Rheological characterization of acid pectin samples in the absence and presence of monovalent ions

Anna Ström [a,b,*], Erich Schuster [c], Suk Meng Goh [d]

[a] Applied Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, 412 96 Göteborg, Sweden
[b] SuMo BIOMATERIALS, VINN Excellence Center, Chalmers University of Technology, 412 96 Göteborg, Sweden
[c] Department of Structure and Material Design, The Swedish Institute for Food and Biotechnology, SIK, Göteborg, Sweden
[d] Singapore Institute of Technology, 10 Dover Drive, Singapore 138683, Singapore

ARTICLE INFO

ABSTRACT

Pectins are traditionally divided into two groups, high methoxy and low methoxy. The groupings determine the charge of the pectin and the gelation mechanism. However, not as yet extensively studied is the impact on gelation of the distribution of the charges as characterized by an absolute degree of blockiness (DB_{abs}). The aim of this study was to characterize rheologically the acid gelation of a pectin with a high DB_{abs} and a degree of methyl esterification of ∼37%, in the absence and presence of monovalent ions. The results obtained suggest that a pectin with a blocky charge distribution at pH conditions close to or below the pKa exhibits weak gel-like properties at intermediate frequencies, despite the absence of a permanent network structure. The addition of monovalent ions changed the rheological behavior to resemble that of a strong gel whose properties depended on the type and concentration of the ions.

1. Introduction

Pectin is a biopolymer used in both the food and pharmaceutical industries, mainly for its gelling and thickening abilities (Thakur, Sing, Handa, & Rao, 1997; Rinaudo, 2008). Pectin describes a family of oligosaccharides and polysaccharides that have common features but are diverse in their fine structures (Willats, Knox, & Mikkelsen, 2006). Commercial pectin contains a high degree of homogalacturonan and a minor amount of neutral sugars (Kravtchenko, Voragen, & Pilnik, 1992). The homogalacturonan is a negatively charged polymer and consists of α-(1,4)-linked D-galacturonate units. The galacturonate units can carry a methyl ester group on the C-6, and the degree of methylation (DM) of the pectin influences its network formation capabilities. Pectins are traditionally divided into two groups, high methoxy (HM) (>50% methyl esters) and low methoxy (LM) (<50% methyl esters). The former forms gels under acid conditions in combination with a high sugar concentration, while the latter forms gels in the presence of most multivalent ions according to the egg-box model (Grant, Morris, Rees, Smith, & Thom, 1973), under acid conditions (Grant et al., 1973; Gilsenan, Richardson, & Morris, 2000) and in the presence of 0.2 M monovalent cations (Yoo, Fishman, Savary, & Hotchkiss, 2003).

Pectins extracted from their sources typically have a high degree of methyl esters in their natural form, and therefore de-esterification is used to produce pectins with lower degrees of methyl esters (May, 1997). De-esterification can be performed using acids, alkalis or enzymes, where the ester groups on the pectin are removed in a random manner. Through the use of certain enzymes, pectin can be de-esterified in a "blockwise" manner, resulting in a blockwise distribution of the galacturonate residues (Garnier, Axelos, & Thibault, 1993; Christensen, Nielson, Kreiberg, Rasmussen, & Mikkelsen, 1998; Hotchkiss et al., 2002).

The distribution pattern of the ester groups and galacturonate residues has been shown to affect the calcium sensitivity of pectin (Hotchkiss et al., 2002) and thus also its gelation behavior (Willats et al., 2006; Ström et al., 2007). The distribution of the methyl esters can be expressed as the degree of blockiness (DB) or the absolute degree of blockiness (DB_{abs}). The DB is calculated from the amount of monomer (1^0), dimer (2^0), and trimer (3^0) of galacturonate residues produced when pectin is incubated with endopolygalacturonase, divided by the amount of free galacturonate residues

* Corresponding author at: Department of Chemical and Biological Engineering, Chalmers University of Technology, 412 96 Göteborg, Sweden. Tel.: +46317723406.
E-mail address: anna.strom@chalmers.se (A. Ström).

present in the pectin sample (Daas, Voragen, & Schols, 2000).

$$DB = \frac{\left(1 \times 1^0 + 2 \times 2^0 + 3 \times 3^0\right) \times M_w^{GalA}}{\left(1 - DM/100\right) \times m_{pectin} \times (m_{uronicacid}/m_{pectin})} \times 100 \qquad (1)$$

The DB_{abs} is based on the same idea, but instead of the amount of mono-, di- and trimer liberated by enzymatic digestion being related to the total amount of free galacturonate residues in the pectin, it is related to the total amount of galacturonate (including methyl-esterified residues) as defined by Guillotin et al. (2005).

$$DBabs = \frac{\left(1 \times 1^0 + 2 \times 2^0 + 3 \times 3^0\right) \times M_w^{GalA}}{m_{pectin} \times (m_{uronicacid}/m_{pectin})} \times 100 \qquad (2)$$

Ström et al. (2007) showed that the DB or the DB_{abs}, has a large impact on the capability of the pectin to form gels.

Polygalacturonate in dilute solutions is thought to change from an extended two-fold (2_1) rotational symmetry to a more compact three-fold (3_1) rotational symmetry in response to pH reduction at a fixed temperature (Cesàro, Ciana, Delben, Manzini, & Paoletti, 1982; Gilsenan et al., 2000). The three-fold rotational symmetry is stabilized by intramolecular hydrogen bonding (Walkinshaw & Arnott, 1981). Intermolecular hydrogen bonds are formed between protonated and unprotonated carboxyl groups and between carboxyl groups and O(3) on a neighboring anti-parallel pectin helix (Walkinshaw & Arnott, 1981).

Acidification of more concentrated LM pectin with a random charge distribution gives strong thermally reversible gels, and it was suggested that the (3_1) rotational symmetry obtained due to pH reduction will reform into a (2_1) rotational symmetry at higher temperature for a fixed low pH (Gilsenan et al., 2000). However, nuclear magnetic resonance and circular dichroism (CD) studies suggest that both (2_1) and (3_1) helices are present in calcium pectate solids and gels (Jarvis & Apperley, 1995). Lootens et al. (2003) reported on the acid gelation of amidated LM pectin and compared it to random charge distribution pectin, and it was observed that the amidated pectin formed a considerably stronger acid gel. It was thought that for the amidated pectins the amide groups reinforce the gel structure via hydrogen bonding (Lootens et al., 2003).

