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Preparation and characterization of *in situ* ionic cross-linked pectin films: Unique biodegradable polymers

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ABSTRACT

The study aimed to investigate the swelling and degradation of calcium pectinate (CaP) films that were cross-linked by the innovative approach of adding aqueous calcium chloride ($CaCl_2$) to pre-formed pectin films *in situ*. The films, cast from low methoxy pectin, were dried and cross-linked by immersion in a selected $CaCl_2$ solution for a selected period. It was found that $CaCl_2$ concentration, immersion time, and temperature affected the films' dissolution and swelling behaviors in simulated intestinal fluid. With lower $CaCl_2$ concentration, more time was needed to form a proper film. Heat accelerated the cross-linking reaction, probably by elevating the cross-linked solution flux into the matrix. Depending upon cross-linking conditions, similar calcium contents in the CaP films resulted in different swelling and degradation behaviors. The degree of pectin esterification (DE) affected the films' degradation rate. The role of pectin molecular weight and DE on the films' mechanical properties was determined by stress/strain analysis.

1. Introduction

The pectins are a class of plant polysaccharides that are mostly utilized for their gelling capabilities. Historically, these polymers have attracted considerable attention for their use in colonic drug delivery systems (Wong, Colombo, & Sonvico, 2011) while recently there has also been progress in their application as nasal excipients (Illum, 2012) or as buccal patch constituents (Juq, Kosalec, Maestrelli, & Mura, 2012).

Structurally, pectins are characterized by a backbone of D-galacturonic acid units linked together by α 1→4 glycosidic linkages. These polygalacturonic chains are arranged in a three-fold helix and are interrupted by links with β, L-rhamnose. The presence of rhamnose in the principal chain results in a deviation from the axis of the helix of ~90°. This facilitates the formation of a 3-D network. Pectins always contain some associated neutral sugars, typically, L-rhamnose, L-arabinose, D-galactose, D-xylose and D-glucose. These sugars are often linked to the hydroxyl groups on the number 2 and 3 carbons of the main chain (Aspinall, Craig, & Whyte, 1968; Colquhoun, De Ruiter, Schols, & Vorgan, 1990; Darvill, McNeil, Albersheim, & Delmer, 1980; Reitsma, Thibault, & Pilnik, 1986).

It is noteworthy that the polygalacturonic acid is partly esterified with methyl groups while the free acid groups maybe partly or fully neutralized with sodium, potassium, or ammonium ions. The ratio between the number of esterified galacturonic acid groups to the total number of galacturonic acid groups is termed the 'degree of esterification' (DE). This parameter has a key influence on the properties of the pectin – especially its solubility, gel-forming capacity and its tendency to undergo crosslinking by divalent ions. A DE value of 50% divides commercial pectins into high ester (high methoxy, HM) and low ester (low methoxy, LM) types. These two groups of pectin gel by different mechanisms (Oakenfull, 1991; Pedersen, 1980, chap. 15; Rolin, 1993).

Calcium ions can react with free carboxylic groups throughout the low methoxy pectin chains to create a water-insoluble cross-linked gel structure (Pedersen, 1980). The gel then contains a 3-D network of Ca^{2+} ion-bridged dimers to induce so-called "egg-box" junction zones (Morris, Powell, Gidley, & Rees, 1982). The size of these junction zones is limited by the presence of sequences containing rhamnose and other neutral sugar residues, which may introduce kinks to interrupt the polygalacturonate blocks (Kravtchenko, Voragen, & Pilnik, 1992). The insolubility of CaP films in aqueous media along with the susceptibility of pectin to specific forms of enzymatic degradation make these films suitable candidates for different delivery systems, especially ones

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Table 1
Pectins used for making calcium pectinate films with different molecular weights (MWs) and degree of esterification (DE).

