in G' (Fig. 14a) and G'' (Fig. 14b) observed during holding at 25 °C for mixtures of HM pectin and 60 wt% sucrose, acidified with GDL to a final pH value of 3.0, at pectin concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 wt%. At the lowest concentration studied (0.25 wt%), the moduli remained roughly constant throughout the holding period, with $G'' > G'$, showing that no gel network was formed. At the other concentrations (0.5–2.0 wt%), however, G' (Fig. 14a) rose steeply above G'' (Fig. 14b).

The mechanical spectra recorded at 0.5 wt% (the lowest concentration at which gelation was observed) and 2.0 wt% (the highest concentration studied) are shown in Fig. 15. At 2.0 wt%, the spectrum has the form typical of a strong gel, with a large separation between G' and G'' throughout the frequency-range studied (0.1–100 rad s^{-1}). At 0.5 wt%, the frequency-dependence of the moduli is greater, and at high frequency G'' rises above G', indicating a substantial sol fraction of sequences that have not been incorporated into the gel network.

The concentration-dependence of G' and G'' at the end of the 16 h holding period at 25 °C is shown in Fig. 16a, in direct comparison with the corresponding curves for samples acidified with citric acid (Fig. 7a) Throughout the concentration range studied, the values of G'' for the samples acidified with GDL are lower than those for the gels formed by cooling with citric acid. For the samples incorporating GDL, G' rises above G'' at a pectin concentration of ~0.3 wt%, indicating that this is the minimum critical concentration at which a continuous network can be formed under the experimental conditions used (final pH of 3.0 after holding for 16 h at 25 °C). At higher concentrations of pectin, the values of G' for the GDL-induced networks increase progressively, converging on the corresponding values for the samples gelled by cooling with

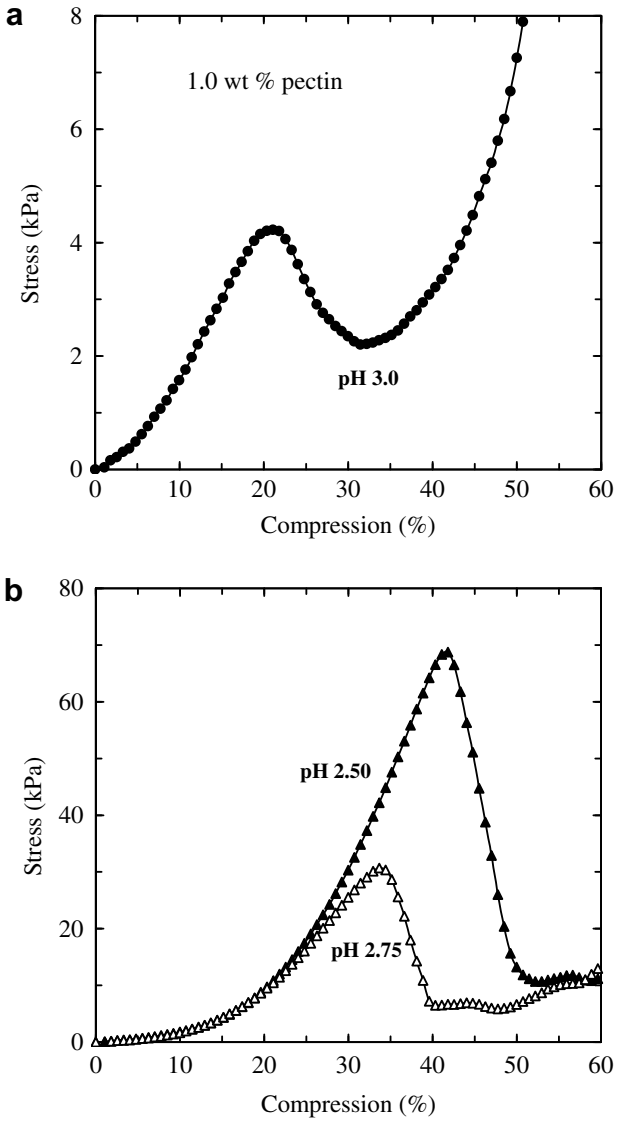

Fig. 12. Compression curves (25 °C; 1 mm/s) for gels of 1.0 wt% HM pectin in the presence of 60 wt% sucrose, acidified with GDL to pH values of (a) 3.0 (●) and (b) 2.75 (△) and 2.50 (▲).