We intended, in this study, to investigate the rheological properties of blocky charge distribution pectin samples, as compared to random charge distribution pectin samples, at pH conditions close to the pKa, in the absence and presence of monovalent ions.

2. Material and methods

2.1. Materials

The pectin used in this study was kindly provided by CP Kelco (Lille Skensved, Denmark) with the following analytical information: DM of ~37% and a galacturonic acid content of ~89%. The pectin had been de-esterified at 45 °C using a PME extracted from papaya, at a pH of 4.5 and an NaCl concentration of 0.4 M. A conventional pectin (LM12 not standardized with sugar) of DM 35% obtained from CP Kelco was also used.

2.2. Methods

The determination of DB and DB_{abs} was performed by incubating the pectin with endopolygalacturonase [EC3.1.2.15] (Bussink, Kester, & Visser, 1990). The oligomers were analyzed using capillary electrophoresis according to a method previously described (Ström & Williams, 2004; Ström et al., 2007), giving a DB of ~57% and DB_{abs} of ~36%. The DB and DB_{abs} of the conventional pectin were 40% and 26%, respectively. The starting pectin solutions were prepared at concentrations of 2–3 wt% and were diluted to the desired end concentrations using either deionized water or salt (NaCl, KCl, LiCl)

solutions. Both pectin and dilution solutions were heated to 45 °C prior to being mixed together. The solutions were then acidified using hydrochloric acid (0.1 N or 1 N) at 45 °C. To achieve higher pH values, 1 M sodium hydroxide (NaOH) was used. Dilution and addition of acid or base was done at elevated temperature (45 °C), as most samples behaved as solutions at this temperature. This minimized the effect of shear or inhomogeneities forming upon acidification or salt addition.

2.2.1. Rotational viscometry

The viscosity of the pectin was measured using an AR 1000 from TA Instruments (Eschborn, Germany). The geometry used was a cone ($\phi = 40$ mm, truncation = 71 μm, angle = 1.59°) and plate. The viscosity was recorded at $T = 20$ °C and between shear rates of $0.03\ s^{-1}$ and $1000\ s^{-1}$.

2.2.2. Oscillatory rheology

Tests of the viscoelastic properties of the samples were performed using the same cone–plate geometry on the same rheometer. The sample loading area was pre-heated to 50 °C prior to loading of the pectin sample, which itself had been preheated to 45 °C. After loading, the sample was quickly quenched to 5 °C and equilibrated at 5 °C for 30 min before measurement started. Oscillatory measurements were conducted at 5 °C to measure the storage and loss moduli (G' and G''). In addition, the response to temperature sweeps (5–40 °C and 40–5 °C) at a rate of 2 °C/min was measured, from which setting and melting temperatures were established. The setting temperature was defined as the temperature at which G' rose above G'' on cooling or dropped below G'' on heating. In these measurements, a constant strain of 0.5% and a constant frequency of 6.28 rad/s were used. Samples were measured in triplicate and variations of the moduli during time sweep and melting and setting temperature were within 10%. A further frequency sweep at $T = 5$ °C was conducted on selected samples. The pH meter used was a Schott CG-825 and was calibrated between pH 7 and pH 4 before each experiment.

3. Results

3.1. Rheological characterization of pectin samples in the absence of monovalent ions

The apparent viscosity as a function of pectin concentration and pH of two pectins that are close in value of DM, ~35%, but differ in
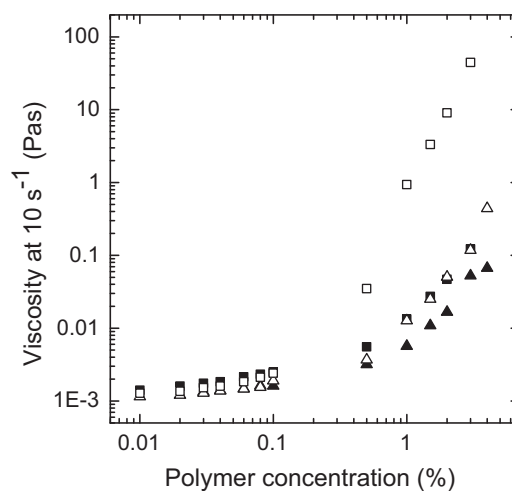