Product	Average molecular weight (Da)	DE (%)
Genu x-2947	122 000	15.0
Genu x-4950	107 000	15.8
Genu x-2947	90 000	16.1
Sanofi – Rouge NA	85 100	27.0
Genu x-4952	114 700	30.6
Genu x-4953	110 000	52.8

involving colonic-targeting (Adkin et al., 1997; Das, Chaudhury, & Ng, 2011; Liu, Fishman, Kost, & Hicks, 2003; Naggar, El-Khawas, Ismail, & Boraie, 1992; Rubinstein, Radai, Ezra, Pathak, & Rokem, 1993; Sande, 2005; Sriamornsak, 2011; Sungthongjeen, Pitaksuteepong, Somsiri, & Sriamornsak, 1999; Yu et al., 2009).

Over the years, many methods have been developed to modulate the calcium salt – pectin reaction rate occurring in an aqueous pectin solution (Garnier, Axelo, & Thibault, 1993, 1994; Thibault & Rinaudo, 1985). These methods have included; the use of an insoluble calcium salt at a selected temperature, control of calcium salt ionization by pH variation, control of calcium salt delivery by using readily soluble sequestrants, and variable solubility of different acids. Although the calcium salt – pectin reaction occurring in an aqueous pectin solution has been extensively studied, no data have been reported on the *in situ* cross-linking of pectin films with calcium salts in solution. Since the reaction between calcium ions and carboxyl groups is rapid, it may be difficult to achieve a homogeneous dispersion of calcium throughout the CaP network. Thus, the final properties of the CaP films formed may be non-uniform.

In the present study, we investigated the kinetics of the reaction between pre-formed pectin film (low methoxy pectin with a known DE) and calcium chloride in aqueous solution. By using this innovative cross-linking approach, the desired aim was to achieve good control over the reaction so as to eventually obtain CaP films exhibiting a range of defined final properties. It was of particular interest to determine the role of pectin DE and pectin molecular weight on ultimate film characteristics.

2. Experimental

2.1. Materials

The citrus-derived pectins (Genu X pectin series) were purchased from Food Gums Division of Hercules Inc. (Copenhagen, Denmark) while the apple-derived pectin was purchased from Sanofi Bio-Industries (Newbury, UK). The DE of each of these pectins was determined by the USP XXII method. Table 1 presents the name, molecular weight and DE of each investigated pectin. The pectin, Genu X-2947 (DE: 15%, MW: 122 000), was used to study cross-linking reaction kinetics. Genu pectin types X-2947 and X-4952 were used for assessing the effect of DE on *in situ* cross-linking. Calcium chloride was purchased from Merck (Darmstadt, Germany). Simulated intestinal fluid (SIF) consisted of 50 mM phosphate buffer solution (PBS) at pH 7.2 that was prepared according to USP XXII (P1789). This solution was diluted to produce a range of solutions exhibiting various concentrations of phosphate (5, 15, and 25 mM).

2.2. Preparation of CaP films

Each film was prepared by depositing the selected aqueous pectin solution (2% (w/v)) into a glass Petri dish. Each solution was allowed to dry at 30 °C until no further loss on drying was detectable and this took ~48 h. Cross-linking was performed by immersing the dry films in aqueous calcium chloride solutions of selected concentrations (0.05, 0.1, 1, 4 and 10% (w/v)), for either 5 s, 10 s, 30 s, 1 min, 5 min or 30 min each. For each cross-linking reaction, the carboxyl to calcium molar ratio was maintained at 1–5. Each cross-linked calcium pectinate (CaP) films thus formed was washed thoroughly with de-ionized water and dried at 40 °C for 24 h.

It was important to determine the role of the pectin molecular weight and DE on the ultimate properties of the final CaP films. To this end, the pectin films were cross-linked by immersion in aqueous calcium chloride solution (4% (w/v)) for 30 min. This was followed by several washings with de-ionized water. These CaP films were dried at room temperature to constant weight.