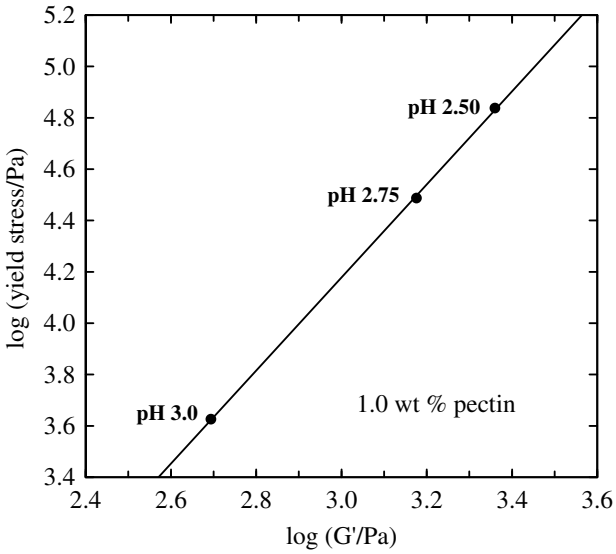

Fig. 13. Correlation of yield stress values from Fig. 12 with values of G' (Fig. 10) for samples of the same composition, after the same holding time (16 h at 25 °C).

citric acid, and then rising slightly above them at the highest pectin concentration studied (2.0 wt%).

Fig. 16b shows the variation of moduli with pectin concentration (c) for the samples acidified with GDL, plotted double-logarithmically. At concentrations well above the minimum critical gelling concentration ($c_o \approx 0.3$ wt%), the slope of $\log G'$ versus $\log c$ approaches a limiting value of ~2 (i.e. c^2-dependence), which is typical (Clark & Ross-Murphy, 1985) of biopolymer gels crosslinked by non-covalent association into ordered junction zones.

3.5. Heating samples acidified with GDL

Fig. 17 shows the variation of G' (Fig. 17a) and G'' (Fig. 17b) during heating from 25 to 90 °C for samples acidified to pH 3.0 with GDL, at pectin concentrations of 0.50, 0.75, 1.0 and 2.0 wt%. At the two lowest concentrations, the curves are broadly similar to the heating curves observed (Figs. 4 and 6) for samples acidified with citric acid and gelled by cooling. At 2.0 wt% pectin, however, there is a large, abrupt, decrease in G' at ~70 °C, and at the same temperature G'' passes through a maximum before also decreasing as the temperature is raised further. A similar, although much smaller, decrease in moduli can be observed (Fig. 17) in the final stages of heating for the sample incorporating 1.0 wt% pectin. A large decrease in G', with an accompanying maximum in G'', can also be seen (Fig. 18) in the heating traces recorded at intermediate concentrations of pectin (1.25, 1.50 and 1.75 wt%).

The concentration-dependence of G' and G'' on completion of heating to 90 °C is shown in Fig. 19, in comparison with the corresponding values (Fig. 16a) at 25 °C. At pectin concentrations above ~0.75 wt%, the values of G'' and, particularly, G' at 90 °C are much lower than at 25 °C, in marked contrast to the behaviour (Fig. 7b) of the gels formed at the same final pH (3.0) by cooling after acidification with citric acid, where, for all concentrations of pectin studied, the moduli at 90 °C were almost identical to those at 25 °C.

Fig. 20 shows the changes in G' (Fig. 20a) and G'' (Fig. 20b) observed during heating for mixtures of 1.0 wt% HM pectin with 60 wt% sucrose gelled by acidification with GDL to final pH values (after 16 h at 25 °C) of 2.25, 2.50, 2.75, 3.00, 3.25, 3.50 and 3.75. The sample acidified to pH 3.0 is common to both series of experiments (i.e. holding pH constant at 3.0 and varying pectin concentration, and holding pectin concentration constant at 1.0 wt% and varying pH). As pointed out above, the heating curves for this sample show a slight downturn in both moduli as the temperature approaches 90 °C. At lower values of pH (2.75, 2.50 and 2.25), there is a sharp reduction in G' during heating, with an accompanying maximum in G'', as was observed (Figs. 17 and 18) at pectin concentrations above 1.0 wt% for samples acidified to a final pH of 3.0. The decrease in G' and maximum in G'' both move to progressively higher temperature (Fig. 20) as the pH is decreased from 2.75 to 2.25.

