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Material properties of concentrated pectin networks

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Abstract—We have examined the mechanical behaviour of different types of pectin at high concentrations (>30% w/w), relevant to the behaviour of pectin in the plant cell wall, and as a film-forming agent. Mechanical properties were examined as a function of counterion type (K^+, Ca^{2+}, Mg^{2+}), concentration and extent of hydration. Hydration was controlled in an osmotic stress experiment where pectin films were exposed to concentrated polyethylene glycol [PEG] solutions of known osmotic pressure. We investigated the mechanical behaviour under simple extension. The results show that the swelling and stiffness of the films are strongly dependent on pectin source and ionic environment. At a fixed osmotic stress, both Ca^{2+} or Mg^{2+} counterions reduce swelling and increase the stiffness of the film.

Keywords: Pectin; Network; Mechanical properties; Hydration; Plant cell walls; Film-forming; Ions

1. Introduction

The primary cell wall of dicotyledenous plants consists of cellulose microfibrils dispersed in a network of pectic polysaccharides. The pectic polysaccharides also form the middle lamella, and are involved in cell adhesion. The cell wall provides different mechanical properties at different times during cell development. During cell growth, the cell wall should be sufficiently plastic to permit cell extension. The middle lamella should also exhibit some plasticity to accommodate cell-wall extension. The mature cell wall needs to resist the forces of turgor, and under these circumstances should behave as an elastic solid. The cell-wall assembly has a major influence on the mechanical properties of cells and tissues, although as yet there is a relatively poor understanding of the effect of specific molecular interactions on wall behaviour. Although there is an established literature on the physical chemistry of pectin gel networks,[1–3] their concentration is generally an order of magnitude less than that found in the cell wall.[4] In many ways it is possible to compare the cell-wall structure to that of a fibre-filled elastomer. In these materials the fibres reinforce the matrix, and the properties of the matrix are important to the strength and stiffness of the material. In this article we examine some of the mechanical properties of the pectic polysaccharide matrix at cell-wall concentrations of the polysaccharide.

Structurally the pectic polysaccharides are a heterogeneous grouping,[5–7] showing substantial diversity with botanical origin. They are based on chains of linear regions of (1→4)-α-D-galacturonosyl units and their methyl esters, interrupted in places by (1→2)-α-L-rhamnopyranosyl units. A fraction of these rhamnopyranosyl residues are branch points for neutral sugar side chains of (1→5)-α-L-arabinofuranosyl or (1→4)-β-D-galactopyranosyl residues. Further structural modification can include xylosyl substitution of the main backbone and a limited acetylation. Recent AFM studies have also shown long chain branching of the main backbone.[8,9] From a polymeric perspective, pectin is a complex, branched polyelectrolyte. Factors such as the charge density, and the way that charge is distributed along the backbone is expected to have an important influence on physicochemical behaviour.

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For any polyelectrolyte, ionic interactions have a big impact on material properties. For the plant cell wall and middle lamella, the ionic environment is provided by the apoplast, which typically contains the inorganic cations, K^+, Mg^{2+} and Ca^{2+}.[10] For a fixed degree of methyl esterification, the affinity of the pectic polysaccharide chain for the counterion increases in the order $K^+ < Mg^{2+} < Ca^{2+}$.[11] It is well established that Ca^{2+} counterions can crosslink moderately concentrated pectin solutions, at junction zones, to form three-dimensional gel networks.[2] A requirement for gelation is that there should be a sufficient number of potential junction zones (or sequences of unesterified residues) per molecule to allow the formation of the network. Generally this requirement means that pectins, which gel in the presence of Ca^{2+} ions, have an average degree of methyl esterification of $<50\%$, although pectins with a higher methoxyl content can also gel in this way.

The material properties of such networks should show a strong concentration dependence. Of particular importance to the in vivo behaviour of the pectin network is the way that the hydration of the network is controlled.[4] The pectin network is exposed to the osmotic stress of the cell contents. The hydration of the network is influenced by the balance between that osmotic stress and crosslinking of the network, which tend to restrict swelling, and the affinity of the network for water, which drives swelling. For a tomato pectin network it was proposed that the galacturonic acid residues of the pectin chain can have a dual role.[12] One fraction is involved in network crosslinking, while another fraction drives swelling through a Donnan-type effect. As a result swelling is influenced by factors such as pH and ionic strength. Typical estimates of the in vivo concentration of pectin in the cell wall and middle lamella are in the region of 30% w/w,[4] this compares to pectin gel networks, which have a typical polymer concentration of only a few percent. There are few studies on the behaviour of concentrated systems ($\sim$30% w/w). In this paper we examine their mechanical properties with the aim of determining the effect of pectin type, and counterion on the observed mechanical behaviour.

