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The environmental response and stability of pectin and poly-L-lysine multilayers

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Abstract

The environmental response of multilayers consisting of pectin and poly-L-lysine (PLL) to changes in pH and ionic strength was studied using Fourier transform infrared-attenuated total reflection spectroscopy (FTIR-ATR) and a quartz crystal microbalance with dissipation monitoring (QCMD). The swelling of the multilayer increased with increasing ionic strength, and was attributed to the screening effect of salt on the attractive forces within the system. Below pH 7.0, reversible swelling decreased with decreasing pH, until at pHs < 3.6 solubilisation of the multilayer occurred. The pH-dependent ionization of the anhydrogalacturonate residues of pectin in the multilayer was examined by FTIR-ATR. It was found there was a substantial shift in their pK_a to more acidic pHs. The deswelling of the multilayer with decreasing pH is associated with small decreases in the ionization of anhydrogalacturonate residues. The solubilisation of the multilayer is associated with a loss of charge on the pectin component.

Keywords: Pectin; Poly-L-lysine; Biopolymer multilayer; Quartz crystal microbalance

1. Introduction

The attraction between oppositely charged polyelectrolytes can create a range of associated structures, including complexes (Mattison, Dubin, and Brittain, 1998), coacervates (e.g. – Weinbreck, de Vries, Schrooyen, and de Kruif, 2003), networks and multilayers (Decher, 1997; Lvov, Ariga, Ichinose, and Kunitake, 1995). Over the past decade, the layer-by-layer technique (Decher, 1997) has been developed for the formation of multilayers through the sequential deposition of oppositely charged polymers. A requirement for multilayer formation is that the addition of an oppositely charged polyelectrolyte to a charged surface results in charge reversal, normally depicted as charged loops and tails of the polyelectrolyte extending from the surface. Charge reversal permits successive deposition of oppositely charged polyelectrolytes. The multilayers formed are non-equilibrium structures, and the structure is dependent on the method of preparation. The formation of the multilayer is influenced by pH (Burke and Barrett, 2003; Kato, Schuetz, Fery, and Caruso, 2002; Shiratori and Rubner, 2000) and ionic strength (Dubas and Schlenoff, 2001a; Kovacevic, van der Burgh, de Keizer, and Stuart, 2002) particularly for weak polyelectrolytes. Important structural variables include the extent to which the two polyelectrolytes interpenetrate (Schlenoff and Dubas, 2001); the extent of crosslinking of individual layers; and the extent of charge neutralisation of one polymer by another. These structural variables lead to differences in functional behaviour, including how the swelling behaviour is influenced by changes in ionic environment.

For neutral polymer networks, the driving force for swelling increases with increasing affinity of the polymer for the solvent (Flory, 1953), until it is balanced by the elastic restorative force of the crosslinked network. For simple polyelectrolyte gels, an additional swelling force arises from the requirement for electrical neutrality which leads to an excess of counterions within the gel compared to

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the external medium. For weakly charged polyelectrolytes, this excess generates an osmotic pressure difference between the gel and external medium, which increases with decreasing ionic strength (Rubinstein, Colby, Dobrynin, and Joanny, 1996). In classical treatments, the effect of electrostatic repulsion between like charges is considered to be small (Flory, 1953). For polyampholyte networks these interactions can become important (Nisato, Munch, and Candau, 1999; Ogawa, Ogawa, and Kokufuta, 2004). If the net charge on the network is large, the networks behave in a similar way to simple polyelectrolyte networks. For systems at low ionic strength, and at charge balance, the attraction between opposite charges provides a force which counteracts swelling. As ionic strength is increased, the screening of the attraction between opposite charges promotes swelling. This ionic strength dependence is the opposite of that observed for polyelectrolyte gels and is known as an antipolyelectrolyte effect (Kokufuta, 2005). The swelling of charge-balanced polyampholyte gels as a function of pH is typically at a minimum at pHs where both basic and acidic residues are fully charged. On shifting pH, the network acquires a net charge which can lead to swelling. Polyampholyte gels which are weakly charged can display both polyelectrolyte and antipolyelectrolyte behaviour, with swelling initially decreasing with increasing ionic strength, going through a minimum, and then increasing with increasing ionic strength. In multilayered networks further complexity is introduced by the heterogeneous nature of the charge distribution. Furthermore the ‘crosslinks’ in multilayered systems involve mainly electrostatic interactions which are sensitive to dissociation through changes in the local ionic environment, with an associated swelling response arising from changes in crosslink density.

