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ORIGINAL CONTRIBUTION

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Influence of mechanical properties of pectin films on charge density and charge density distribution in pectin macromolecule

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Abstract The hydration and mechanical properties of citrus pectin films were examined in conditions relevant to those in the plant cell wall. The pectins used for this study varied in the degree of esterification (DE) (high or low) and charge distribution on the backbone (random or block). The hydration of the films was controlled in an osmotic pressure experiment using polyethylene glycol solutions (PEG 20000). Hysteresis tests at constant deformation rate (stress vs deformation) were used for investigating the mechanical behaviour of films. Mechanical and hydration properties of pectin films were examined as a function of charge density, charge density distribution and counterion environment—K^+, Ca^{2+}, Mg^{2+}. Swelling decreased with increasing counterion concentration. The effect is stronger in the case of Ca^{2+} and Mg^{2+} for low esterified pectins and therefore crosslinks from divalent ions could be assumed. The crosslink effect is confirmed in mechanical experiments where an increase in the film tensile modulus is observed with increasing counterion concentration. It is shown for the first time that in case of highly concentrated pectin solutions Mg^{2+} cations also act as a crosslinker for pectin macromolecules.

Keywords Cell wall · Pectin · Swelling · Rheology · Tensile

Introduction

Pectin is one of the major structural polysaccharides of higher plant cells. The primary cell wall of dicotyledonous plants consists of cellulose microfibrils partially coated with xyloglucan, dispersed within a matrix of pectic polysaccharides, which also form the middle lamella [1]. Pectins are charged polysaccharides 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. Fractions 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 [2, 3]. Naturally occurring pectins are polysaccharides with a high degree of esterification (DE, about 75–80%). They could be further deesterified and based on the type and conditions of deesterification (chemically or enzyme deesterification), random or block distributions of carboxyl groups could be achieved [4]. Non-esterified carboxyl groups can be dissociated in pectin water solution and random or block distributed charges appear on the macromolecule. From a physico-chemical point of view pectin is an anionic polyelectrolyte. Therefore ionic interactions have a big impact on its behaviour in solutions. Factors such as ionic strength, counterion type and concentration, as well as charge density and charge density distribution on macromolecule are expected to influence its physico-chemical properties. It is well known that divalent metal ions (Ca^{2+}) can act as crosslinking agents in a semi-concentrated pectin solution to form three-dimensional gel networks [5, 6]. A requirement for gelation is that there should be sufficient number of blocks of charge per molecule to form junction zones and the three-dimensional network. The affinity for Ca^{2+} and other cations increases with increasing charge density along the polymer backbone and increasing ‘blockiness’ [7]. The divalent cations (Sr^{2+}, Ba^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}) usually interact with polygalacturonate (or pectin) similarly as calcium cations, and the subtle differences are interpreted as reflecting variations in binding site geometry to accommodate ions of different sizes. The only exception among divalent cations seems to be magnesium ions (Mg^{2+}). Contrary to the crosslinking effect of Ca^{2+}, the addition of Mg^{2+} does not lead to any significant chain association in semi-concentrated pectin solutions [8]. In dilute salt-free solutions a simple electrostatic interaction of Mg^{2+} counterions is observed [9]. This phenomenon does not still have a theoretical explanation.

Swelling of the polyelectrolyte network is its important characteristic. The theory of the swelling behaviour of charged polymers was described by Flory [10]. This is governed predominantly by three factors: the interaction between the polymer and the solvent; Donnan type of membrane equilibrium, expressed as increasing the concentration of mobile ions in the network in comparison with the concentration of the surrounding solution; and the elasticity of the network. The first two factors tend to extend the network and the last one restricts the swelling. As a result the total osmotic pressure in the polymer network is given by:

$$\pi_{\text{t}} = \pi_{\text{mix}} + \pi_{\text{ion}} + \pi_{\text{el}}, \tag{1}$$

where π_{mix} is the osmotic pressure, due to the polymer–solvent interaction:

$$\pi_{\text{mix}} = \frac{RT}{V_1}\left[\ln\left(1-\frac{1}{q}\right)+\frac{1}{q}+\chi\frac{1}{q^2}\right] \tag{2}$$

π_{ion} is the contribution of the polyelectrolyte effect:

$$\pi_{\text{ion}} = \frac{RT(ic_2)^2}{2\omega v C_s *}, \tag{3}$$

π_{el} is the osmotic pressure, correlated to the elastic deformation:

$$\pi_{\text{el}} = -G. \tag{4}$$

In these formulas R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature, V_1 is the molar volume of the solvent ($V_1 = 18\times10^{-3}$ l mol^{-1}), q is the swelling ratio (ratio between the final and initial volume of the polymer network), χ is the Flory interaction parameter, i is the degree of ionisation, multiple on the valence charge on the polymer, c_2 is the concentration of the polymer charge (moles of repeating unit), ω is the valence factor of the electrolyte, v is the number of cations and anions into which the electrolyte dissociated, C_s* is the concentration of the strong electrolyte and G is the shear modulus.