2. Experimental

2.1. Source of materials

Pectins were obtained from CP Kelco, which were enzyme de-esterified, E (de 71.2%), and chemically de-esterified, C (de 70.6%). Pectin was also prepared from apples, A (Jonagold), using a buffered phenol extraction as described.[1] The cell-wall residue was exhaustively washed with water to remove phenol and the pectin was extracted with 50 mM CDTA in sodium acetate buffer (pH 7.5) at room temperature.

2.2. Characterisation of pectins

The neutral sugar content of the pectins was determined, after hydrolysis, by conversion of the neutral sugars to their alditol acetates, followed by GC.[13] The pectins were fragmented using a chemical procedure to obtain oligomers containing contiguous uronic acid residues.[14] The analysis of the oligomeric products by high-pressure anion exchange chromatography (HPAEC), and electrospray-ionisation mass spectroscopy was carried out as described.[14]

2.3. Viscometry

Measurements of specific viscosity as a function of concentration of pectin (0.01–0.1% w/w) in 50 mM acetate buffer (pH 5.6) were carried out using an Ubbelohde viscometer at 20 °C. The efflux time for buffer was 115 s and no shear rate corrections were made. The intrinsic viscosity $[\eta]$ was calculated from the extrapolation of reduced viscosity to zero concentration.

2.4. Preparation of pectin films

Cast films were prepared by drying 2–3% w/w solutions in 50 mM acetate buffer (pH 5.4) at 42 °C. The dry weight of the air-dried films was determined by vacuum drying at 60 °C over P_2O_5 for 16 h. Films were allowed to hydrate for 24 h in concentrated polyethylene glycol solutions (PEG 20,000), of known osmotic pressure and counterion composition, at room temperature.

2.5. Swelling measurements

Preliminary measurements of the volumetric swelling of the films using a travelling microscope established that the swelling was isotropic. Swelling was determined from the change in mass of the film.

2.6. Mechanical properties

The tensile modulus of films (1 cm × 0.4 cm × 0.005 cm) was determined from force versus deformation measurements in simple extension using a Stable Microsystems® Texture Analyser (TAXT2i). The films were glued to metal supports with a cyanoacrylate adhesive. The mechanical properties were examined in uniaxial extension as a function of deformation, up to a maximum of 0.03, at a rate of $0.002\,s^{-1}$. After the loading phase, the deformation was maintained and the force required to maintain that deformation over 30 s recorded. Finally after 30 s the deformation was allowed to recover at a rate of $0.002\,s^{-1}$, and the force monitored during this unloading phase.

Table 1. Characteristics of the pectins

Sample	Neutral sugar composition (%)					% De	% GA	[η] (mL/g)
	Rha	Ara	Xyl	Gal	Glc			
E	7	41	tr	49	3	71.2	90	532
C	6	29	1	62	3	70.6	85	230
A	7	41	7	26	19	70.0	90	1160

3. Results and discussion

The characteristics of the pectins used in this study are shown in Table 1. Two commercial pectins were used, which were enzyme de-esterified (E), and chemically de-esterified (C), and their behaviour compared to that of a chelator-extractable apple pectin (A). The pectins have a similar uronic acid contents, and average degrees of esterification (~70%). The major neutral sugars found are characteristic of pectins and include arabinose and galactose. The intrinsic viscosities of the pectins were in the range 230–1160 mL g^{-1}, with the apple pectin, extracted under mild conditions, having the largest average size in solution as indicated by its very large intrinsic viscosity. The values obtained indicate that the pectins adopt an expanded conformation in aqueous solution.