Fig. 1. Viscosity at a shear rate of $10\ s^{-1}$ as a function of pectin concentration at pH 5 (triangles) and pH 3 (squares) for pectin with similar degree of methylation but with random (solid symbols) and blocky (open symbols) charge distribution.
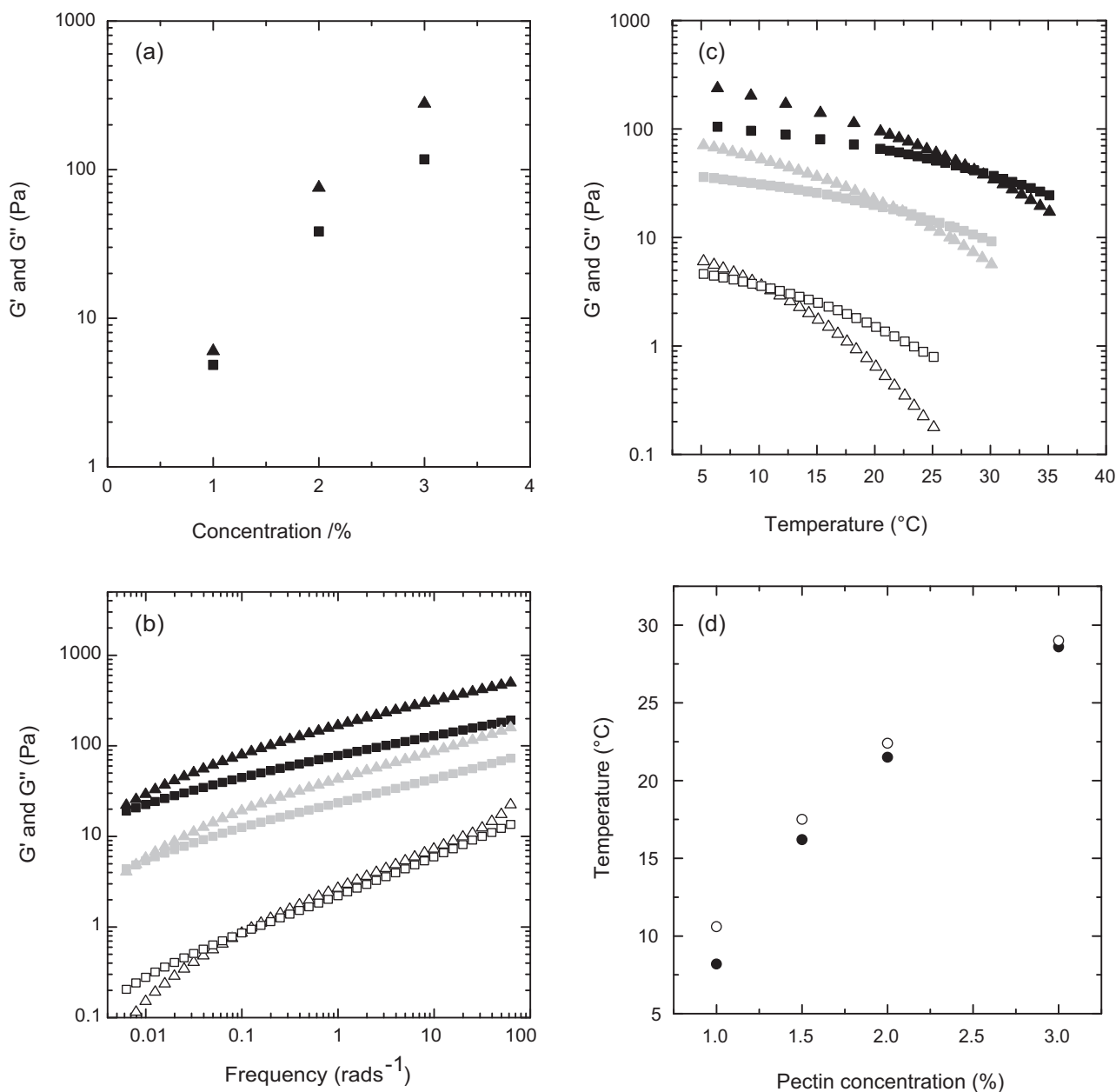
Fig. 2. Oscillatory rheology on blocky pectin gels, where G' (triangles) and G'' (squares) of pectin samples at pH 3 and as a function of (a) pectin concentration at $T = 5\,°C$; (b) frequency sweep where black symbols represent 3% pectin, gray 2% pectin and open 1% pectin at $T = 5\,°C$; (c) temperature reduction where black symbols represent 3% pectin, gray 2% pectin and open 1% pectin; and (d) temperature at which $G' = G''$ upon temperature reduction (filled symbols) and temperature increase (open symbols) of pectin samples. All tests were performed at $T = 5\,°C$, $f = 6.28\,rad/s$, pH = 3 and strain = 0.5%, unless otherwise indicated.

their absolute degree of blockiness (36% and 26%, respectively), is shown in Fig. 1. It can be observed that the slope of the apparent increase in viscosity is higher for the blocky pectin compared to the less blocky pectin at pH 3. Similar behavior of the two pectins was observed at pH 5, which indicates that the behavior at this pH was independent of their absolute blockiness. The estimated overlap concentration c* obtained for the blocky pectin at pH 5 is 0.4%, close to the value obtained for the random charge density pectin at the pH and temperature. At pH 3, however, the value of c* of the blocky pectin was close to 0.25%.

Oscillatory measurements show that the moduli of the sample prepared using the blocky pectin increased as the pectin concentration increased from 1.0% to 3.0% at a fixed pH value of 3.0 (Fig. 2a). At the same time, tan δ (tan $\delta = G''/G'$) decreased from 0.63 to 0.35, showing an increased importance of the elastic component with increasing polymer concentration. On the other hand, loss moduli

were always higher than storage moduli for the non-blocky pectin at the pH and temperature tested (data not shown).

The mechanical spectrum at the lowest pectin concentration tested (1%) is typical of a gelling sample where the degree of crosslinks is just sufficient to form a continuous network. Log G' and log G'' varied linearly with log ω over the frequency range studied and with the same slope (Fig. 2b). Upon increase in pectin concentration, the value of G' was higher than G'' at frequencies between 0.02 and 80 rads^{-1}. It appears that the slopes of log G' were slightly higher than the slopes of log G''. The mechanical spectra further suggest a crossover of G' and G'' at low frequency. Pre-shearing the samples at different shear rates prior to oscillatory measurements showed a fast recovery independent of pre-shear speed (1 s^{-1}, 10 s^{-1}, 100 s^{-1}), and the moduli recovered to the same absolute values regardless of the pre-shear rate (data not shown).
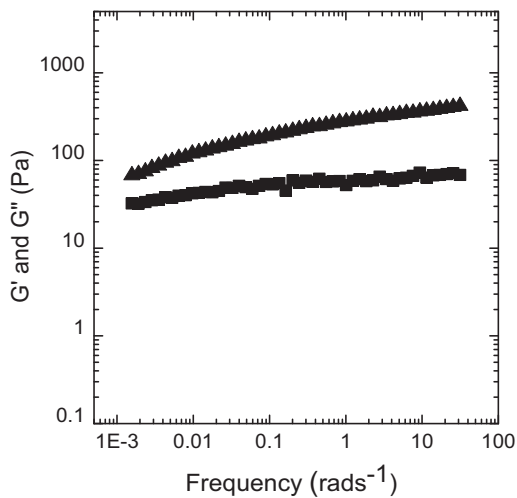
Fig. 3. Frequency sweep of G' and G'' at a strain of 0.5%. $T = 5\,°C$, pH = 2.5 and pectin concentration = 2%.