The equilibrium condition of the freely swollen network is determined by the condition that the total osmotic pressure inside the network is equal to the osmotic pressure of the surrounding solution. In many cases addition of salts can drastically change the swelling properties of the polyelectrolyte network. In gels salt addition may cause a volume transition. In concentrated systems the divalent Ca^{2+} is very effective in inhibiting swelling and promoting network formation, even for the weakly charged polysaccharides [11].

Pectins are important components of the plant cell walls, and contribute many of the functions that cell walls perform in plant tissues. These include physiological aspects related to growth, the determination of cell size and shape, the integrity and rigidity of tissues, ion transport, water holding, and defence mechanisms against infections by plant pathogens and wounding [12].

The metabolic environment of the plant cell and middle lamella is the apoplast, which contains several of the inorganic (containing Ca^{2+}, Mg^{2+}, K^{+}, etc.) and organic salts [13, 14]. This region is also subject to the osmotic stress of the cell, typically in the region of 0.1–0.4 MPa [15]. From the cell wall perspective it is important to understand the behaviour of highly concentrated pectin solutions (films) under different ionic and water activity conditions, found in the cellular environment. In this contribution we examine the hydration and mechanical behaviour of films (the pectin concentration is relevant to that in the plant cell wall), prepared from isolated pectic polysaccharides as a function of counterion type (Ca^{2+}, Mg^{2+}, K^{+}, which are found in the apoplast) and concentration and pectin structure—charge density and charge density distribution.

Experimental methods

Citrus pectins, with a different DE, which are chemically or enzyme deesterified were obtained from CP Kelco Company, Denmark. The neutral sugar content of the pectins was determined, after hydrolysis, by conversion of the neutral sugars to their alditol acetates, followed by GC [6]. The charge density distribution was examined by chemical fragmentation. The pectins were fragmented using a chemical procedure to obtain oligomers containing contiguous uronic acid residues [16]. These residues are converted, with hydroxylamine, to hydroxamic acids, and then, with a carbodiimide, to isoureas; the latter undergoes a Lossen rearrangement on alkaline hydrolysis. The isocyanates formed are hydrolyzed to 5-aminoarabinopyranose derivatives, which spontaneously ring open to give 1,5-dialdehydes. The latter are reduced, in situ, to avoid peeling reactions with sodium borohydride to give substituted arabitol residues. Thus, overall, partially esterified pectins are specifically cleaved to generate a series of oligogalacturonic acids bearing an arabitol residue as aglycone. Analysis of oligomers thus generated discloses the pattern of contiguous non-esterification in a variety of pectins of differing degrees and type of esterification. The analysis of the oligomeric products by high-pressure anion exchange chromatography (HPAEC) and electrospray-ionization mass spectroscopy was carried out as described [16]. The viscosity average molecular weight, DE and galacturonic acid content (GA) are used in accordance with the manufacturer's specifications.

Films were prepared by dispersing 3% w/w pectin in 50 mM acetate buffer (pH 5.6), spreading a thick layer (5 g) of the solution on Teflon-lined Petri dishes, 46 mm diameter, and allowing it to dry in an airdraft at 42 °C for 16 h. The pectin content of the air-dried films was determined by vacuum drying at 60 °C over P_2O_5 for 16 h from small samples of every dish. No microphase separation was recognised during film-forming.

For swelling experiments the films were cut out with a punch and bisected with a razor. The sizes (4 cm×0.4 cm×0.005 cm) were measured with travelling microscope to 10 μm. Sections of the films were hydrated for 24 h in concentrated polyethylene glycol solutions (PEG 20000), of known osmotic pressure ($\log \pi = 6.7$) [17] and counterion composition (10, 30 and 50 mM Ca^{2+}, Mg^{2+} and K^{+}), at room temperature.

Preliminary experiments from measurements of the change of linear dimensions of the film (using travelling microscope) established that the swelling is isotropic. In the present experiments swelling was calculated as a ratio of film weights, after and before the hydration, and was expressed in percentage.