2.3. Polymer swelling studies

Pre-weighed CaP films were stirred in a 50 ml volume of SIF maintained at 37 °C. Aliquots of 2 ml were withdrawn at 1, 2, 3, 4, 5 and 20 or 25 h. After each withdrawal, the SIF volume was replenished with an equal volume of fresh medium. Equilibrium swelling properties in SIF were determined gravimetrically and expressed as a % weight-gain over the initial dry weight.

2.4. Degradation studies

Degradation of the films was assessed by measuring both free pectin and calcium concentrations in the 2 ml aliquots obtained in the manner described above.

Pectin concentrations in the dissolution medium, indicative of film degradation, were assayed by gel filtration chromatography. This required use of a 5-point calibration curve (0.01, 0.05, 0.10, 0.25 and 0.5% (w/v)). The GFC system consisted of an HPLC pump (Waters 510), a differential refractometer (Waters 410) warmed to 40 °C and an autosampler (Waters 717). The column was a Shodex® OH pak KB-806 warmed to 35 °C. The mobile phase consisted of the SIF, degassed, sonicated and pre-filtered through an Alltech® 0.45 μm membrane filter and an on-line Rheodyne® inlet filter placed before the column. The flow rate was 1 ml/min. The samples were filtered through a 0.45 μm syringe filter prior to injection.

The amount of calcium ions (% (w/w)) in each CaP film, or in the dissolution medium, was quantified by ion exchange chromatography (IEC). To this end, a 6-point calibration curve (10, 20, 40, 60, 80, and 100 ppm) was constructed. The standards were prepared by dissolving calcium chloride in purified water and acidifying by adding 1% of 0.5 M hydrochloric acid. The IEC system consisted of an HPLC pump (Waters 510) and a conductivity detector (Waters 431). An Alltech® Universal Cation 7 μm (100 mm × 4.6 mm) cartridge column was used in conjunction with an Alltech® cation guard column. The mobile phase consisted of 4 mM nitric acid (pH at 2.4 ± 0.1) that had been filtered through a 0.45 μm nylon membrane filter, degassed with helium and sonicated before use. The flow rate was 1 ml/min. Film samples were digested in 1 ml of nitric acid for 24 h, followed by the addition of 100 ml of 0.005 M hydrochloric acid. Each sample was passed through a 0.45 μm syringe filter prior to injection (20 μl) into the HPLC system.

2.5. Measurements of mechanical properties

Dry film strips were initially soaked in water for 2 h, after which their dimensions were measured. Film elasticity was measured using a Series IX Automated Materials Testing System 1.15, which is a stress/strain apparatus developed by Instron (High Wycombe, UK). The device was used under the following settings: specimen gauge length of 15–20 mm, gauge width of 6 mm, gauge thickness of 100–450 μm, crosshead speed of 20 mm/min and relative humidity of 40–50%. Three different specimens of the same type of film were analyzed in order to yield one mean elasticity measurement.