Even though the average degree of esterification of the pectins was similar, variations in the way that the unesterified uronic acids was distributed could affect the observed physicochemical behaviour. The pattern of distribution of the uronic acid in the pectin was examined using a chemical fragmentation procedure. The partially esterified pectins were specifically cleaved to leave a series of oligogalacturonic acid residues bearing an arabitol residue as aglycone deriving from an esterified galacturonic acid residue. A chromatographic analysis of the fragments produced from the chemically de-esterified pectin, C, is shown in Figure 1. The chromatogram shows a series of major peaks, progressively decreasing in size. The retention times of the major components, and the masses of their negative ions observed on HPAEC ESI-MS, are shown in Table 2. The major components belong to a series of oligomers of $(GalA)_n$-1,5-di-D-Araol. The structure of the minor components is discussed in Needs et al.[14] For values of n between 2 and 6, the mass fraction of GalA for each oligomer was calculated and presented as a ratio to $GalA_2$ for each of the different pectins. For the pectins,

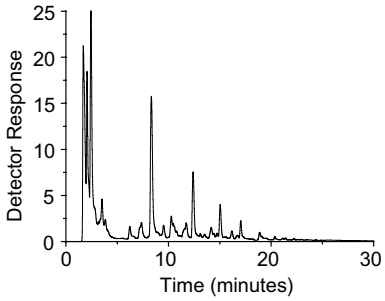

Figure 1. Chromatogram of the products of the chemical fragmentation of pectin C.

the observed ratios are very similar, and differ somewhat from that predicted for a random distribution of galacturonic acid residues.

3.1. Swelling behaviour

The mechanical properties of pectin networks have generally been examined for gel systems. To prepare the concentrated systems, which are representative of the concentrations found in the plant cell wall, an osmotic stress approach was used. A similar approach was used to examine the swelling of cell-wall material extracted from tomato.[4]

The dried pectin films were allowed to hydrate in concentrated polyethylene glycol solutions with an osmotic pressure of 5 MPa, containing the inorganic counterions, K^+, Mg^{2+} and Ca^{2+}, which are found in the apoplast. The swelling of polyelectrolytes is generally dominated by a Donnan-type effect, which decreases with increasing ionic strength and counterion valency. For pectin networks the counterions may also facilitate network crosslinking, which will oppose the swelling force. The swelling behaviour of the pectin films is shown in Table 3. Some general trends can be noted. Irrespective of counterion type and pectin, the swelling

Table 2. Characteristics of products of chemical fragmentation

$(GalA)_n$-1,5-di-D-Araol			z	Mass ratio of products			
n	R_T (s)	m/z		Pectin C	Pectin E	Pectin A	Predicted
2	484	503	−1	1	1	1	1
3	767	679	−1	0.49	0.46	0.44	0.34
4	912	855	−1	0.28	0.26	0.26	0.20
5	1032	515	−2	0.14	0.14	0.10	0.03
6	1131	603	−2	0.06	0.06	0.05	0.01

Table 3. Swelling behaviour of pectin films, expressed as g swollen film/g dry film, at an osmotic stress of 5 MPa

Sample	Ca^{2+} (mM)			Mg^{2+} (mM)			K^{+} (mM)		
	10	30	50	10	30	50	10	30	50
E	2.51	2.30	2.11	2.60	2.44	2.34	2.65	nd	2.46
C	3.06	2.91	2.85	3.12	3.08	3.00	3.20	3.13	3.04
A	1.79	1.46	1.17	2.03	1.75	1.46	2.14	1.92	nd

decreases with increasing ionic strength. This effect is typical of polyelectrolytes and was observed for weakly charged pectic polysaccharides extracted from orange pith[15] and tomato parenchyma.[4] It indicates that the charge on the pectin has a major impact on observed swelling behaviour. At a fixed ionic strength the observed swelling decreases in the order, $K^{+} > Mg^{2+} > Ca^{2+}$. Although it is expected that monovalent counterions generate a higher swelling pressure than divalent counterions, the observed difference in swelling behaviour between films, which have Mg^{2+} and Ca^{2+} counterions, suggests that Ca^{2+} is potentially a more effective crosslinking agent than Mg^{2+}. For a particular counterion at a fixed concentration, swelling increases in the order apple < enzyme de-esterified < chemically de-esterified. Generally the interaction of counterions with a polyelectrolyte is dependent on charge distribution along the polymer backbone.[16] At high charge densities counterions condense on the backbone and therefore do not participate in a Donnan-type swelling effect. For the highly esterified pectins used in the present study with their relatively short sequences of contiguous uronic acid residues this effect should be small. The presence of long sequences, or blocks, of contiguous uronic acid residues is also associated with the ability to form junction zones and crosslinking in gel systems. This crosslinking would also reduce swelling. At the higher pectin concentrations used in the current study, shorter sequences of uronic acid residues might have the potential to function as junction zones. These data demonstrate that the swelling of pectin networks is dependent on pectin type, counterion type and concentration, and that by changing these parameters a range of behaviour is observed. Polymer network concentration has an important influence on the mechanical behaviour of synthetic polymer networks. Through the use of the osmotic stress approach it was possible to prepare pectin networks, which are representative of cell-wall concentrations of pectin.