The data for the temperature sweep of the pectin samples are presented in Fig. 2c. Reducing the temperature increased the moduli of all samples, and increasing pectin concentration increased the setting temperature of the pectin system at pH 3. There was little hysteresis between the setting and melting temperatures at pH 3 (Fig. 2d).

A frequency sweep at pH 2.5 shows similar mechanical spectra as at pH 3, where $\log G'$ and $\log G''$ varied linearly with $\log \omega$ over the frequency range studied, and with the same slope. One can observe the tendency of a cross-over ($G'' > G'$) at low frequencies (<0.001 rad/s) (Fig. 3).

The acid pectin sample containing 3% pectin shows a power law response as $\log \eta^*$ is plotted against $\log \omega$ (Fig. 4). The slope of the dynamic viscosity is -0.77, thus giving a power law relation of $\eta^* = a\omega^{-0.77}$.

Visually, all samples were transparent and clear; no indication of coarsening or large-scale heterogeneity was observed. This is in contrast to what has been reported for random LM pectin (Lootens et al., 2003).
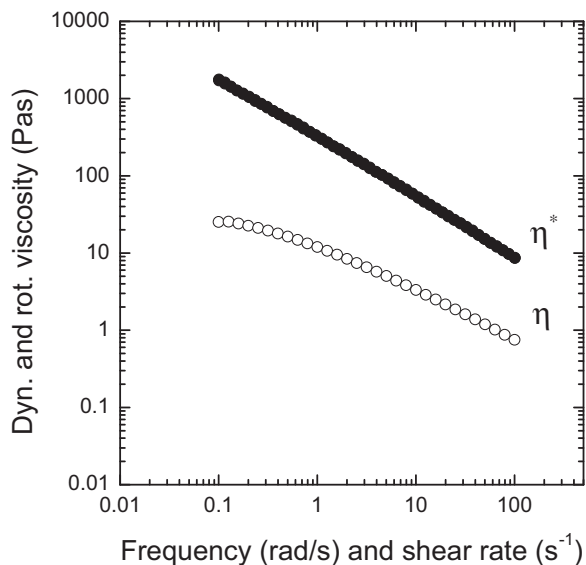

Fig. 4. η^* and η as a function of frequency and shear rate. $T = 5\,°C$, pH = 3.0, pectin concentration = 3% and strain of 0.5%.

3.2. Rheological characterization of acid pectin samples in the presence of monovalent ions

The addition of NaCl to the acid pectin gels at pH 3 increased G', while $\tan \delta (G''/G')$ was reduced from 0.65, 0.47 and 0.14 to 0.07 upon addition of 0, 0.05, 0.1 and 0.2 M NaCl, respectively (Fig. 5a). The mechanical spectra of the pectin sample with increasing amount of salt (Fig. 5b) showed high absolute values of moduli, and more importantly, a larger difference between G' and G'' and a lower frequency dependence. In addition to the increase in G' and G'', the gelation and melting temperatures also increased (Fig. 5c), as did the thermal hysteresis of the sample where NaCl was present. In some cases, such as that corresponding to pH 2.45 with 0.05 M NaCl, the melting temperature of the gel was above the preparation and testing temperature of $45\,°C$. These gels could be visually observed to be syneretic and inhomogeneous. Rheological tests on these conditions were not done, since the high temperature required to melt the gel could cause evaporation (cone and plate geometry used) and lead to unreliable data. The use of concentric cylinder geometry would reduce the effect of evaporation; however, this would have required quantities of pectin sample larger than those available in this study.

The effect of the addition of 0.05 M of LiCl, NaCl and KCl on G' as a function of time is shown in Fig. 6. The sample is loaded on the rheometer at a temperature above gel formation for the different systems ($40\,°C$) and subsequently quenched to $5\,°C$ with a rate of $8\,°C/min$. KCl is shown to be most effective in promoting network formation where the strength of the network measured in G' is higher and the difference between G' and G'' is larger ($\tan \delta = 0.27$) than observed for the addition of NaCl and LiCl ($\tan \delta = 0.40$ and $\tan \delta = 0.44$, respectively). LiCl had the least impact on the absolute value of G' and is also the system where G' and G'' are the closest. Furthermore, there is a continuous increase in G' over time in the presence of KCl. Fig. 7 shows the effect of pH on the values of G' measured for 1% blocky pectin after holding for 2400 s at $5\,°C$ in the presence of 0.05 M LiCl, NaCl or KCl. There is a progressive increase in G' with decreasing pH, which is particularly evident for the mixtures of pectin with KCl. At all pH values studied, KCl gave the highest moduli and LiCl the lowest (i.e. the same order as in the time curves shown in Fig. 6 for a fixed pH of 3.0). With the exception of LiCl at pH 2.5, the mixtures had higher G' than pectin alone at the same values of pH (by a factor of ~5 for KCl).

As pH was reduced from 3 to 2.5, $\tan \delta$ varied from 0.26 to 0.22 for the samples containing KCl, 0.45 to 0.47 for the samples containing NaCl, 0.44 to 0.45 for the samples containing LiCl and 0.77 to 0.5 for the pectin samples at 1% with no added salt. It is further worthwhile to note that upon addition of urea, G' was reduced from 16 to 3 Pa and the $\tan \delta$ was increased from 0.47 to 1 for the 1% pectin samples containing 0.05 M NaCl (data not shown).