Mechanical experiments were examined in the simple extension mode using a Stable Microsystems Texture Analyzer (TAXT2i). The samples (support length 1–1.5 cm) were glued to metal sample holders with a cyanoacrylate adhesive. A hysteresis test was carried out (Fig. 1).

The sample was extended and compressed with a constant deformation rate of 0.02 mm s^{-1}. The tensile modulus was determined from the linear part of the force–deformation dependence during the extension. The area of the hysteresis curve could be used to estimate the viscoelastic behaviour of the films—for pure elastic solids this area should be zero.

Results

The physico-chemical properties and composition of pectins chosen for the study are outlined in Table 1. Four commercial pectins were used, which are chemically and enzyme deesterified. The pectins could be categorized into two groups based on the DE—low esterified (DE~36%) and high esterified (DE~70%).

The reported values for molecular weight (54,000–122,000) indicate a large molecular size. In concentrated systems (>30% w/w) these materials will be heavily entangled.

The pectins not only have high uronic acid content, but also contain some neutral sugars—predominately

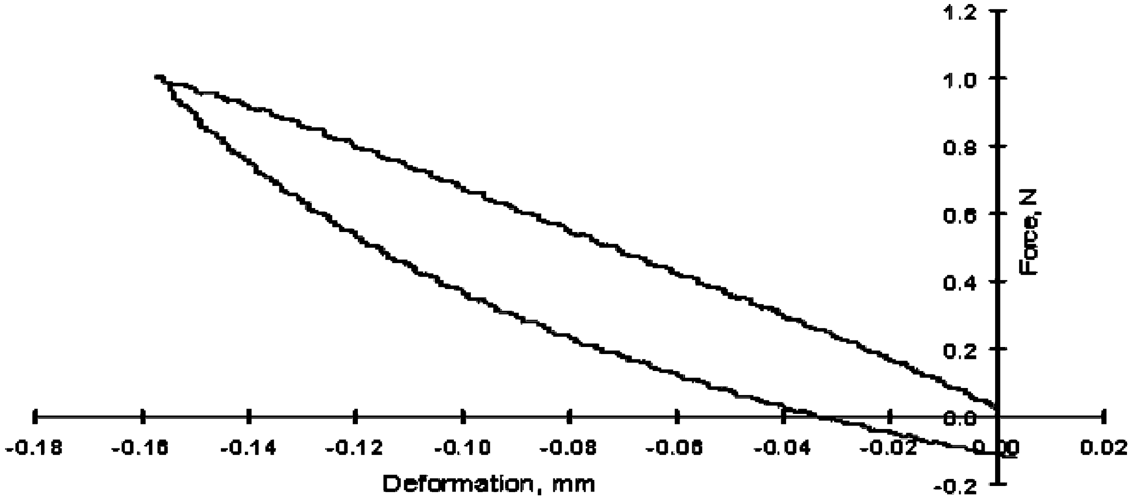

Fig. 1 Hysteresis method for determination of tensile modulus

Table 1 Physico-chemical properties and composition of pectins

Pectin type and note on figures and in other tables	Molecular weight (g mol^{-1})	Anhydrous sugar/mg dry weight (µg)						Neutral sugar (% w/w)	DE[a] (%)	GA[b] (%)	Type of deesterification (charge distribution)
		Rha	Ara	Xyl	Man	Gal	Glc				
812634-40 Block, high	113,000	7.0	36.5	0.8	0.6	44.5	3.9	9.33	71.2	90.3	Chemically (Block)
98246-5-A Random, high	55,000	4.3	19.7	0.7	0.2	42.2	1.8	6.89	70.6	85.4	Enzyme (Random)
0001-8-F Block, low	122,000	7.1	15.6	1.7	0.2	49.0	3.6	7.80	36.7	89.1	Chemically (Block)
98246-5-E Random, low	54,000	6.9	2.1	1.3	0.0	37.0	2.7	5.00	35.6	92.4	Enzyme (Random)

[a] Degree of deesterification
[b] Galacturonic acid content

rhamnose, galactose and arabinose. Because the total amount of neutral sugars varied from 5 to 10%, we can estimate that the pectin molecule is linear.

Variations in the distribution of non-esterified GA residues could affect the observed physico-chemical behaviour. The pattern of distribution of uronic acid in the pectin was examined using the chemical fragmentation procedure. Results of this research have been already discussed in previous studies and here we use just the final conclusions [18, 19].

The dry content of pectin films before swelling varies from 74.8% for block, high esterified pectin to 88.0% for random, low esterified pectin, which corresponds to a pectin concentration of 4.25 mol l^{-1} for block, high esterified pectin to 5 mol l^{-1} for random, low esterified. The charge density was calculated based on pectin concentration, DE and uronic acid content (Table 2). The dry content of films decreases with increasing DE and blockness of charge in pectin macromolecules.