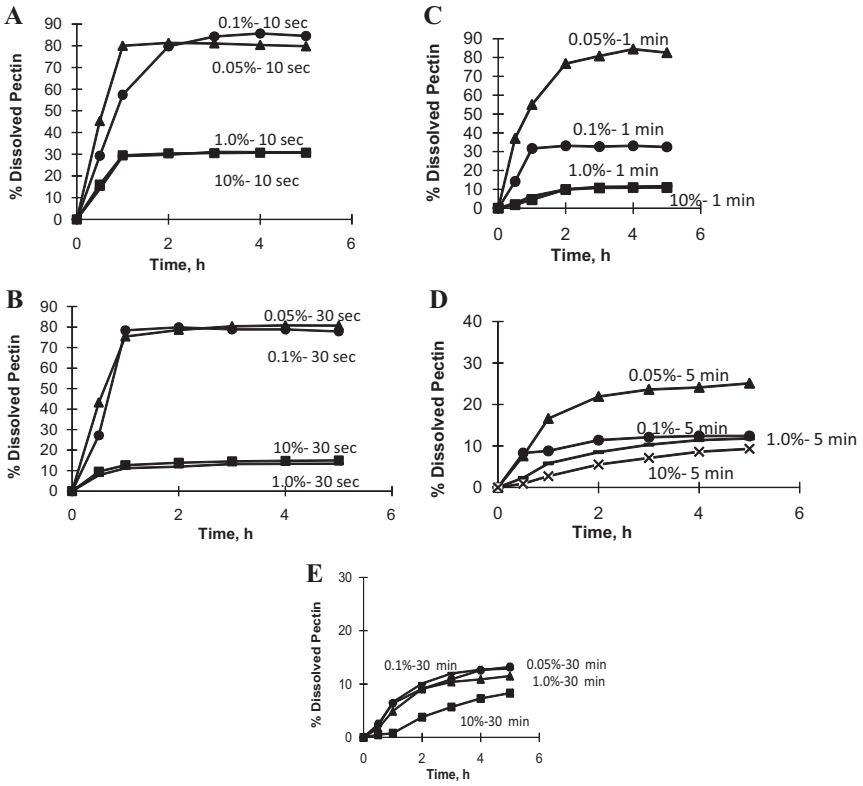

Fig. 1. The effect of immersion time (A – 10, B – 30, C – 60 s, D – 5 min and E – 30 min) in increasing concentrations of $CaCl_2$ (triangles: 0.05, circles: 0.1, rectangles: 1.0, squares: 10.0% (w/v)) on the stability of the *in situ* formed CaP films, as expressed by free pectin release into SIF. Shown are the mean values with $n = 3$. SD values were negligible.

3. Results and discussion

3.1. The kinetics of the cross-linking reaction

Fig. 1 presents the graphs of pectin dissolution versus time that were obtained when CaP films were soaked in SIF. Fig. 1A–E shows dissolution data for immersion times of 5 s, 10 s, 5 min, 10 min and 30 min, respectively. Furthermore, each of these 5 graphs contains 4 plots representing different $CaCl_2$ concentrations. It can be seen that the dissolution of free pectin depends on both the solution's $CaCl_2$ concentration as well as the immersion time. Raising the $CaCl_2$ concentration tends to decrease the dissolution of free pectin. This feature was more pronounced at shorter immersion times. Lengthening the immersion period also tends to decrease pectin dissolution. Generally, the dissolution of free pectin from a cross-linked film is inversely correlated to the extent of cross-linking in the polymer. Less pectin dissolution usually means there is greater cross-linking and thus smaller degradation rates. Hence, the results suggest that the *in situ* cross-linking of pectin is a kinetic process, in which beyond the chemical affinity of calcium ions for galacturonic acid, there is a diffusion of $CaCl_2$ into the pectin/CaP film. A very brief immersion time (~30 s) was needed for the higher range of concentrations (1% or 10%) of $CaCl_2$ solution to show a constant value of dissolved pectin in SIF, as opposed to a 30 min period needed for the lowest concentration (0.05%).

Fig. 2 shows the graph of total pectin dissolution versus time that was obtained when the CaP films were soaked in SIF. The influence of 3 different immersion times and 2 different temperatures is shown. The results indicated that providing both the calcium ion concentration and immersion time were fixed, soaking the films at a higher temperature enhanced the cross-linking of pectin. For example, in a 10% $CaCl_2$ solution, film immersion for 10 s at 40 °C produced a similar pectin dissolution to that obtained following immersion for 30 s at room temperature. It is likely that the rate of calcium ion diffusion into the film is directly enhanced by higher temperatures.