There have been a number of recent studies on the swelling behaviour of multilayered systems (Gergely et al., 2004). Dubas and Schlenoff (Dubas and Schlenoff, 2001a) showed that a system containing one weak and one strong polyelectrolyte swelled at high salt and subsequently dissolved at concentrations greater than 0.6 M NaCl. Other studies have been carried out on the swelling behaviour of systems containing two weak polyelectrolytes and found that the swelling was also strongly dependent on the pH of formation and the swelling medium (Burke and Barrett, 2003; Hiller and Rubner, 2003; Salloum and Schlenoff, 2004). When multilayers are formed at a pH where one polyelectrolyte is in an extended form, a swollen structure is created that can exhibit a relatively large equilibrium swelling. Rapid transitions in swelling can occur over a narrow pH range due to changes in the extent of association of the oppositely charged polymers.

In this article we wish to consider the swelling response of multilayers formed from two oppositely charged weak biopolyelectrolytes, pectin and poly-L-lysine. The pectic polysaccharides are weak polyelectrolytes based on a backbone of (1→4)-α-D-galacturonosyl units and their methyl esters, interrupted in places by (1→2)-α-L-rhamnopyranosyl units. The average spacing between charges along the galacturonosyl backbone is inversely related to the methyl ester content. Pectins can form network structures with divalent counterions such as Ca^{2+}, with the affinity for the counterion increasing with decreasing degree of esterification, DE, (Kohn, 1975). Gel networks and multilayers may be formed with basic biopolyelectrolytes such as chitosan and poly-L-lysine (Marudova, MacDougall, and Ring, 2004a, 2004b). Networks formed from pectins with a low DE have a tendency to form collapsed structures, as DE increases, the pectin networks have a greater potential for swelling, until at high DE there are insufficient charged junction zones to form network structures.

In this study we examine the response of multilayers of pectin and poly-L-lysine (Krzeminski et al., 2006) to changes in pH and ionic strength. We have used Fourier transform infrared-attenuated total reflection spectroscopy (FTIR-ATR) to obtain information on the formation and chemical characteristics of the deposited layers, and a quartz crystal microbalance with dissipation monitoring (QCMD) to obtain information on the hydration and swelling behaviour.

2. Materials and methods

2.1. Polyelectrolyte solutions

Poly-L-lysine hydrobromide (PLL) with a mean degree of polymerisation of 70 was obtained from Sigma; citrus pectin, with a DE of 70.6%, was obtained from CP Kelco. D_2O (99.9%) was obtained from Sigma. Reagents were analytical grade. Polymer solutions, at a concentration of 0.6 mg mL^{-1}, were prepared in the buffer of desired pH and ionic strength. HCl/KCl, acetate and phosphate buffers were used to cover the pH range 1.5–8.0; NaCl was used as a supporting electrolyte.

2.2. FTIR-ATR spectroscopy

Infrared spectra were collected, over the range 4000–800 cm^{-1}, on a Nicolet 860 FTIR spectrometer (Thermo Electron Corporation, Madison, USA) fitted with a Micro-Circle liquid ATR cell (SpectraTech, Warrington, UK). The ATR crystal was a cylindrical Si rod, with 11 internal reflections, mounted in a thermostatted steel jacket maintained at 20 °C. PLL and pectin solutions were prepared as described above using D_2O instead of H_2O. Multilayers were fabricated by the alternate deposition of PLL and pectin layers, with PLL forming the base layer. 1 mL of biopolymer solution was injected into the cell and left for 16 min. After each deposition step the cell was rinsed with 2 mL of deuterated buffer. Spectra were accumulated by co-adding 1024 scans at a resolution of 2 cm^{-1} and referenced against a background of buffer alone. The determination of the mass of PLL and pectin in the surface layer and the ester content of the pectin was determined as described (Krzeminski et al., 2006), from the characteristic absorbances of the PLL at 1645 cm^{-1} (amide I) (Jackson, Haris, and Chapman, 1989) and pectin at 1610 cm^{-1} (uronate) and 1730 cm^{-1} (ester) (Monsoor, Kalapathy, and Proctor, 2001a; Monsoor, Kalapathy, and Proctor, 2001b).