Swelling behaviour

Figure 2a–c shows the variation of the swelling degree (q) on pectin films in different salt solutions as a function of the salt concentration. As a reference ($c_{\text{salt}}=0$) the swelling in the PEG solution without added salts was used. The ionic strength of the buffer solution (50 mM acetate buffer) is used in the calculations for osmotic stress in these films.

Table 2 Pectin content and charge concentration in dry pectin films

Pectin type	Dry content (% w/w)	Pectin concentration (mol l^{-1})	Charge concentration (mol l^{-1})
Block, high	74.77	4.25	1.10
Random, high	81.16	4.61	1.16
Block, low	76.80	4.36	2.46
Random, low	88.00	5.00	2.98

In general, irrespective of the counterion and pectin type, salt addition decreases film swelling because of ion screen repulsive interactions between charge monomer units. The behaviour of pectin films exhibits substantial differences in solutions of 1:1 electrolyte (KCl) and 2:1 electrolytes ($CaCl_2$ and $MgCl_2$). In KCl solutions the variation in the swelling degree is weaker. For each pectin, at a fixed ionic strength the observed swelling decreases in the order $K^+ > Mg^{2+} > Ca^{2+}$. The decrease in swelling with increasing counterion valence could be associated with the increasing polyelectrolyte type effect (higher ionic strength for multivalent counterions at the same salt concentration) or effect of network crosslinks. The pectin type also affects the observed swelling. For a particular counterion at a fixed concentration the swelling increases in the order random, low esterified < block, low esterified≈block, high esterified < random, high esterified. This fact appears to be in contradiction with the theory of polyelectrolytes, as far as it might be expected that increasing charge will lead to increasing swelling (Eq. 3). In fact the opposite is observed here—swelling is much less for low esterified pectins. Generally the swelling depends on the charge distribution along the backbone.

Mechanical behaviour

The study focussed on the short time behaviour of the pectin network. Figure 2d–f shows the dependence of the Young modulus (E; MPa) on salt concentration for different types of pectin films in different salt solutions. The constant-strain-rate tensile modulus ranged from 34 to 660 MPa for the film examined. Large differences were observed in the values of E for films from different pectins, swollen in PEG solutions without added salts. The modulus in that case increases in the order random, high < block, high < block, low < random, low. For all pectin types, irrespective of the added counterions, the tensile modulus increases with increasing salt concentration. The observed reason for this dependence is the reduction of swelling with increasing salt concentration and the increasing of film dry content. The