4. Discussion

The term "weak gel" was introduced (Ross-Murphy, 1984) to describe systems that give a gel-like response to small deformations but show a solution-like response to higher stress. These gels are distinguish from conventional ("true") gels that are weak only in the sense of having low moduli. Usually, weak gels give mechanical spectra with greater frequency-dependence of G' and G'' and smaller separation between the two moduli (i.e. higher $\tan \delta$) than true gels. Additional distinction of true and weak gels is that true gels fracture under stress, with little, if any, subsequent recovery of a continuous network, whereas weak gels yield and flow, with rapid recovery of their gel-like character when the stress is removed Another characteristic feature of weak gels is that, unlike solutions of entangled polymer coils, they violate the Cox–Merz
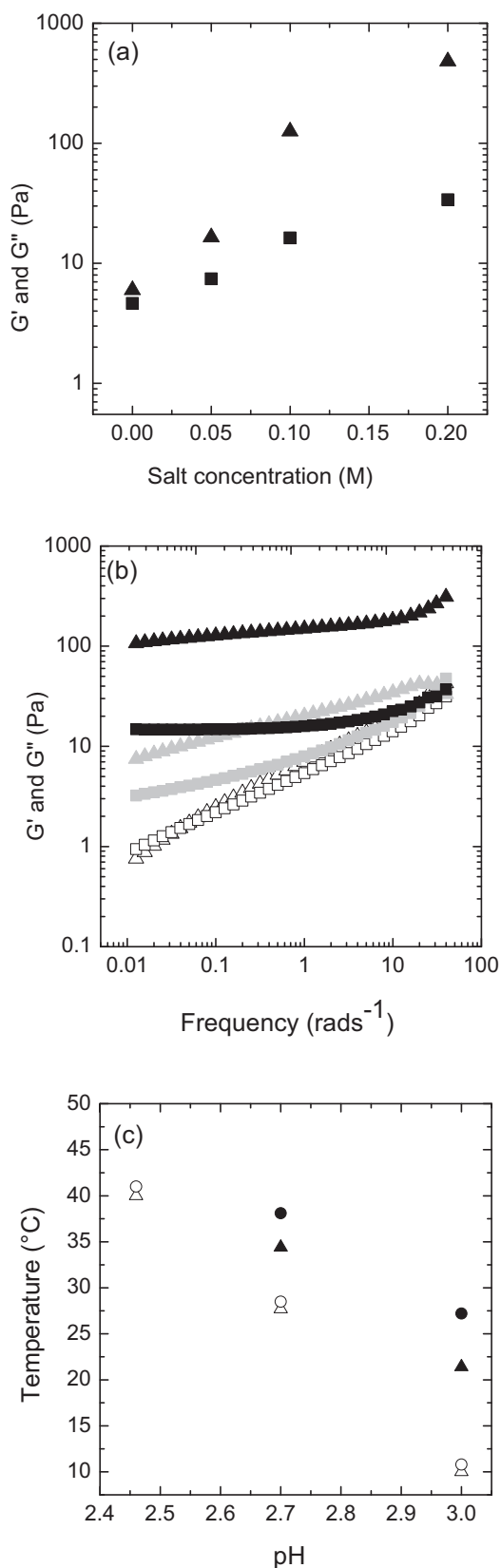
Fig. 6. Storage moduli as a function of time for 1% pectin samples at pH 3 with the addition of 0 M salt (open symbol) and 0.05 M of LiCl (light gray), 0.05 M NaCl (dark gray) and 0.05 M KCl (black). The temperature to the left of the vertical dotted line in the symbol is decreasing from 40 °C to 5 °C over time, while the temperature is kept constant at $T = 5$ °C to the right of the dotted line. Measurements were done at a strain = 0.5% and $f = 6.28$ rad/s.

rule (Cox & Merz, 1958), giving complex dynamic viscosity (η^*) greater than steady-shear viscosity (η^*) at equivalent numerical values of frequency (rad/s) and shear rate (1/s). By these criteria, the blocky pectin studied in the present investigation show weak gel like properties.

One interpretation of the properties of weak gels, proposed initially for ordered xanthan, is that they have a network structure similar to that of true gels but that the junctions are weaker and more dynamic (e.g. Ross-Murphy, 1984; Morris, Nishinari, & Rinaudo, 2012). However, in an investigation of ordered kappa carrageenan in the presence of NaI, which inhibits the helix-helix aggregation required to give true gels, Ikeda and Nishinari (2001) observed weak gel-type properties of the sample (that is violation of the Cox–Merz rule, frequency-dependence of G' and G'' and rapid recovery of the mechanical spectra even after shearing at $1000 \, s^{-1}$ for 30 s). They conclude that even nonaggregated

Fig. 7. The influence of type of ions added at a concentration of 0.05 M to a 1% pectin sample as a function of pH and at $t = 2400$ s, where the line between the symbols is added to aid the eye; addition of KCl (black), NaCl (dark gray), LiCl (light gray) and no added salt (open symbol). Measurements were done at $T = 5$ °C, strain = 0.5% and $f = 6.28$ rad/s.