2.3. Quartz crystal microbalance

Measurements were carried out using a D300 quartz crystal microbalance with dissipation monitoring (QCMD) (Q-Sense AB, Västra Frölunda, Sweden) with a QAFC 302 axial flow measurement chamber and gold-coated sensor crystals (QSX-301). The sensing element is a disc-shaped, AT-cut piezoelectric quartz crystal sandwiched between two gold electrodes. Material deposited on the sensor surface alters the resonance characteristics of the sensor. For elastic materials there is a simple, (Sauerbrey, 1959) relationship between frequency change and mass deposited, for viscoelastic materials the frequency change was modeled using the Voigt model (Voinova, Rodahl, Jonson, and Kasemo, 1999) to obtain the hydrated mass of material deposited on the sensor surface and its viscoelastic characteristics (Krzeminski et al., 2006). The swelling ratio was calculated from the ratio of the hydrated masses of the multilayer, obtained before and after a specific treatment.

A multilayer was prepared at 20 °C by sequential deposition of PLL (0.5 mL) and pectin (0.5 mL). After multilayer formation, buffers of either different ionic strength or pH were allowed to flow into the cell and the response examined. Responsiveness was investigated for multilayer structures composed of 10 and 11 layers. The 10 layer structures have pectin as the final deposited layer and for the 11 layer structures PLL is the final layer. Between each experiment the sensor chip was cleaned by bathing in a 2% w/w Hellmanex® solution (Hellma UK) for 30 min. followed by 15 min. in a UV–ozone cleaner (Bioforce Nanosciences), followed by rinsing in distilled water and drying in a filtered N_2 stream. The measurement head and associated tubing were cleaned with a 2% w/w Hellmanex® solution.

3. Results and discussion

3.1. Multilayer formation – effects of ionic strength and pH

In earlier studies we have shown that pectins with a range of DE can form multilayer structures with PLL (Krzeminski et al., 2006) or chitosan (Marudova, Lang, Brownsey, and Ring, 2005) as the cationic polymer. With the low molecular weight PLL, the layers exhibited an exponential-type growth. This type of growth is a consequence of the ability of PLL to diffuse to the surface of the growing multilayer and capture progressively more anionic polymer with increasing layer number (Picart et al., 2002). The DE of the pectin had little effect on multilayer growth (Krzeminski et al., 2006). The amount of pectin deposited in each layer increased with increasing concentration of polymer in solution, a common feature of polymer adsorption at surfaces, associated with the ability of higher concentrations of adsorbed polymer to reduce structural rearrangement at a surface. The overall charge of the multilayer network was positive, and increased with increasing DE of the pectin. The formation of a 10 layer pectin/PLL multilayer was first investigated at 30 mM and 200 mM NaCl using both FTIR-ATR spectroscopy and QCMD. Table 1 summarises the masses of the systems obtained from FTIR and QCMD data. From the separate absorbances of pectin (uronate) and PLL (amide I) it was possible to estimate the charge ratio. The multilayers formed had a net positive charge as observed previously. There was also some subfractionation of the pectin on deposition. At pH 7.0 the deposited pectin had a DE of ~55%, increasing to ~65% at pH 3.6, which is closer to the DE of the pectin preparation of ~71%. The solids concentration was expressed as the mass obtained from FTIR-ATR as a fraction of the mass obtained from QCMD data. The conditions of preparation had small effects on the characteristics of the multilayers formed.