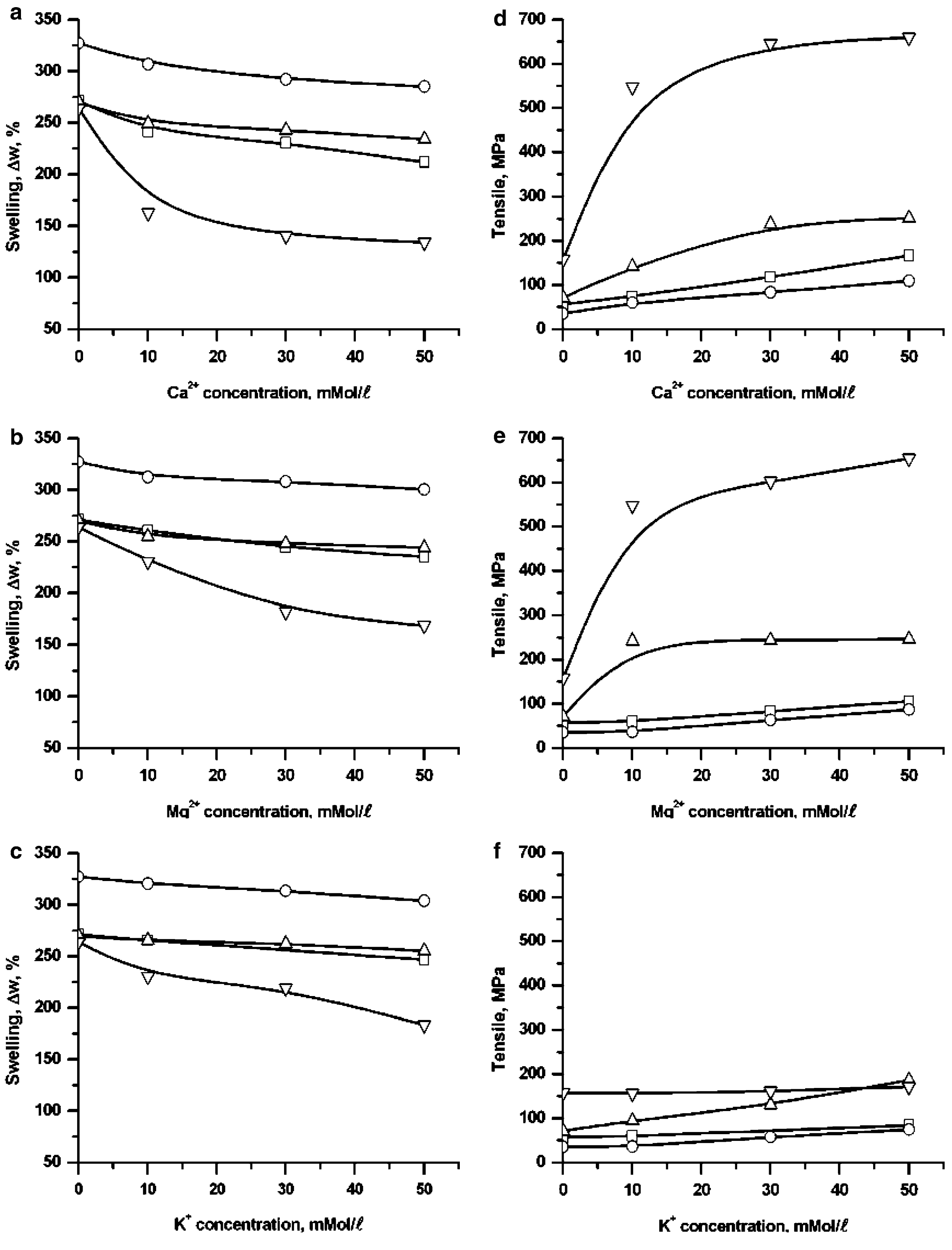

Fig. 2 Effect of counterion type and concentrations on swelling behaviour and tensile properties of high, block (*open square*); high, random (*open circle*); low, block (*open triangle*); and low, random (*open inverted triangle*) pectin. Effect of counterion types on swelling: **a** Ca^{2+}, **b** Mg^{2+} and **c** K^{+}. Effect of counterion types on tensile: **d** Ca^{2+}, **e** Mg^{2+} and **f** K^{+}

increasing of stiffness is counterion dependent, increasing the order $K^{+} < Mg^{2+} < Ca^{2+}$. In addition the constant-rate tensile modulus shows a dependence on pectin type, with the values, increasing in the order random, high < block, high < block, low < random, low. The faster increase of E for low esterified pectins in 2:1

electrolyte solutions could be associated with the effect of network crosslink.

Discussion

The main subject of recent research is to explain somehow the observed swelling and mechanical behaviour of hydrated pectin films in different salt environments, which are found in the apoplast. The first contradiction in the swelling of pectin films, prepared from pectins that varied in DE and charge distribution, is the reduced swelling of low esterified pectin films in comparison with the prediction of the polyelectrolyte effect. There are two main reasons which decrease the swelling: counterion condensation and network crosslinks.

For polyelectrolytes in different solvents it is observed that increasing charge on the macromolecule can lead to a collapse phenomenon. At high charge density counterions condense on the backbone and therefore do not participate in the Donnan type swelling effect. To estimate the contribution of counterion condensation on reduced swelling of low esterified pectin films, a Manning dimensionless parameter ξ could be used [20]. It is calculated from:

$$\xi = \frac{e^2}{\varepsilon\,\varepsilon_0 4\,\pi\,kTb}, \tag{5}$$

where ε is the dielectric constant of the medium, ε_0 is the dielectric constant, e is the elementary charge, k is the Boltzmann's constant and b is the spacing between charges. For many polyelectrolytes, counterion condensation is observed above a value of $\xi = 1$. The parameter ξ is a good estimation for the counterion condensation when the charge on the polymer backbone has a relatively random distribution. In the case of water as the solvent, at 25 °C, $\xi = (0.71/b)$, if b is expressed in nm. With charge spacing along the polygalacturonic acid chain 0.435 nm [21], the calculated values of ξ for the different used pectins are: 0.39 for random, high esterified; 0.36 for block, high esterified; 0.96 for random, low esterified; 0.87 for block, low esterified. Values for ξ parameters are less than one for all types of pectins used. The greatest is for random, low esterified pectin, where one could expect counterion condensation, but even in that case we can assume it as negligible, especially as the charge distribution in this pectin is predominantly random and areas with higher charge concentration occur much rarely.