Fig. 5. Influence of NaCl concentration on acid pectin samples where the pectin concentration is 1%. (a) G' (triangles) and G'' (squares) at pH 3 at a strain of 0.5% and $f = 6.28$ rad/s at $T = 5$ °C. (b) Frequency dependency of samples containing different amount of NaCl from 0 M (open symbols), 0.05 M (gray symbols) to 0.1 M (black symbols) at $T = 5$ °C. (c) Gelation (triangle) and melting (circle) temperatures in the absence of NaCl (open symbols) and in the presence of 0.05 M NaCl (solid symbols) at various pH.
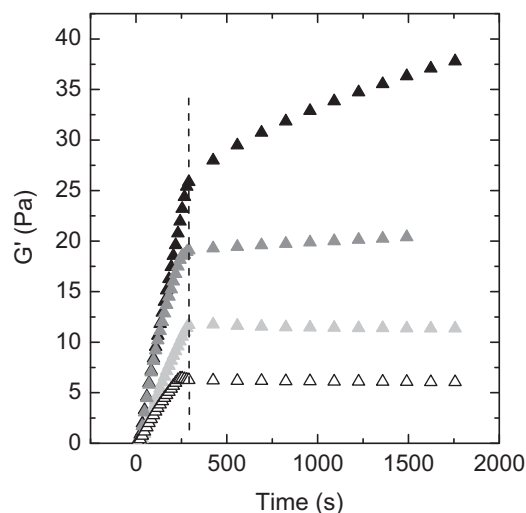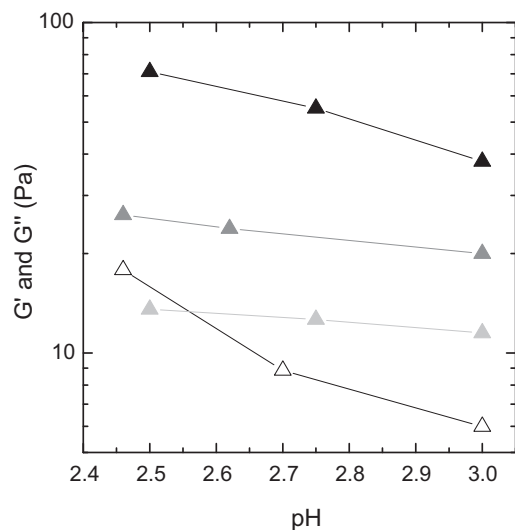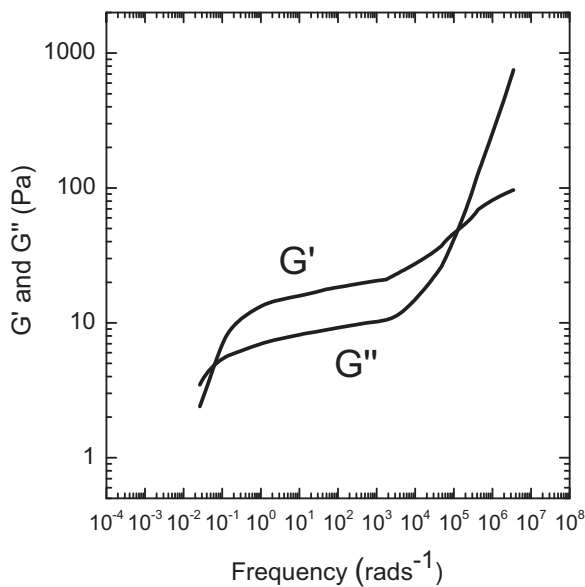
Fig. 8. The frequency-dependent storage modulus G' and loss modulus G'' for a 1% DM 42 blocky pectin gel at pH 2 reported by Vincent et al. (2013). The MSD of the microrheological data (Vincent et al., 2013) was mapped onto G' and G'' using estimation method for the generalized Stokes–Einstein equation by Mason (2000).

helical conformers of carrageenan are rigid enough for their entanglements to exhibit gel-like visco-elasticity without the formation of a permanent network structure (Ikeda & Nishinari, 2001). Similar behavior is indeed observed for the pectin sample at pH 3, albeit at lower shear rates than the suggested $1000\,s^{-1}$. It cannot be concluded from this study alone whether the weak gel like rheological properties of the current blocky pectin sample arise from weak side-by-side associations arising from e.g. co-operative attraction, or via entanglement of rigid structures as proposed in the case of kappa carrageenan in the presence of NaI (Ikeda & Nishinari, 2001). The common feature for the two proposals is that the polymer must be conformationally ordered.

The analysis of microrheological data on acid gels at pH 2 reported by Vincent, Mansel, Kramer, Kroy, and Williams (2013) indicate further the presence of transient structures or junctions (although measured using a DM 42 blocky apple pectin. Microrheology enabled the authors to measure the mean square displacement (MSD) of 500 nm—sized polystyrene beads over a time scale of more than 8 orders of magnitude. The MSD data obtained were converted into the mechanical spectra G' and G'', using the generalized Stokes–Einstein equation (Mason, 2000) (Fig. 8). The data show systems which were slightly weaker but still of an order of magnitude similar to that found in the current work; furthermore, a cross-over of G' and G'' at low frequencies was observed. Finally, the slopes of G' and G'' were found to be similar to the pH 2.5 gels investigated in this paper. In addition to measuring the mechanical spectrum, the high frequency regime of the microrheological data yielded information about the relaxation and dynamics of the constituent polymer chains in the gel. Vincent et al. (2013) found that MSD for pectin chains at pH 2 follows at short times a power law of 3/4, which is characteristic for semiflexible polymers. In other words, the observed pectin at pH 2 is in a stiff conformation, responding faster than flexible chains, which would follow a power law close to 1/2, as described by the Rouse model (Rubinstein & Colby, 2003). The authors concluded that the networked strands of an acid gel are not permanently cross-linked; rather, they described the observed mechanical response by the sliding of crosslinks at intermediate time scales.

The lower c^* obtained for the blocky pectin at pH 3 and the higher increase in viscosity as a function of polymer concentration once above c^* (Fig. 1) would support the hypothesis of a stiffer chain, that is a higher degree of conformational order, in the case of the blocky pectin. However this is only true at low pH, as the two pectins behave similarly at pH 5. It is further noteworthy that while the DM of pectins influences its pKa, a random or blocky charge distribution appears to not influence pKa (Ralet, Dronnet, Buccholt, & Thibault, 2001); thus, the protonation degree of the pectin cannot explain the behavior observed in this study. Instead, the bulk rheology behavior of the pectin with blocky charge distribution could be explained in similar terms, exhibiting transient or sliding crosslinks at intermediate time scales, as observed using microrheology for an acid apple pectin sample (Vincent et al., 2013).

HM pectin, in dilute solutions, is prone to forming aggregations of different kinds, for example, microparticles consisting of microgels, rods and kinked rods. Such structures have been visualized using electron microscopy for pectins extracted from peaches (Fishman, Cooke, Hotchkiss, & Damert, 1993) and later confirmed via AFM studies for pectins extracted from oranges, which further gave evidence to the presence of lateral aggregations of pectin chains (Fishman, Cooke, Chau, Coffin, & Hotchkiss, 2007). Fishman and co-workers suggested that the various aggregations are governed by hydrophobic interactions and hydrogen bonding. While LM pectin has a higher charge than the HM pectin at neutral pH, which reduces the tendency of self-aggregation of pectin chains at neutral pH, the self-aggregation may again be favored at pH under pKa of the pectin where the overall charge is reduced due to protonization of the carboxyl groups. Indeed, the formation of lateral aggregations of pectin chains, as reported in the work performed by Fishman et al. (2007), could explain the proposed stiffened structure, or the higher degree of conformational order, of pectin with blocky charge distribution at low DM and low pH. It would be interesting to further shed light on the potential difference in aggregation behavior between an LM pectin of a blocky charge distribution with one of a random charge distribution at low pH and dilute solutions, following the study performed by Fishman and co-workers using AFM (Fishman et al., 2007).