3.2. Response to ionic strength changes

After multilayer formation, the swelling of the multilayers was examined at pH 7.0 as a function of ionic strength of the supporting electrolyte over the range of 10–200 mM (Fig. 1) The swelling ratio was calculated from the ratio of the frequency change (3rd overtone at 15 MHz) observed on changing ionic strength to the frequency change observed for the formation of the multilayer. Both 10 and 11 layer multilayer structures were examined which had been fabricated in either 30 (Figs. 1a and b) or 200 mM NaCl at pH 7.0 (Figs. 1c and d). The observed effects were relatively small. For the multilayers formed in 200 mM NaCl the swelling decreased with decreasing ionic strength and was essentially reversible for both 10 (Fig. 1c) and 11 (Fig. 1d) layer structures. The change in salt concentration resulted in no detectable loss of either pectin or PLL as observed by FTIR. The observed dependence of swelling on ionic strength was consistent with the antipolyelectrolyte type swelling of a polyampholyte

Table 1
Characteristics of 10 layer pectin/PLL multilayers formed under different conditions

Condition of formation	FTIR mass (ng cm^{-2})	QCMD mass (ng cm^{-2})	Solids conc. (% w/w)	Charge ratio (−/+)
pH 7.0, 30 mM NaCl	740	5500	14	0.63
pH 7.0, 200 mM NaCl	860	5100	17	0.55
pH 3.6, 30 mM NaCl	790	7500	11	0.82

Charge ratio was calculated when both PLL and pectin were fully ionised.

networks (Baker, Blanch, and Prausnitz, 1995; Nisato et al., 1999), although rather small. This effect has also been observed for other multilayered structures (Burke and Barrett, 2005; Dubas and Schlenoff, 2001b). For the multilayers formed in 30 mM NaCl (Figs. 1a and b), although there was an underlying trend of increased swelling with increasing ionic strength, this was complicated by the non-reversibility of the swelling behaviour. Non-reversible behaviour is indicative of the solubilisation of polymer. For the multilayers formed in 30 mM NaCl, there was a mass loss of 8–9% w/w of adsorbed polymer, as a result of increasing NaCl concentration to 200 mM, with a preferential loss of polymer which formed the uppermost layer. This loss of polymer has opposite effects on the hydrated mass of the 10 and 11 layer structures. Loss of pectin from the 10 layer structure results in an expected loss of hydrated mass. However, an increase in hydrated mass is observed for the 11 layer system. This is a result of a reduction in the mass of the PLL crosslinker creating a more open, hydrated structure.

3.3. Response to pH changes

The swelling of the multilayers was examined in 30 mM NaCl, as a function of pH in the range 3.6–7.0. Both 10 and 11 layer multilayer structures were examined which had been fabricated at either pH 7.0 or 3.6 (Fig. 2). In the ranges examined, the effect of change in pH on swelling was larger than the effects observed through changing ionic strength. The swelling of structures formed at pH 7.0 decreased with decreasing pH, and was essentially reversible. When pectin formed the uppermost layer (10 layers), the observed effect of pH on swelling was larger than that observed when PLL was the uppermost layer (11 layers). A characteristic feature of the formation of pectin/PLL multilayers with a pectin of DE 71% was a deswelling of the multilayer when the PLL was added, an effect ascribed to a crosslinking of the structure with PLL (Krzeminski et al., 2006). The reduced dependence of swelling on pH for structures containing PLL as the uppermost layer is consistent with this type of effect – the structure is more crosslinked and has a reduced tendency to swell. The form of the dependence of swelling on pH was similar to that observed for pectin gels (Tibbits, MacDougall, and Ring, 1998). In the latter case this was ascribed to a suppression of the ionization of anhydrogalacturonate residues with decreasing pH, with a resulting decrease in the associated polyelectrolyte swelling effect. The multilayers have a more complex structure with an overall net positive charge (Table 1) and alternative explanations need to be considered. The dependence of swelling on ionic strength suggests that the attraction between oppositely charged residues on