At pH values above 3.0, the heating curves show an initial decrease in moduli which, as discussed above (Section 1), can be attributed to dissociation of hydrophilic junctions, followed by an increase in G' attributable to hydrophobic association. The increase occurs over roughly the same temperature range as the sharp reductions in G' observed at pH values below 3.0, and be-

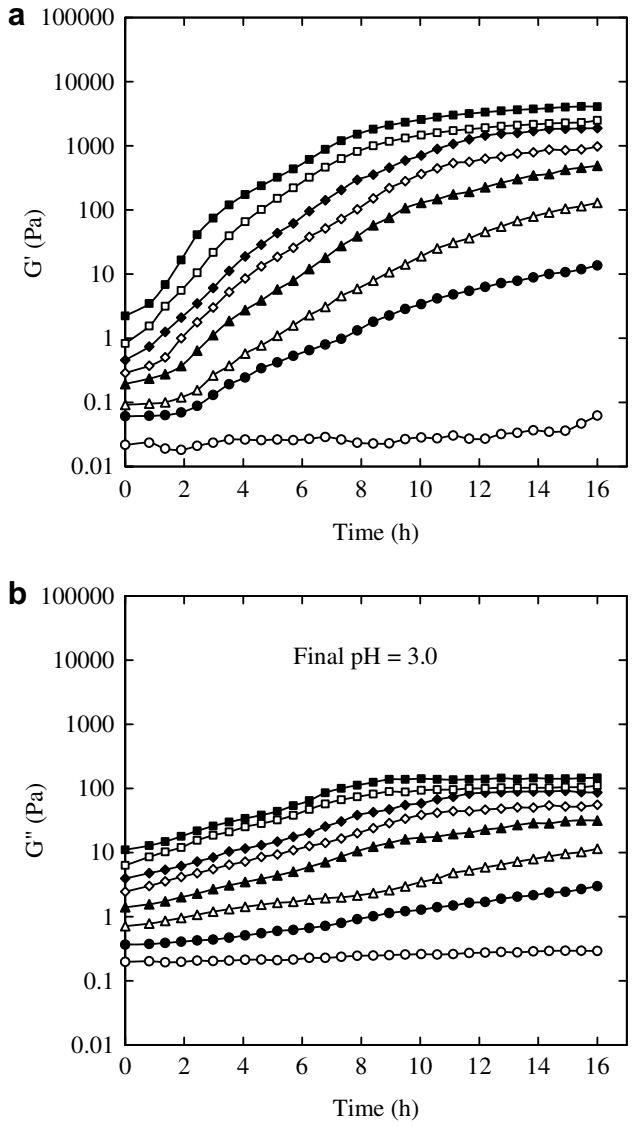

Fig. 14. Variation of (a) G' and (b) G'', measured at 1 rad s^{-1} and 0.5% strain during holding at 25 °C, for mixtures of HM pectin and 60 wt% sucrose, acidified with GDL to give a final pH (after 16 h at 25 °C) of 3.0 at pectin concentrations (wt%) of: 0.25 (○), 0.50 (●), 0.75 (△), 1.00 (▲), 1.25 (◇), 1.50 (◆), 1.75 (□) and 2.00 (■).