The graphs in Fig. 3 present the bound calcium ion content in CaP films as a function of both immersion time and $CaCl_2$ concentration. It was observed that lengthening the film's immersion time in the $CaCl_2$ solution elevated the film's bound calcium ion

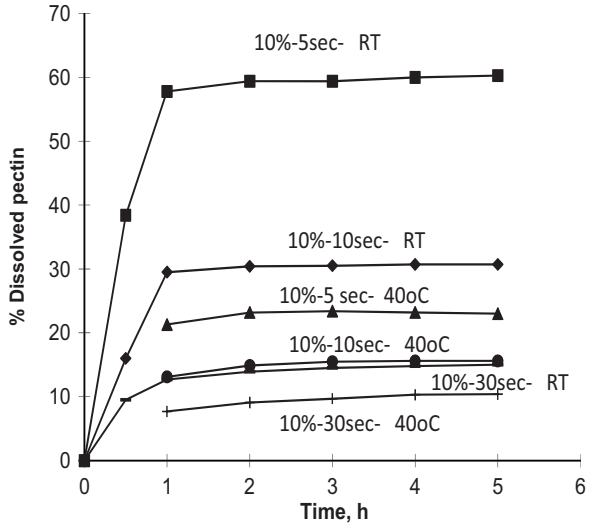

Fig. 2. The effect of $CaCl_2$ solution temperature [immersion times 5 (squares), 10 (rhombi) and 30 (rectangles) s at room temperature or 5 (triangles), 10 (circles) and 30 (crosses) s at 40 °C] on the stability of the *in situ* formed CaP films, as expressed by free pectin release into SIF. Shown are the mean values with $n = 3$. SD values were negligible.

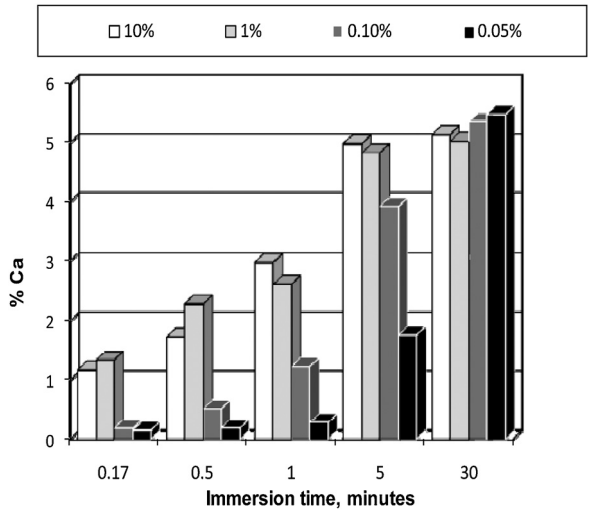

Fig. 3. Bound Ca content (% (w/w)) in CaP film as a function of $CaCl_2$ solution concentration and immersion times. Shown are the mean values with $n = 3$. SD values were negligible.

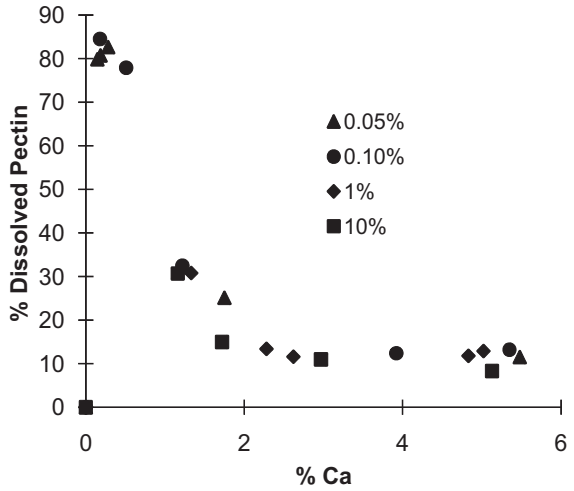

Fig. 4. The correlation between the percentage of bound Ca and dissolution of free pectin from CaP film. Shown are the mean values with $n = 3$. SD values were negligible.