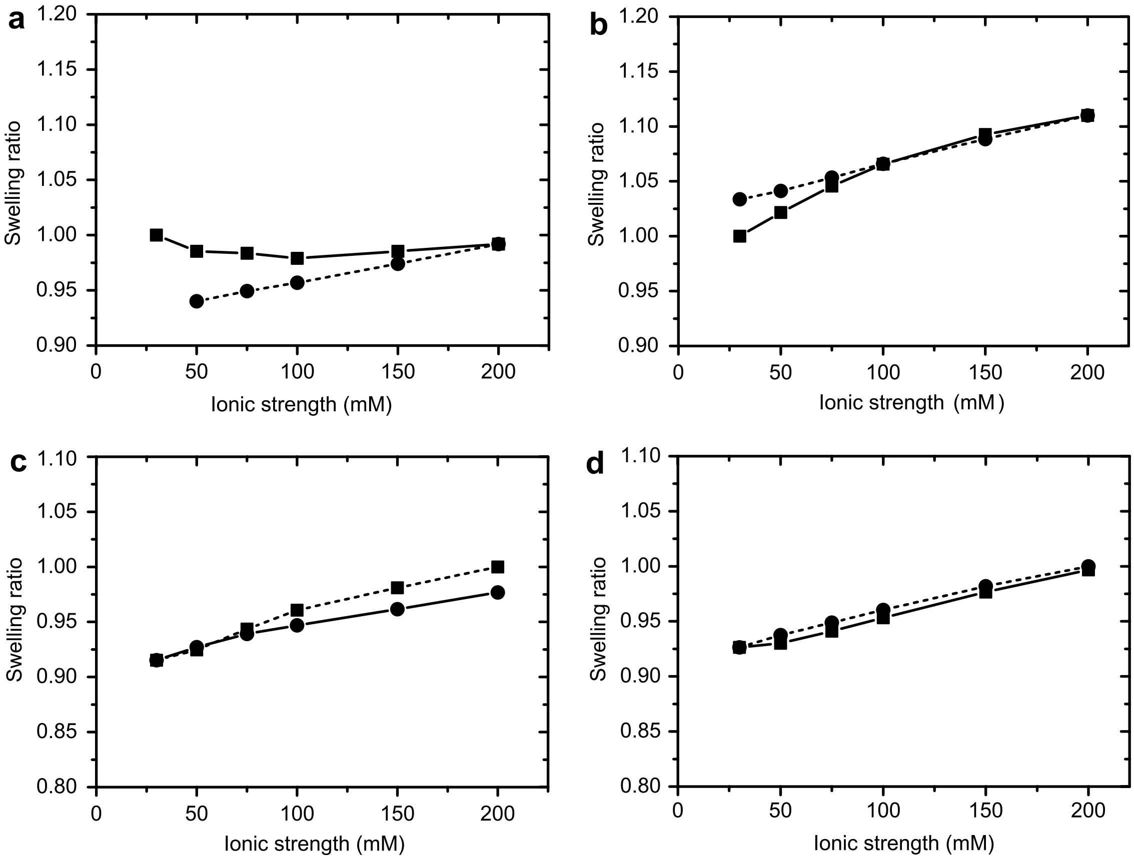

Fig. 1. Swelling ratio as a function of ionic strength, over the range 30–200 mM for (a) 10 and (b) 11 layers formed at 30 mM NaCl, and (c) 10 and (d) 11 layers formed at 200 mM NaCl ((——) ionic strength increase; (----) ionic strength decrease).