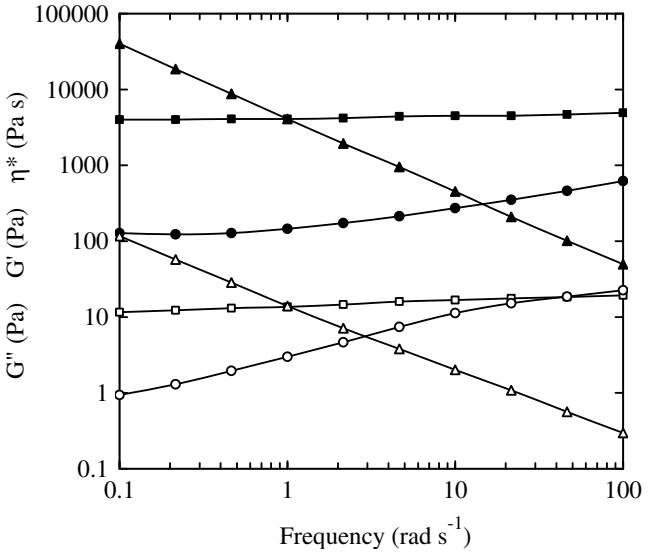

Fig. 15. Mechanical spectra (25 °C; 0.5% strain) showing the frequency-dependence of G' (squares), G'' (circles) and η^* (triangles) for gels of HM pectin in the presence of 60 wt% sucrose, acidified to pH 3.0 with GDL at pectin concentration (wt%) of 2.0 (filled symbols) and 0.5 (open symbols).

comes progressively greater as the pH is decreased from 3.75 to 3.25. Fig. 21 shows the values of G' and G'' recorded at the end of the heating scans in Figs. 17, 18 and 20 (at 90 °C) divided by the corresponding values at the start of heating (i.e. at 25 °C). On decreasing pH at 1.0 wt% pectin or increasing concentration of pectin at pH 3.0 (Fig. 21b) both ratios show an initial increase, before the ratios for G'' level out and those for G' drop sharply below them (corresponding to the steep decreases shown in Figs. 17, 18 and 20). The significance of these observations is discussed below.

4. Discussion and conclusions

As shown in Fig. 16b, the concentration-dependence of G' for the samples acidified with GDL to a final pH of 3.0 has the form typical of a gelling biopolymer, with the plot of $\log G'$ versus $\log c$ approaching a limiting slope of 2 at high values of pectin concentration (c). For samples acidified to the same final pH by the conventional procedure of adding citric acid at high temperature, however, the moduli (Fig. 7a) level out, or decrease slightly, at pectin concentrations above ~1.5 wt%. The most likely interpretation of this behaviour is that it arises from the "pregelation" phenomenon discussed in Section 1.

Two opposing factors will operate in solutions of HM pectin at high temperature: the drive to formation of intermolecular junctions by hydrophobic association of methyl ester groups; and electrostatic repulsion between the charged carboxylate groups of the polymer chains, resisting intermolecular association. For solutions at or around neutral pH, where the chains are essentially fully charged, electrostatic repulsion outweighs hydrophobic interactions and no gelation occurs. The balance between the two opposing factors can, however, be tipped in favour of hydrophobic association by lowering the pH, which suppresses repulsion by reducing charge density, and can therefore allow an intermolecular network to form.

Comparison of the relative values of G' and G'' shown in Figs. 4 and 6 for the samples gelled by acidification with citric acid at high temperature demonstrates that network structure had indeed formed at the loading temperature of 90 °C. In all cases, the initial values of G' at the start of the cooling curves are higher than the corresponding values of G''. For the samples prepared at pH 3.0 (Fig. 6), the separation of the two moduli increases progressively with increasing polymer concentration, from less than a factor of 2 at 0.25 wt% pectin ($G' \approx 0.2$ Pa; $G'' \approx 0.12$ Pa) to more than an order of magnitude at 2.0 wt% ($G' \approx 750$ Pa; $G'' \approx 60$ Pa). The separation of G' and G'' at 2.0 wt%, and the absolute values of both moduli, are in the ranges seen for comparatively strong biopolymer gels (Ross-Murphy, 1984). It seems reasonable to conclude, therefore, that the downturns in moduli (Fig. 7a) at high concentrations of pectin arise from formation and disruption of network structure ("pregelation") at high temperature during mixing with citric acid and loading of samples onto the rheometer. For samples of lower pectin concentration or higher pH, however, the separation of the initial moduli at 90 °C indicates a "weak gel" structure (Ross-Murphy, 1984), capable of network re-arrangement and flow, rather than irreversible fracture. As discussed below, these "weak gel" networks