content. This relationship held for all concentrations of $CaCl_2$ solution tested in this study. The highest levels of bound calcium ion content were 5.13–5.48% and these were only achieved following 30 min of immersion. Considering a DE of 15%, a molecular weight of 122 000 Da (Table 1) and the weight of pectin film taken for cross linking, a calcium content of 5% corresponds to a molar ratio of ~0.4 calcium ions to 1 galacturonic acid. So regardless of $CaCl_2$ concentration, a 30 min immersion time was sufficient for more or less all the available carboxyl groups to be bound to calcium ions. In other words, cross-linking can reach the highest level, regardless of $CaCl_2$ concentration, if the film is allowed for a sufficient time to be immersed in $CaCl_2$ solution. This means that cross-linking density in the resulting films will probably be similar for all concentrations at longest immersion time. Based on the findings from Figs. 2 and 3 one can also conclude that the diffusion of $CaCl_2$ into the film is based on a kinetic process in which the parameters such as concentration of $CaCl_2$ solution, immersion time and temperature of the $CaCl_2$ solution can affect the content of calcium ion in the film.

It seems that a higher concentration of $CaCl_2$ solution can promote rapid absorption of $CaCl_2$ solution into the pectin films, thus allowing a more homogeneous Ca-pectin reaction throughout the film even at the beginning of the immersion *i.e.* in a short immersion time. This fact can also be seen in Fig. 1 where low values of dissolved pectin are resulted upon exposure to a higher concentration of $CaCl_2$ solution even for a short immersion time. On the contrary, lower concentration of $CaCl_2$ solution can generate rapid absorption of $CaCl_2$ solution at the pectin film surface. Also, it is noteworthy that the rapid cross-linking occurring at the surface of pectin films upon the use of a low concentration of $CaCl_2$ solution does not prevent the further absorption of $CaCl_2$ solution with time.

Fig. 4 plots the dissolution of free pectin from the CaP film as a function of the bound calcium ion content. It is apparent that the two parameters are roughly inversely proportional to each other. This is because the more calcium cross-linking occurs, the less pectin from the film dissolves in the SIF. Interestingly, the graph indicates that beyond a critical concentration of 2% (w/w) Ca in CaP, further increases in bound calcium content, do not affect pectin dissolution in SIF. This feature is noteworthy, given the above estimate that a calcium content of ~5% is required to achieve maximum cross-linking (considering a molar ratio of 0.4 Ca:COOH). This is explainable by the fact that the pectin backbone, which is composed of both linear sequences (regular uronic regions, "smooth") as well as branched regions (rhamnose-rich regions, "hairy") (Durand, Bertrand, Clark, & Lips, 1990; Garnier et al., 1994), can form molecular entanglements between tied junctions during the cross-linking process. Such entanglements can prevent the dissolution of free pectin which remains either not cross-linked or cross-linked to a low degree.

In studies aimed at elucidating the role of temperature, CaP films were immersed for 30 s in $CaCl_2$ solutions (10% (w/v)) maintained at either room temperature or at 40 °C. It was discovered that bound calcium ion content was 1.6% following cross-linking at room temperature as opposed to 2.6% following cross-linking at 40 °C. This reflects direct thermal acceleration of calcium ion diffusion.

The graph in Fig. 5 depicts the swelling behaviors of two different CaP films in SIF at 37 °C. The two films had been cross-linked under different calcification conditions. One film was calcified by immersion in 10% (w/v) $CaCl_2$ solution for 30 s while the other film was calcified by immersion in a 0.05% (w/v) $CaCl_2$ solution for 5 min. Crucially, both films had an identical bound calcium content of ~1.7%. Yet it is obvious that they exhibit different SIF uptake profiles. This probably reflects a structural difference in terms of the homogeneity and dispersion of cross-linking bonds. It seems that cross-linking occurring upon with exposure to a more concentrated calcium ion solution enables more homogeneous cross-linking throughout the film even if the film is exposed to $CaCl_2$ solution