It was shown, on calcium induced commercial LM pectin gels, that a reduction in pH led to a reduction in CD ellipticity characteristic of formation of egg-box junctions decreased (Gidley, Morris, Murray, Powell, & Rees 1980). The CD ellipticity characteristic of formation of egg-box junctions was replaced progressively by the increase in ellipticity seen for acid-induced gelation of HM pectin, where junctions are formed by sequences in the three-fold conformation. At pH values around the pKa (e.g. pH 3.5) the gels were weakened or abolished by incorporation of either (i) 8 M urea, which inhibits acid-induced gelation but not cation-mediated (egg-box) association, or (ii) polygalacturonate blocks, which inhibit cation-induced gelation by occupying binding sites on intact pectin chains, but have no effect on the junctions induced by acidification. The authors concluded that the dominant mechanism of intermolecular association shifts progressively from cation-mediated association of two-fold chain sequences to acid-induced association with decreasing pH, and that contributions from both types of association are necessary for gelation at pH values close to the pKa.

As illustrated in Fig. 5, true gels (those with low-frequency dependence of G' and G'' and increased temperature hysteresis) were formed on incorporation of monovalent salt at pH 3. As the pH was increased, gelation was suppressed, and at pH 5, neither of the pectin samples studied formed a gel in the presence of monovalent ions, indicating a requirement for some acid-induced association in addition to cation-mediated junctions, in line with the results outlined above.

At pH 3, the influence of the ions was found to increase in the following order: $Li^+ < Na^+ < K^+$. This trend is also observed for the

effectiveness of the same ions in promoting gelation of carrageenan (Morris, Rees, & Robinson, 1980) and gellan (Morris et al., 2012), and in the magnitude of CD change observed (Seale, Morris, & Rees, 1982) on addition of monovalent cations to solutions of alginate poly-L-guluronate sequences, which are closely similar in structure to the poly-D-galacturonate sequences of pectin. In the investigation by Yoo et al., 2003), a similar trend was observed, but at pH 5, where KCl gave the strongest gels; those formed with NaCl were substantially (∼3.3×) weaker, and LiCl gave negligible gel strength, similarly as shown in this study at pH 3 (Figs. 6 and 7). Differences in effectiveness of the monovalent ions to promote load bearing junctions have been explained (Morris et al., 2012) by the efficiency with which cations can occupy specific binding sites on the polymer chains, which will depend on ionic radius or by the alternative explanation based on degree of hydration of the cations (Yoo et al., 2003).

It is clear from this study and others that the mechanism behind gelation of different pectins at slightly different conditions is still not totally explained. The results presented in this particular study show different rheological behavior for LM pectin with a blocky charge distribution at pH below pKa compared to previously known behavior of conventional LM pectin at pH below pKa (Gilsenan et al., 2000; Lootens et al., 2003). Furthermore, the pectin studied here did not form gel in the presence of monovalent ions at pH 5, which has been reported for other blocky pectins of low DM (Yoo et al., 2003, 2009). Instead, the presence of both hydrogen bonds and monovalent ions is required to form a gel exhibiting strong gel characteristics, that is, with no frequency dependence. The differences in rheological properties may originate from differences in length and distribution patterns of free carboxyl groups (Yoo et al., 2009; Kim et al., 2013) as well as variation in tertiary distribution of de-esterified pectin blocks (Fishman et al., 2007).

5. Conclusions

The rheological behavior of a blocky low-methoxy pectin was studied at low pH (2–4) and in the absence and presence of monovalent ions. This study suggests that the pectin at low pH (<3.5) does not form strong gels in the sense that the gel consists of disordered flexible polymer chains linking in ordered junction zones. Instead, the rheological characterization of the acid pectin samples shows the characteristics of a weak gel ($G' > G''$ coupled with frequency dependence) at intermediate frequencies. However, at low frequencies, a shift in rheological properties appears to happen, where G'' becomes larger than G', thus indicating a system without a permanent network structure but with characteristics of a weak gel at intermediate frequencies.

Upon addition of monovalent ions the pectin sample turned into a typical true gel. Little influence of frequency on the moduli and larger hysteresis during temperature sweeps suggests larger aggregates are formed (also observed visually by the sample turning turbid instead of clear and the occurrence of syneresis as the salt concentration increased). It is suggested that the presence of monovalent ions reduces the repulsive charges between pectin chains but the monovalent ions do not induce sufficient salt bridges as the presence of urea reduced the gel strength, indicating that the presence of hydrogen bonds is still required for gelation to occur.

Acknowledgments

References

Bussink, H. J. D., Kester, H. C. M., & Visser, J. (1990). Molecular cloning, nucleotide sequence and expression of the gene encoding pre-pro-polygalacturonase II of *Aspergillus niger*. *FEBS Letters, 273*, 127–130.

Cesàro, A., Ciana, A., Delben, F., Manzini, G., & Paoletti, S. (1982). Physicochemical properties of pectic acid. I. Thermodynamic evidence of a pH-induced conformational transition in aqueous solutions. *Biopolymers, 21*, 431–449.

Christensen, T. M. I. E., Nielson, J. E., Kreiberg, J. D., Rasmussen, P., & Mikkelsen, J. D. (1998). Pectin methyl esterase from orange fruit: Characterization and localization by in-situ hybridization and immunohistochemistry. *Planta, 206*, 493–503.

Cox, W. P., & Merz, E. H. (1958). Correlation of dynamic and steady flow viscosities. *Journal of Polymer Science, 28*, 619–622.

Daas, P. J., Voragen, A. G., & Schols, H. A. (2000). Characterization of non-esterified galacturonic acid sequences in pectin with endopolygalacturonase. *Carbohydrate Research, 326*, 120–129.