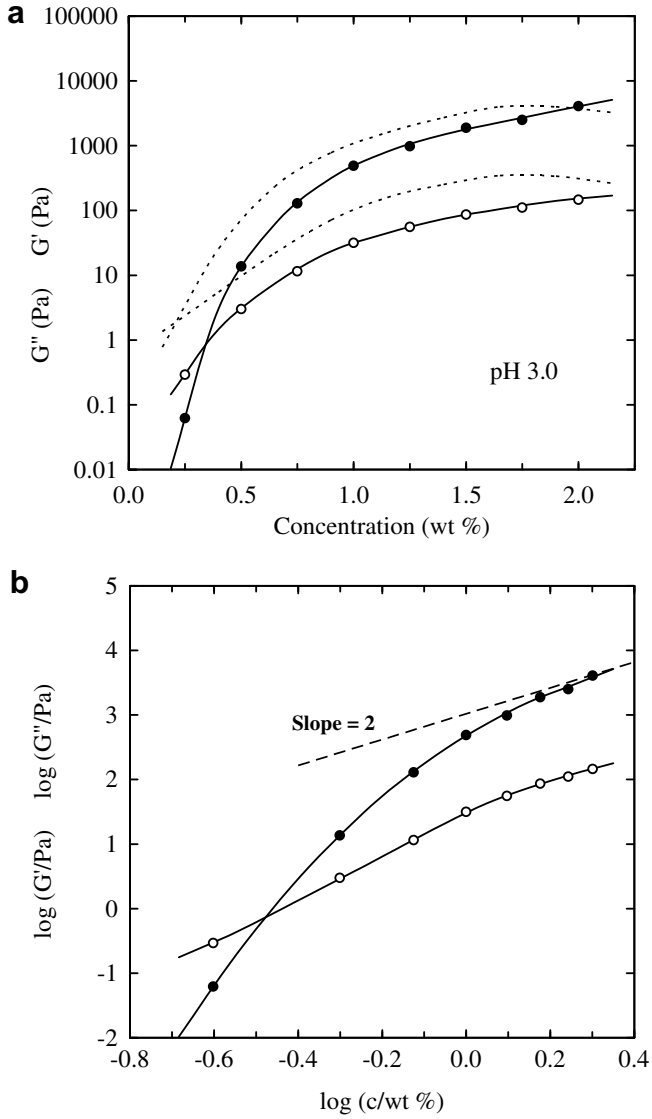

Fig. 16. (a) Concentration-dependence of G' (●) and G'' (○), measured at 1 rad s^{-1} and 0.5% strain, for gels of HM pectin in the presence of 60 wt% sucrose, acidified with GDL to pH 3.0. (b) The same values plotted double-logarithmically. The dotted lines in (a) show the corresponding moduli (25 °C) for samples acidified with citric acid and cooled from 90 °C.