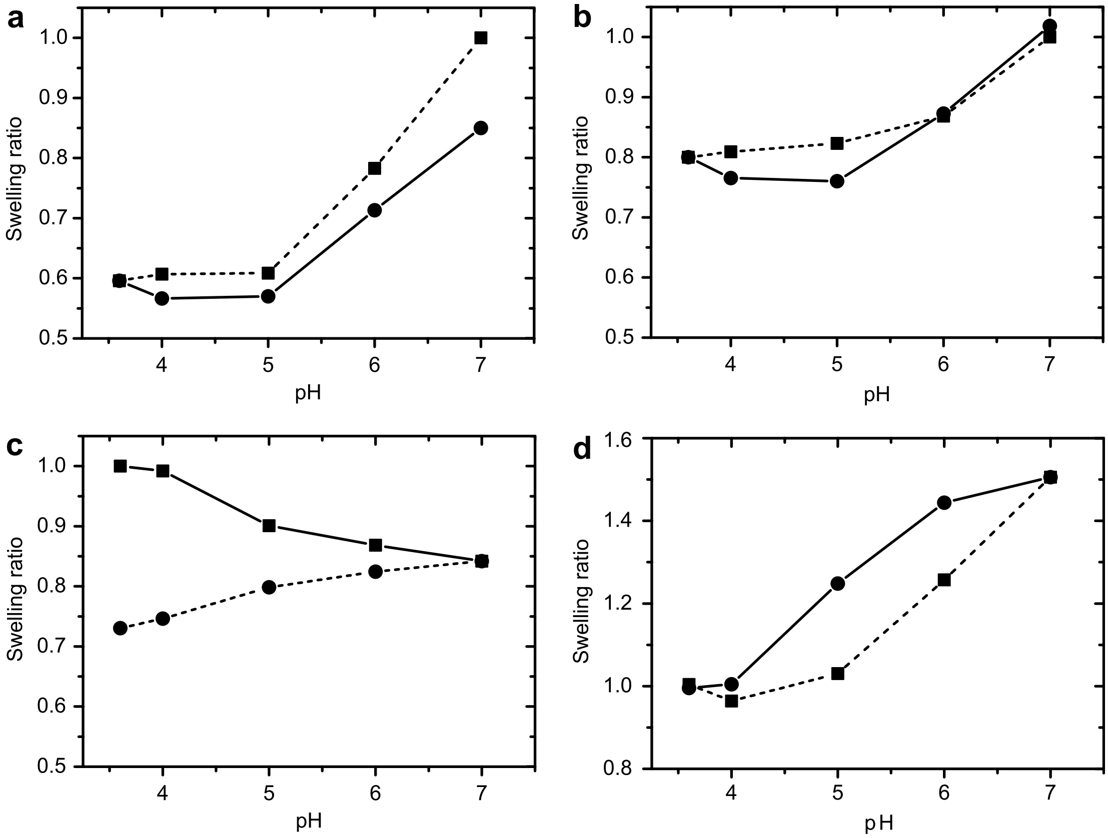

Fig. 2. Swelling ratio as a function of pH, over the range 3.6–7 for (a) 10 and (b) 11 layers formed at pH 7.0, and (c) 10 and (d) 11 layers formed at pH 3.6 ((——) pH increase; (----) pH decrease).

the polymer chains can counteract swelling. If ionization of the anhydrogalacturonate was suppressed then it might be expected that this would decrease the attraction between oppositely charged polymer chains and therefore lead to an increase in swelling – the reverse of what is observed. The observed decrease in swelling with decreasing pH, suggests that pectin chains are the main structural element affecting behaviour, with the extent of ionisation of the anhydrogalacturonosyl residues having an important effect.

The multilayers formed at pH 3.6 have a larger proportion of pectin (Table 1) than the systems formed at pH 7.0. For the 11 layer system (Fig. 2d), swelling increases with increase in pH as before and the observed swelling is largely reversible. The observed effect of pH on swelling is larger than that of the systems formed at pH 7.0 and is consistent with the pectin fraction having a major influence on swelling behaviour. When pectin is the uppermost layer (10 layers, Fig. 2c), non-reversible behaviour is observed with changing pH. On increasing pH to 7.0 there is a decrease in hydrated mass which falls further on subsequent decrease in pH to 3.6. The irreversible nature of the behaviour strongly suggests that there is a solubilisation of polymer with change in pH.

FTIR was used to examine the response to changes in pH in more detail. The transfer of a 10 layer multilayer from pD 7.0 (Fig. 3a) to pD 3.6 resulted in a relatively small increase in the absorbance in the region of 1700–1725 cm^{-1}. The solution spectra of polygalacturonic acid at acidic pH showed a prominent feature with an absorbance at 1715 cm^{-1}, associated with the carbonyl stretching mode of the carboxyl group. The molar absorbances of carboxyl (1715 cm^{-1}) and carboxylate (1600 cm^{-1}) groups are approximately equivalent (Xie and Granick, 2002). Although there is some conversion of anhydrogalacturonate to anhydrogalacturonic acid on change in pD from 7.0 to 3.6, the effect is small. The pK_a of D-galacturonic acid is 3.23 and that of anhydrogalacturonic acid in pectins is in the range 3.0–4.5 (Ralet, Dronnet, Buchholt, and Thibault, 2001). As a consequence it would be expected that change in pD to 3.6 would result in a large increase in the carboxyl absorbance. The small increase observed shows that within the multilayer the acidity of the carboxylate group has increased with a shift in the pK_a to more acidic pHs. The effect of local environment on pK_a has been well documented for the charged residues in proteins and multilayer structures and may have several origins (Xie and Granick, 2002). In the present case it can be attributed to the overall net positive charge balance of the multilayer. For both 10 and 11 layer multilayer structures, examined at pD 7.0, changing the pD in the sequence 7.0 to 3.6 to 7.0 resulted in no detectable spectral change in