3.2. Mechanical behaviour

The study focused on the short time behaviour of the pectin networks. The mechanical properties of the hydrated pectin films was investigated in uniaxial extension for deformations <0.03 applied at a constant deformation rate of $0.002\,s^{-1}$. After the loading phase the deformation was maintained and the force required to maintain that deformation recorded as a function of time. Finally after 30 s the deformation was allowed to recover at a rate of $0.002\,s^{-1}$, and the force monitored during this unloading phase. Over the timescales examined the materials behaved as viscoelastic solids. It was experimentally convenient to determine a constant-strain-rate tensile modulus, $F(t)$.

For these small deformations, at the strain rates used, there was a linear relationship between applied force and deformation during the initial loading phase. $F(t)$ was calculated from this linear behaviour and is given in Table 4 for the hydrated pectin films. The modulus was examined as a function of counterion type and concentration and pectin type. The constant-strain-rate tensile modulus ranged from 11 to 166 MPa for the films examined. Some general trends were noted. Irrespective of counterion, the stiffness of the films increased with the ionic strength of the bathing solution. The stiffness of the films was counterion dependent, increasing in the order $K^{+} < Mg^{2+} < Ca^{2+}$. In addition, the constant-strain-rate tensile modulus showed a dependence on pectin type, with the modulus increasing in the order apple < chemically de-esterified < enzyme de-esterified.

An important variable in these experiments is the extent of hydration of the pectin films, which is dependent on type of pectin, counterion and its concentration. The data from the tensile experiments are summarised in Figure 2 where the constant-strain-rate tensile modulus is plotted as a function of the extent of swelling of the pectin film. For all the pectins, the modulus shows a simple inverse linear relationship with the extent of swelling of the film. For a particular pectin, the counterions present influence the extent of swelling of the film, and through this effect, there is an influence on mechanical behaviour. When examined in this way the different pectins show clearly different behaviour, with the apple pectin showing reduced swelling and reduced modulus, and the chemically de-esterified pectin showing comparatively high moduli at comparatively large

Table 4. Tensile modulus (MPa) of pectin films as a function of pectin type and counterion

Sample	Ca^{2+} (mM)			Mg^{2+} (mM)			K^{+} (mM)		
	10	30	50	10	30	50	10	30	50
E	73	117	166	59	82	105	59	nd	83
C	60	83	109	36	63	86	35	57	74
A	15	33	46	16	34	53	11	18	nd

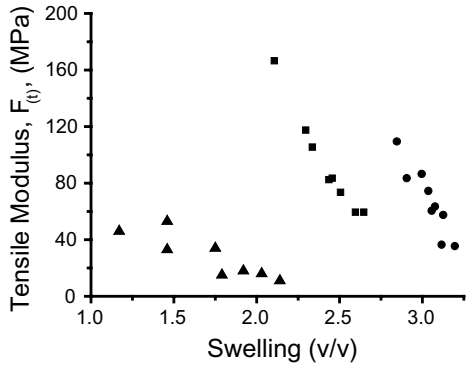

Figure 2. Dependence of tensile modulus, $F(t)$, on swelling of chemically de-esterified (●); enzyme de-esterified (■) and apple (▲) pectins.

extents of swelling. In these systems, the mechanical behaviour at short times <30 s, is mainly influenced by the type of pectin present and its hydration. Counterion type and concentration exert an effect through their effect on the hydration of the network at constant osmotic stress.