Fishman, M. L., Cooke, P. H., Hotchkiss, A., & Damert, W. (1993). Progressive dissociation of pectin. *Carbohydrate Research, 248*, 303–316.

Fishman, M. L., Cooke, P. H., Chau, H. K., Coffin, D. R., & Hotchkiss, A. T. (2007). Global structures of high methoxyl pectin from solution and in gels. *Biomacromolecules, 8*, 573–578.

Garnier, C., Axelos, M. A. V., & Thibault, J. F. (1993). Phase diagrams of pectin–calcium systems: Influence of pH, ionic strength, and temperature on the gelation of pectins with different degrees of methylation. *Carbohydrate Research, 240*, 219–232.

Gidley, M. J., Morris, E. R., Murray, E. J., Powell, D. A., & Rees, D. A. (1980). Evidence for two mechanisms of interchain association in calcium pectate gels. *International Journal of Biological Macromolecules, 2*, 332–334.

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

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

Guillotin, S. E., Bakx, E. J., Boulenguer, P., Mazoyer, J., Schols, H. A., & Voragen, A. G. J. (2005). Populations having different GalA blocks characteristics are present in commercial pectins which are chemically similar but have different functionalities. *Carbohydrate Polymers, 60*, 391–398.

Hotchkiss, A. T., Jr., Savary, B. J., Cameron, R. G., Chau, H. K., Brouillette, J., Luzio, G. A., & Fishman, M. L. (2002). Enzymatic modification of pectin to increase its calcium sensitivity while preserving its molecular weight. *Journal of Agricultural and Food Chemistry, 50*, 2931–2937.

Ikeda, S., & Nishinari, K. (2001). Weak gel type rheological properties of aqueous dispersions of nonaggregated κ-carrageenan helices. *Journal of Agriculture and Food Chemistry, 49*, 4436–4441.

Jarvis, M. C., & Apperley, D. C. (1995). Chain conformation in concentrated pectic gels: Evidence from ^{13}C NMR. *Carbohydrate Research, 275*, 131–145.

Kim, Y., Williams, M. A. K., Galant, A. L., Luzio, G. A., Savary, B. J., Vasu, P., & Cameron, R. G. (2013). Nanostructural modification of a model homogalacturonan with a novel pectin methylesterase: Effects of pH on nanostructure, enzyme mode of action and substrate functionality. *Food Hydrocolloids, 33*, 132–141.

Kravtchenko, T. P., Voragen, A. G. J., & Pilnik, W. (1992). Analytical comparison of three industrial pectin preparations. *Carbohydrate Polymers, 18*, 17–25.

Lootens, D., Capel, F., Durand, D., Nicolai, T., Boulenguer, P., & Langendorff, V. (2003). Influence of pH, Ca concentration, temperature and amidation on the gelation of low methoxyl pectin. *Food Hydrocolloids, 17*, 237–244.

Mason, T. G. (2000). Estimating the viscoelastic moduli of complex fluids using the generalized Stokes–Einstein equation. *Rheologica Acta, 39*, 371–378.

May, C. D. (1997). Pectins. In A. Imeson (Ed.), *Thickening and gelling agents for food* (pp. 124–152). London: Blackie Academic and Professional.

Morris, E. R., Rees, D. A., & Robinson, G. (1980). Cation-specific aggregation of carrageenan helices: Domain model of polymer gel structure. *Journal of Molecular Biology, 138*, 349–362.

Morris, E. R., Nishinari, K., & Rinaudo, M. (2012). Gelation of gellan—A review. *Food Hydrocolloids, 28*, 373–411.

Ralet, M. C., Dronnet, V., Buchholt, H. C., & Thibault, J. F. (2001). Enzymatically and chemically de-esterified pectins; characterisation, polyelectrolyte behavior and calcium binding properties. *Carbohydrate Research, 336*, 117–125.

Rinaudo, M. (2008). Main properties and current applications of some polysaccharides as biomaterials [Review]. *Polymer International, 57*, 397–430.

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

Rubinstein, M., & Colby, R. H. (2003). *Polymer physics*. Oxford: Oxford University Press.

Seale, R., Morris, E. R., & Rees, D. A. (1982). Interactions of alginates with univalent cations. *Carbohydrate Research, 110*, 101–112.

Ström, A., Ribelles, P., Lundin, L., Norton, I., Morris, E. R., & Williams, M. A. K. (2007). Influence of pectin fine structure on the mechanical properties of calcium–pectin and acid–pectin gels. *Biomacromolecules, 8*, 2668–2674.

Ström, A., & Williams, M. A. K. (2004). On the separation, detection and quantification of pectin derived oligosaccharides by capillary electrophoresis. *Carbohydrate Research, 339*, 1711–1716.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin—A review. *Food Science and Nutrition, 37*, 47–73.

Vincent, R. R. R., Mansel, B. W., Kramer, A., Kroy, K., & Williams, M. A. K. (2013). Micro-rheological behaviour and nonlinear rheology of networks assembled from polysaccharides from the plant cell wall. *New Journal of Physics, 15* (art. no. 035002).

Walkinshaw, M. D., & Arnott, S. (1981). Conformations and interactions of pectins 1. X-ray diffraction analysis of sodium pectate in neutral and acidified forms. *Journal of Molecular Biology, 153*, 1055–1073.

Willats, W. G. T., Knox, J. P., & Mikkelsen, J. D. (2006). Pectin: New insights into an old polymer are starting to gel. *Trends in Food Science & Technology, 17*, 97–104.

Yoo, S. H., Fishman, M. L., Savary, B. J., & Hotchkiss, A. T., Jr. (2003). Monovalent salt-induced gelation of enzymatically deesterified pectin. *Journal of Agriculture and Food Chemistry, 51*, 7410–7417.

Yoo, S.-H., Lee, B.-H., Savary, B. J., Lee, S., Lee, H. G., & Hotchkiss, A. T. (2009). Characteristics of enzymatically-deesterified pectin gels produced in the presence of monovalent ionic salts. *Food Hydrocolloids, 23*, 1926–1929.