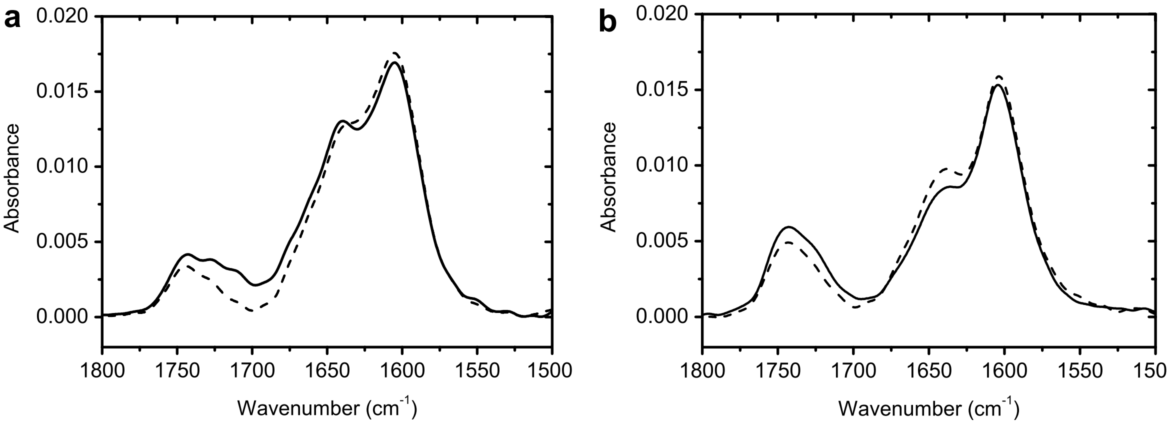

Fig. 3. Comparison of spectra of a 10 layer multilayer formed at pH 7.0 and examined at pD 3.6 (——) and pD 7.0 (----) (3a); and formed at pH 3.6 and examined at pD 3.6 (——) and pD 7.0 (----) (3b).

the range examined, indicating there was no loss of polymer. The observed changes in QCMD response are therefore due to changes in hydration of the multilayer. The decrease in swelling with decrease in pH is associated with suppression in the ionization of a small fraction of anhydrogalacturonate residues.

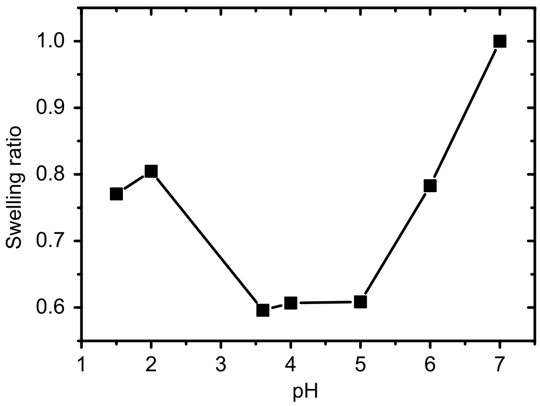

Fig. 4. Swelling ratio as a function of pH in the range 7.0–1.5 for a 10 layer multilayer formed at pH 7.0.

In a similar way the sensitivity of multilayers fabricated at pH 3.6 were also examined. Fig. 3b shows the spectra of the multilayer fabricated at pD 3.6 and examined at pD 3.6 and 7.0. The composition of the multilayer formed at pD 3.6 is somewhat different to that formed at pD 7.0, more particularly there is an enhanced relative absorbance in the region of 1700–1750 cm^{-1}. This is associated with an increase in both the anhydrouronic acid ester and anhydrouronic acid content of the pectin fraction, although the fraction of anhydrouronic acid is less than might be expected from the known pK_a of pectin in solution. Increasing the pD to 7.0 results in a preferential loss of esterified pectin, and a reduction in the anhydrouronic acid content of the multilayer. The non-reversibility of multilayer swelling for a 10 layer multilayer formed at pH 3.6 is therefore associated with a loss of polymer. For the 11 layer multilayer formed at pH 3.6 no detectable loss of polymer was observed when pD was changed in the sequence 3.6 to 7.0 to 3.6. The observed changes in QCMD response are therefore associated with a reversible swelling.