The viscoelastic behaviour of the pectin films was examined in more detail through analysis of the stress relaxation. All the hydrated films, which were tested behaved similarly. On maintaining a constant deformation there was a partial relaxation of the applied force. After 30 s this relaxation was approaching a limiting value. Over this timescale the materials behaved as viscoelastic solids. In Figure 3a is shown the relaxation behaviour of the chemically de-esterified pectin in 50 mM concentration of counterion at an osmotic stress of 5 MPa. Under these conditions the relaxation behaviour is similar with no significant effect of counterion type. In Figure 3b is shown the effect of pectin type on the observed relaxation in 50 mM Mg^{2+} at an osmotic stress of 5 MPa. Again the observed differences are not experimentally significant. On removal of the applied stress the deformation was substantially recovered. The form of the relaxation indicates a spread of relaxation times. The data could be fitted to relationships of the form of

$$\phi(t) = \exp[-(t/\tau_0)^{\beta}] \qquad (1)$$

where τ_0 is a relaxation time and β $(\beta > 0 \leqslant 1)$ is a measure of its nonexponentiality, with the applied force required to maintain the deformation approaching a constant value after 100 s. Typical values of τ_0, which were obtained on fitting the short-term relaxation were in the region of 14 s with a value of β of ~0.6. The relaxation indicates a partial structural rearrangement of a heavily entangled polymer network.

A conventional view of the pectin network of the plant cell wall and middle lamella is that the network is crosslinked by Ca^{2+} counterions at junction zones, and that the other ions present in the apoplast do not participate to a significant extent in network crosslinking. The data obtained in this paper suggest an alternative view. Pectin structure as well as counterion type and concentration, affect the swelling response of the pectin network to a fixed osmotic stress. It is this swelling response, which determines the mechanical behaviour in extension of the pectin network. At short times (<30 s) concentrated networks show a similar viscoelastic response on deformation, with the tensile modulus increasing with increasing polymer concentration. The tensile modulus of the most concentrated systems approach the measured moduli of the plant cell wall,[17] suggesting that the pectin network can have a major influence on cell-wall mechanical behaviour. The data further suggest that the short-term mechanical response of the pectin network of the plant cell wall may be modulated through the control of the ionic composition of the apoplast.

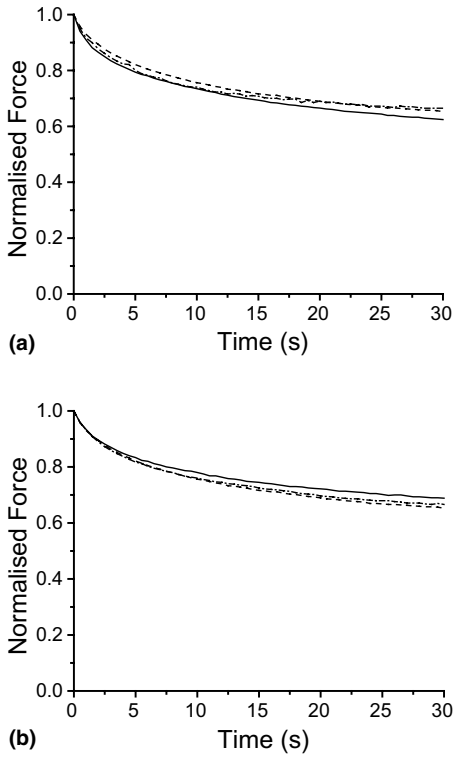

Figure 3. (a) Relaxation behaviour of chemically de-esterified pectin at a counterion concentration of 50 mM Ca^{2+} (—); Mg^{2+} (- - -) and K^{+} (-·-·-). (b) Relaxation behaviour of pectin at a counterion concentration of 50 mM Mg^{2+} for chemically de-esterified (- - -); enzyme de-esterified (—) and apple pectin (-·-·-).

4. Conclusions

The swelling of high methoxyl pectin films at a constant osmotic stress is dependent on source of the pectin. Swelling is also dependent on counterion type and

concentration. The swollen films behave as viscoelastic solids with a simple proportionality between polymer concentration and tensile modulus. The data suggest that the mechanical properties of the pectin network of the plant cell wall may be modulated through the control of the response of the pectin network to the osmotic stress of the cell contents, and the composition of the apoplastic sap.

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