The second factor, which reduces the swelling effect, is the interactions between pectin macromolecules and metal cations, expressed in terms of crosslinking. In order to examine the role of 1:1 and 2:1 electrolytes in crosslink formation, it is useful to estimate the number of crosslinks in pectin films. From the theory of rubber elasticity for crosslinked synthetic polymers, the dependence of tensile modulus on molecular weight between crosslinks, M_c (g mol^{-1}), is given by [8, 22]:

$$E = E_0\left(\frac{1}{q}\right)^{1/3} \sim \frac{cRT}{M_c}\left(\frac{1}{q}\right), \tag{6}$$

where c (g m^{-3}) is the polymer concentration, E_0 is the tensile modulus in the case of non-swelled network, and R and T have their usual meaning. According to this theory the mechanism of energy storage is entropic, arising as a result of the constraint of the number of accessible configurations on the polymer as the network is stretched. The dependences of the E_0 modulus on counterion concentration for different types of pectins are shown in Fig. 3. There are significant differences not just in the values of the E_0 modulus, but in the type of network behaviour through increasing electrolyte concentration, which are connected with both DE and charge density distribution. It is apparent that the effect of KCl is much less detectable in comparison with 2:1 electrolytes. For both high esterified pectin and low esterified pectin with block charge distribution E_0 modulus increases linearly with increasing K^+ concentration. It maintains a constant value in the case of low esterified pectins with random charge distribution. The effect of increasing E_0 modulus could be due to a decrease in water activity with increasing ionic strength and an increase in hydrophobic interactions between pectin macromolecules, leading to a stronger network.

Both divalent counterions strongly affect the young modulus. The effect of Mg^{2+} and Ca^{2+} strongly depends on pectin type and increases in the order random, high < block, high < block, low < random, low. While almost no differences could be observed between E_0 modulus of pectin films from high esterified pectin in the present of K^+ and Mg^{2+}, there is a tremendous increase in film hardness for low esterified pectins with Mg^{2+}. The effect of Mg^{2+} in that case is even greater than that of Ca^{2+}. This finding implies that there is no strong specific interaction between K^+ and pectin. When K^+ is replaced with Mg^{2+} or Ca^{2+}, the value E_0 becomes considerably higher and increases more rapidly with salt concentration. A possible explanation is the complex formation between polymer and metal ions, leading to network crosslinking. Contrary to the effect of Mg^{2+} in dilute and semi-concentrated solutions, where, based on the literature available, just a simple electrostatic interaction between pectin chains and Mg^{2+} cations is observed, at high concentration the Mg^{2+} crosslink pectin network and chain association is realised. It seems that Mg^{2+} is a more effective crosslinker for low esterified pectins than Ca^{2+}.

It is expected that specific interactions between cations and hydroxyl groups affect the mixing term in the osmotic pressure. The mixing pressure is given by [23]:

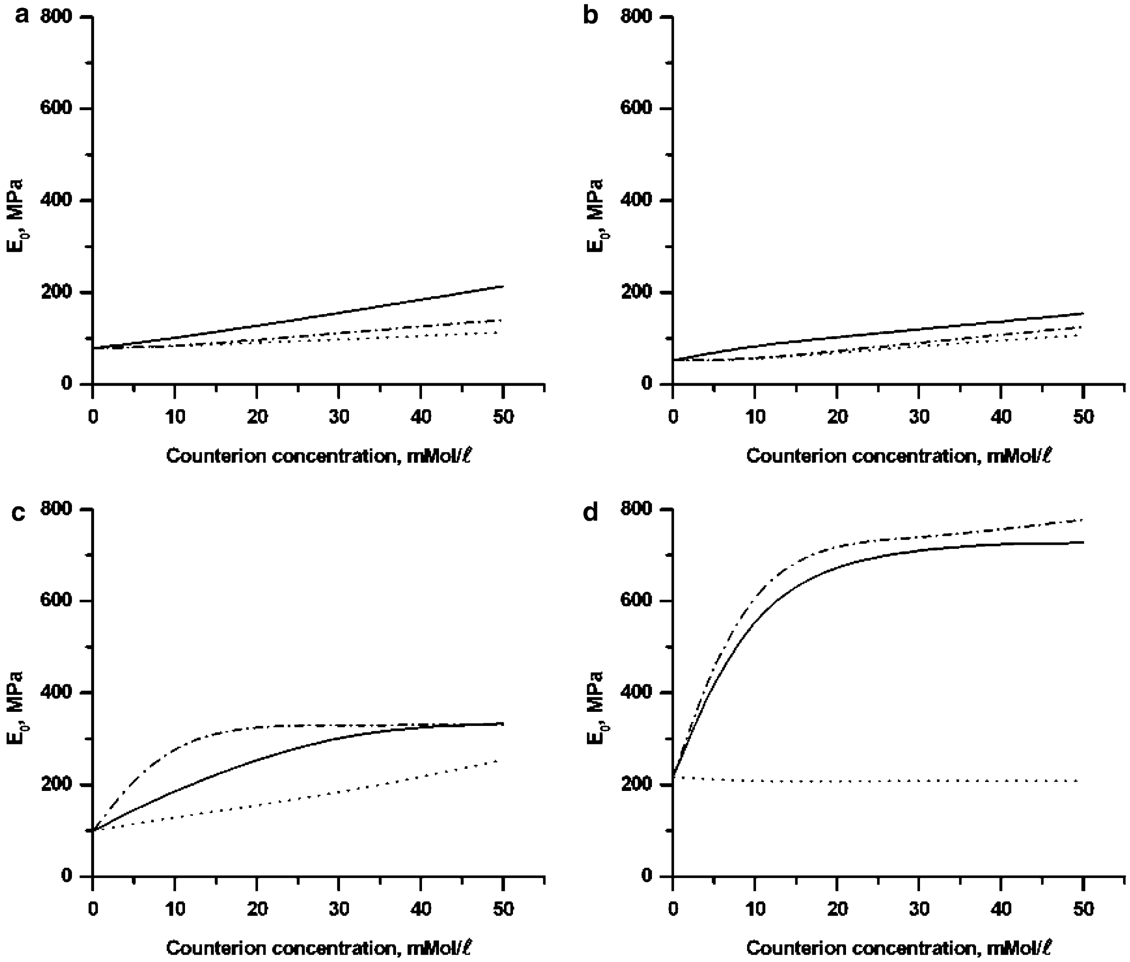

Fig. 3 Effect of counterion type (*solid line* Ca^{2+}; *line with dots* Mg^{2+}; *dotted line* K^+) and concentration on Young modulus for **a** block high, **b** random high, **c** block low and **d** random low pectin

$$\pi_{\text{mix}} = \frac{RT}{V_1}\left[\ln\left(1-\frac{1}{q}\right)+\frac{1}{q}+\chi_0\frac{1}{q^2}+\chi_1\frac{1}{q^3}\right], \qquad (7)$$

where χ_0 and χ_1 are constants. The dependency of χ_0 and χ_1 on cations is shown in Table 3. They greatly depend on pectin DE. While for high esterified pectin these

Table 3 Values of the Flory–Huggins interaction parameters for pectin films in salt solutions

Salt solution	Pectin type							
	Block, high		Random, high		Block, low		Random, low	
	χ_0	χ_1	χ_0	χ_1	χ_0	χ_1	χ_0	χ_1
Without salt	0.250	0.000	0.148	0.000	0.310	0.000	0.345	0.000
10 mM $CaCl_2$	0.283	0.084	0.187	0.120	0.329	0.045	0.419	0.118
30 mM $CaCl_2$	0.316	0.153	0.214	0.193	0.335	0.055	0.437	0.128
50 mM $CaCl_2$	0.344	0.199	0.227	0.224	0.343	0.074	0.442	0.129
10 mM $MgCl_2$	0.268	0.049	0.177	0.089	0.323	0.028	0.365	0.034
30 mM $MgCl_2$	0.295	0.110	0.185	0.113	0.330	0.044	0.403	0.100
50 mM $MgCl_2$	0.309	0.140	0.199	0.153	0.334	0.053	0.414	0.112
10 mM KCl	0.260	0.027	0.161	0.042	0.313	0.009	0.365	0.046
30 mM KCl	–	–	0.175	0.083	0.316	0.015	0.373	0.062
50 mM KCl	0.290	0.101	0.193	0.135	0.323	0.029	0.401	0.105

parameters are around 0.2–0.3, for low esterified random pectin they reach the value of 0.4 and water becomes a relatively poor solvent. The values for χ_1 are higher in divalent salt solutions, which indicate that both Ca^{2+} and Mg^{2+} act as crosslinking agents. They increase with increasing salt concentration.