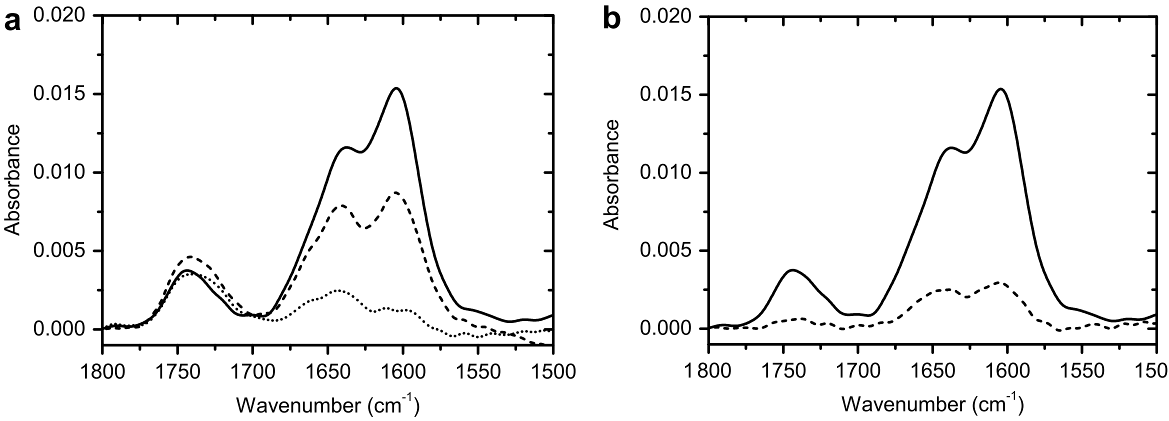

Fig. 5. FTIR-ATR spectra of a 10 layer system formed at pH 7.0 as a function of pD (at pD 7.0 (——)), pD 2.0 (----) and pD 1.5 (······) (5a); and at pD 7.0 before (——) and after (----) exposure to acid pH (5b).

Of relevance to the practical application of these structures is the response to different pHs in sequence. Fig. 4 shows the swelling ratio, as a function of pH, for a 10 layer multilayer which was formed at pH 7.0. Following a decrease in swelling with decreasing pH, an increase in swelling was observed as the pH was lowered to from 3.6 to 2.0. Pectin gels crosslinked with Ca^{2+} also show this form of behaviour, with a peak in swelling, following a progressive decrease, being observed at pH 3.25 (Tibbits et al., 1998). FTIR analysis of the effect of these lower pHs show increasing solubilisation of polymer as the pH is reduced (Fig. 5). At pH 2 there is a loss of 32% w/w of the PLL and a decrease in the anhydrogalacturonate component of pectin of 44% w/w. There is also a relatively small increase in the anhydrogalacturonic acid component. Further decrease in pH to 1.5, results in further loss of PLL, (79% w/w in total) and anhydrogalacturonate, (91% w/w). Subsequent increase in pH to 7.0, shows that this pH sequence has resulted in a total solubilisation of 79% w/w of the PLL, and 83% w/w of the pectin. As the decrease in pH in the range 3.6–2.0 leads to an increased swelling, this suggests that the loss of polymer is accompanied by the formation of a more open structure. On subsequent return to pH 7.0 $>$ 50% of the hydrated mass of the multilayer was lost.

4. Conclusions

The environmental response of multilayers of pectin and PLL to changes in ionic strength and pH was investigated. Systems formed at high ionic strength tended to form more stable structures with a lower hydrated mass than systems formed at low ionic strength. These systems showed a weak reversible increase in swelling with increasing ionic strength characteristic of polyampholyte gels. Response to changing pH was more complex with both reversible and irreversible effects being observed. The reversible pH response was consistent with pectin being the main structural component of the network. Its ionisation was influenced by PLL leading to shifts in the pK_a of the pectin arising from the overall positive charge of the multilayer. Irreversible swelling behaviour was associated with polymer solubilisation and extensive disassembly was observed on returning to neutral pH after initially solubilising the PLL at low pHs.

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