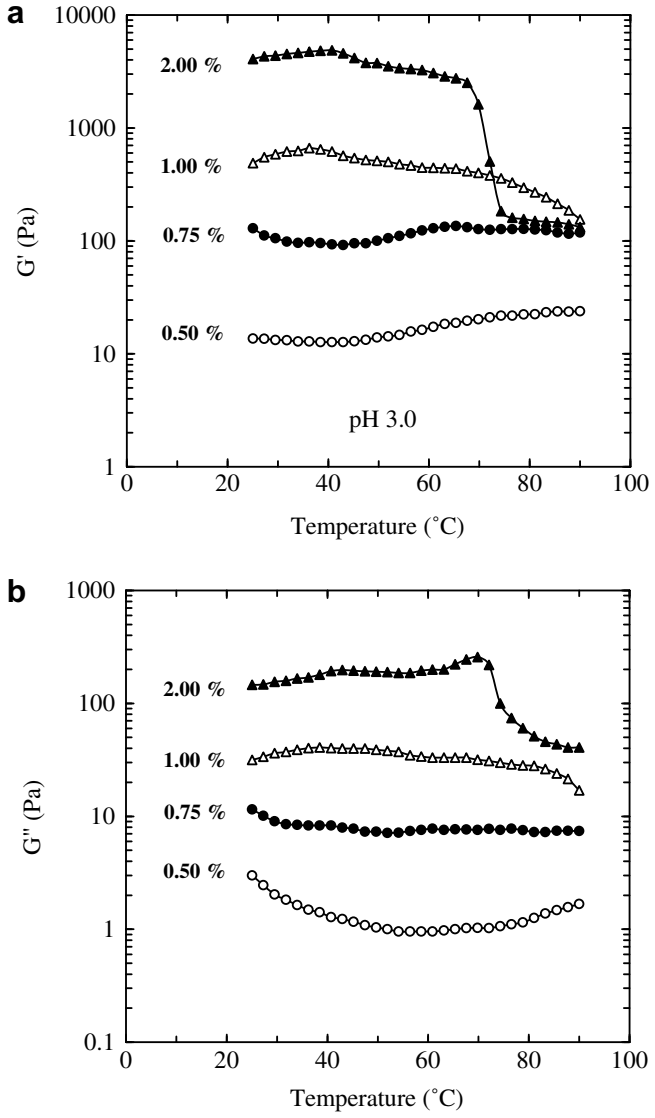

Fig. 17. Variation of (a) G' and (b) G'', measured at 1 rad s^{-1} and 0.5% strain during heating at 1 °C/min, for preparations of HM pectin with 60 wt% sucrose acidified to pH 3.0 with GDL, at pectin concentrations (wt%) of 0.50 (○), 0.75 (●), 1.00 (△) and 2.00 (▲).

formed at high temperature may have an important role in determining the structure of the strong gels obtained on cooling, and their response to re-heating.

On cooling, the hydrophobic junctions formed at high temperature dissociate, and hydrophilic junctions are formed. Analysis of thermograms from differential scanning calorimetry (DSC) studies of acidified preparations of HM pectin in the presence of various cosolutes (Tsoga et al., 2004a) indicates that the temperature ranges over which these two processes occur overlap with one another, and that the converse processes on heating (dissociation of hydrophilic junctions and formation of hydrophobic junctions) occur over essentially the same, overlapping, ranges of temperature. Thus the pectin does not revert to the solution state at any stage of cooling or heating, introducing a mechanism by which structures formed in one range of temperature may influence those formed over the other range.

It seems likely that hydrophobic association at high temperature will occur preferentially between chain segments with a high content of methyl ester substituents, particularly under conditions that are least favourable to intermolecular association (low concentrations of pectin or pH values above pK_o). It would follow that the un-associated segments will have a higher than average content of free (i.e. non-esterified) carboxyl groups, making them particularly suited to formation of hydrophilic junctions (Gilsenan et al., 2000). This may explain why, except at high concentrations of pectin and low values of pH, where "pregelation" occurs, the gels formed by cooling from high temperature after acidification with citric acid have higher moduli (Figs. 10 and 16) than those obtained by acidification with GDL at 25 °C, where gelation will occur by random percolation from the solution state, with no pre-existing network to direct formation of long hydrophilic junctions between sequences of low ester content.

For conventional biopolymer networks that form on cooling and dissociate on heating, the sharp reduction in G' during thermal dissociation is often accompanied by a maximum in G'', which arises from transient formation of a large sol fraction of network fragments, before these also dissociate. Similar behaviour was ob-

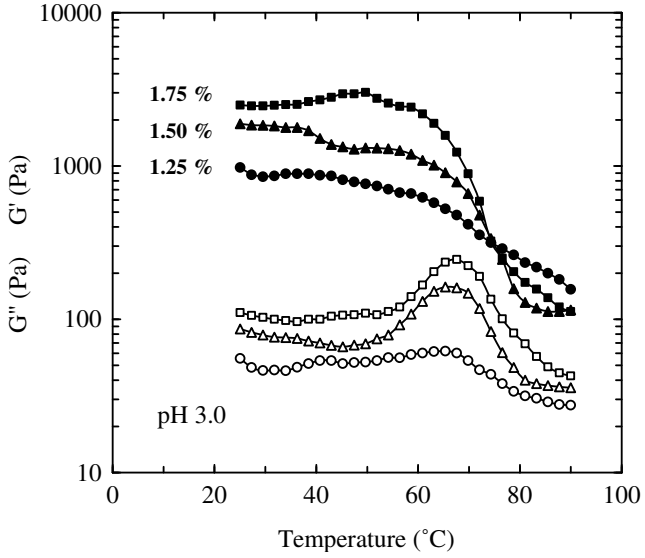

Fig. 18. Variation of G' (filled symbols) and G'' (open symbols), measured at 1 rad s^{-1} and 0.5% strain during heating at 1 °C/min, for preparations of HM pectin with 60 wt% sucrose acidified to pH 3.0 with GDL, at pectin concentrations (wt%) of 1.25 (circles), 1.50 (triangles) and 1.75 (squares).