Conclusion

Monovalent metal ions, K^+, do not affect considerably the equilibrium hydration and elastic properties of pectin films. Their effect can be explained within the theory of Donnan type of equilibrium between the polyelectrolyte network and its surrounding solution and polymer theory of elasticity. Addition of both the divalent metal ions, Mg^{2+} and Ca^{2+}, drastically changes the swelling behaviour and tensile moduli of pectin films. They affect both the ionic and mixing contribution of the total swelling pressure and significantly increase the equilibrium tensile modulus. The most likely explanation of this effect is the process of specific binding of divalent cations to the pectin macromolecule and crosslinking of the pectin network. This result is well known for Ca^{2+} ions, and the experimental results confirm the same effect for the first time for Mg^{2+}. The crosslinking of pectin depends on the charge density and charge density distribution on the backbone. Ca^{2+} and Mg^{2+} are more effective with increasing charge density (low esterified pectins) and blockness in charge distribution. They act efficiently even for high esterified pectins with block distribution of the charge. In the case of low esterified pectin Mg^{2+} appears to be a more effective crosslinker.

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References

1. Cosgrove DJ (1999) Enzymes and other agents that enhance cell wall extensibility. Annu Rev Plant Physiol Plant Mol Biol 50:391–417
2. Schols HA, Voragen AGJ (1994) Hairy (ramified) regions of pectins. 4. Occurrence of pectic hairy regions in various plant-cell wall materials and their degradability by rhamnogalacturonase. Carbohydr Res 256:83–95
3. Schols HA, Voragen AGJ (1994) Hairy (ramified) regions of pectins. 5. Isolation and characterization of rhamnogalacturonan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. Carbohydr Res 256:97–111
4. Taylor AJ (1982) Intramolecular distribution of carboxyl groups in low methoxyl pectins—a review. Carbohydr Polym 2(1):9–17
5. Thibault J-F, Rinaudo M (1985) Interactions of monovalent and divalent counterions with alkali-deesterified and enzyme-deesterified pectins in salt-free solutions. Biopolymers 24(11):2131–2143
6. Macdougall AJ et al (1996) Calcium gelation of pectic polysaccharides isolated from unripe tomato fruit. Carbohydr Res 923:235–249
7. Bystricky S et al (1990) Interaction of alginates and pectins with cationic polypeptides. Carbohydr Polym 13:283–294
8. Thibault JF, Rinaudo M (1986) Chain association of pectin molecules during calcium-induced gelation. Biopolymers 25(3):455–468
9. Malovikova A, Rinaudo M, Milas M (1994) Comparative interactions of magnesium and calcium counterions with polygalacturonic acid. Biopolymers 34:1059–1064
10. Flory PJ (1953) Principles of polymer chemistry. Cornell University Press, London, pp 464–469 (see also pp576–581)
11. Ryden P et al (2000) Hydration of pectic polysaccharides. Biopolymers 154(6):398–405
12. Basic A, Harris PJ, Stone BA (1988) Structure and function of plant cell walls. In: Preiss J (ed) The biochemistry of plants, a comprehensive treatise, vol 14. Academic, London, pp 297–369
13. Macdougall AJ et al (1995) Nonaqueous fractionation to assess the ionic composition of the apoplast during fruit ripening. Plant Physiol 108:1679–1689
14. Almeida DP et al (1999) Apoplastic pH and inorganic ion levels in tomato fruit: a potential means for regulation of cell wall metabolism during ripening. Physiol Plant 105:506–512
15. Tomos AD et al (1999) The pressure probe: a versatile tool in plant cell physiology. Annu Rev Plant Physiol Plant Mol Biol 50:447–472
16. Needs PW et al (2001) Specific degradation of pectins via a carbodiimide-mediated Lossen rearrangement of methyl esterified galacturonic acid residues. Carbohydr Res 333:47–58
17. Parsegian VA et al (1995) Macromolecules and water probing with osmotic stress. Methods Enzymol 259:43–95
18. Marudova MG et al (2004) Physicochemical studies of pectin/poly-L-lysine gelation. Carbohydr Res 339(2):209–216
19. Zsivanovits G et al (2004) Material properties of concentrated pectin networks. Carbohydr Res 339(7):1317–1322
20. Manning GS (1996) The critical onset of counterion condensation: a survey of its experimental and theoretical basis. Berenges Phys Chem 100:923–928
21. Kohn R (1975) Ion binding on polyuronates—alginate and pectin. Pure Appl Chem 42:371–399
22. Oakenfull DG (1984) A method for using measurements of shear modulus to estimate the size and thermodynamic stability of junction zones in noncovalent crosslinked gels. J Food Sci 49(4):1103–1104
23. Horkay F et al (2000) Osmotic swelling of polyacrilate hydrogels in physiological salt solutions. Biomacromolecule 1:84–90