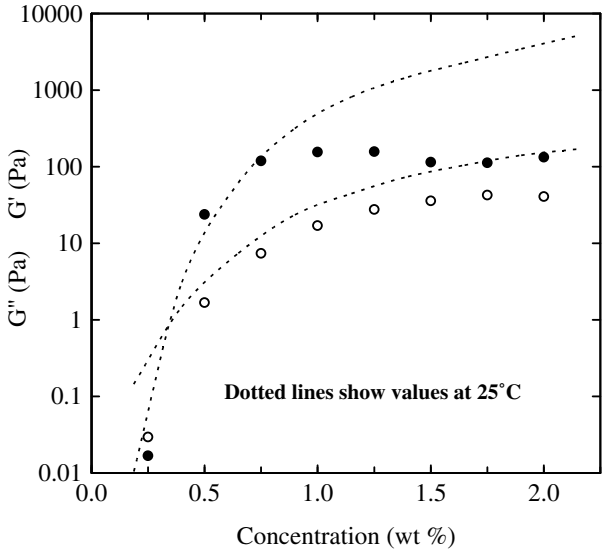

Fig. 19. Concentration-dependence of G' (●) and G'' (○), measured at 1 rad s^{-1} and 0.5% strain after heating to 90 °C, for gels of HM pectin in the presence of 60 wt% sucrose, acidified with GDL to pH 3.0. The dotted lines show the corresponding values after completion of holding for 16 h at 25 °C (Fig. 16a).

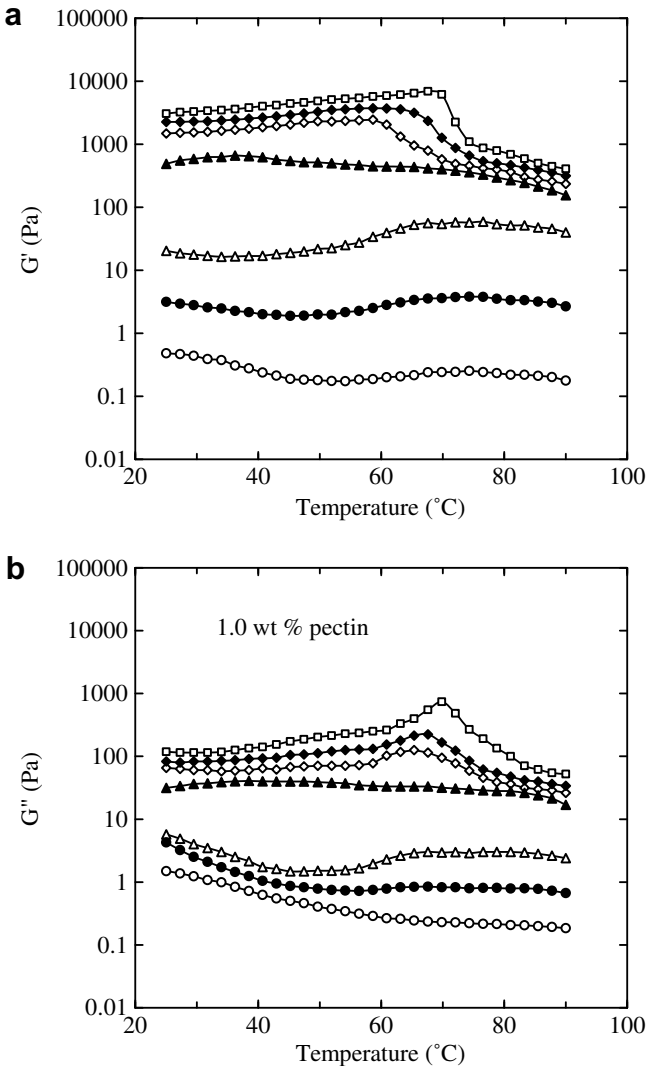

Fig. 20. Variation of (a) G' and (b) G'', measured at 1 rad s^{-1} and 0.5% strain during heating at 1 °C/min, for gels of 1.0 wt% HM pectin in the presence of 60 wt% sucrose, acidified with GDL to give final pH values (after 16 h at 25 °C) of: 3.75 (○), 3.50 (●), 3.25 (△), 3.00 (▲), 2.75 (◇), 2.50 (♦) and 2.25 (□).

served, over the temperature range ~60–80 °C, when the HM pectin gels formed with GDL at 25 °C were then heated to 90 °C at pectin concentrations above ~1.0 wt% for samples acidified to pH 3.0 (Figs. 17 and 18) and at pH values below ~3.0 for samples incorporating 1.0 wt% pectin (Fig. 20).

One interpretation might be that the extent of hydrophobic association during heating is insufficient to compensate for loss of the hydrophilic junctions formed during holding at 25 °C, and that the networks dissociate towards solutions of individual pectin chains. This, however, seems unlikely, for two reasons. First, the analysis of DSC traces by Tsoga et al. (2004a) indicates that thermal dissociation of hydrophilic junctions is complete at temperatures well below the range of the steep reductions in G' observed (Figs. 17, 18 and 20) for the GDL-induced networks. More directly, however, the reductions were seen only under conditions that would be expected to promote, rather than limit, intermolecular association (high concentrations of pectin and low values of pH). At lower concentrations (Fig. 17a) and higher pH (Fig. 20a), increases in G' were observed over roughly the same temperature range as the sharp decreases seen under conditions more favourable to association of the pectin chains. The obvious interpretation is that the decreases come, not from insufficient hydrophobic association, but from excessive association of chains into large aggregates, leading to collapse of network structure.

However, no sharp reductions in G' during heating were observed (Figs. 4a and 6a) for the gels formed by cooling after acidification with citric acid at high temperature, indicating that segregation of the pectin chains into regions of low ester content (giving strong hydrophilic junctions) and regions of high ester content (suited to hydrophobic association) does not only increase gel

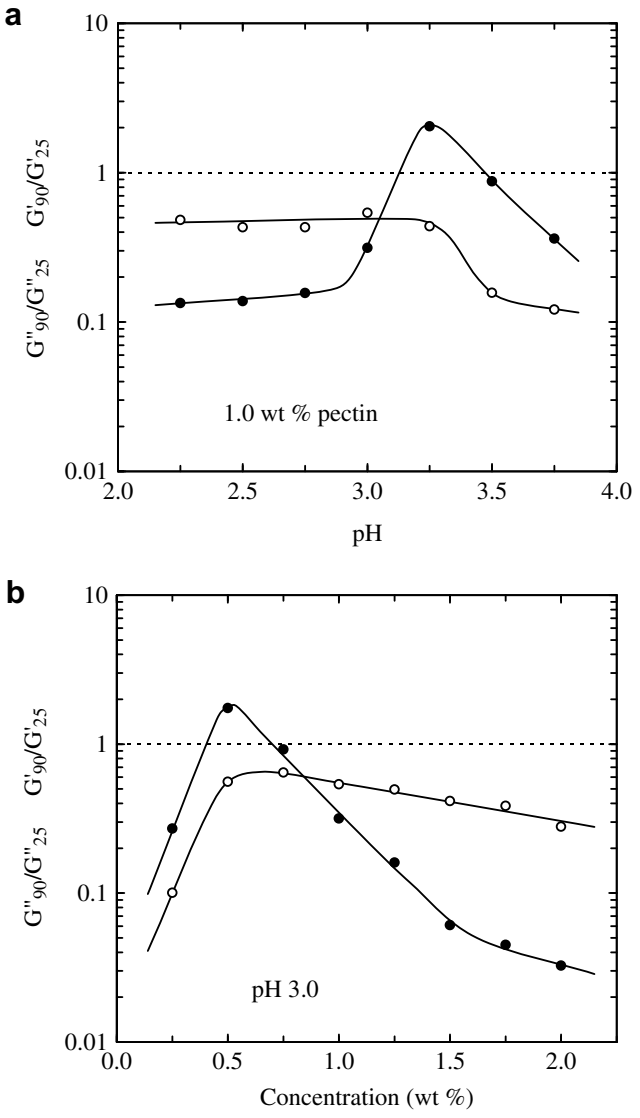

Fig. 21. Values of G' (filled symbols) and G'' (open symbols) after heating to 90 °C divided by the corresponding values at 25 °C before heating, for gels of HM pectin in the presence of 60 wt% sucrose, acidified with GDL. (a) Variation with pH at fixed pectin concentration of 1.0 wt%. (b) Variation with pectin concentration at a fixed pH of 3.0.

moduli, but also gives networks that are more resistant to collapse during heating.

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