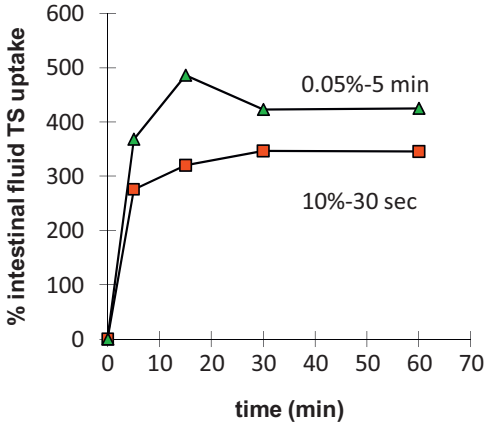

Fig. 5. Swelling kinetics of two different CaP films containing identical bound Ca content. Shown are the mean values with $n = 3$. SD values were negligible.

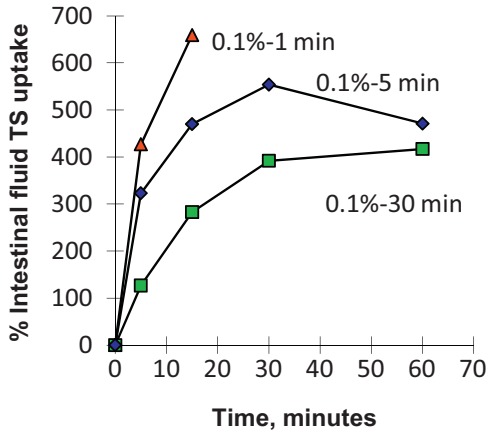

Fig. 6. Swelling kinetics of CaP films made by immersing pectin films in 0.1% (w/v) $CaCl_2$ solution for 1 (squares), 5 (rectangles) and 30 (triangles) min in SIF. Shown are the mean values of 3 measurements. Standard deviation values were negligible.

for a shorter time. This forms a denser network that relatively suppresses the absorption of SIF diffusion into the film. Conversely, slower cross-linking associated with exposure to more dilute calcium ion solutions, specially in a shorter immersion time, generates proportionally more cross-linking bonds specially at the film surface. This in turn leaves uncross-linked polymer chains in the bulk of the film allowing more SIF flux into the bulk of the film and thus more SIF uptake is resulted. Also it is noteworthy, that the uncross-linked polymer chains in the bulk of the film upon exposure to more dilute calcium ion solutions may still dissolve to leave the film. This may explain the decrease occurs in SIF uptake following 30 min as compared to 15 min as seen in Fig. 5 for the film resulting from 0.05%, 5 min.

Fig. 6 presents the SIF uptake profiles of three different films. Each film had been subjected to different immersion times in 0.1% (w/v) $CaCl_2$ solution. Hence, the films had a different bound calcium ion content. It is apparent that the higher the degree of cross-linking, the lower the SIF uptake and hence film swelling. Thus, there is a direct correlation between the swelling of the film at equilibrium and the bound calcium ion content which corresponds to the degree of cross-linking.

3.2. Degradation of CaP films in PBS

The stability of CaP films exposed to PBS of differential phosphate concentrations was determined from pectin dissolution assays. Two types of CaP films were investigated. Both of these films had been originally prepared from low methoxy pectins (type X-2947 and type X-4952) that had been immersed in 10% $CaCl_2$ solution for 30 min. Tables 2 and 3 summarize the derived dissolution data for the films with DE of 16% and DE of 30%, respectively.

Table 2
Pectin dissolution as a function of phosphate concentration for a DE of 16%.

Time (h)	Phosphate concentration (mM)				
	0	5	15	25	50
	Pectin dissolved (%)				
1	0.1	0.9	0.9	0.2	0
2	0.7	3.33	1.6	1.2	1.3
3	0.7	4	1.9	1.4	1.9
4	0.1	4	2.1	1.7	2.4
5	0.3	4.7	2.6	1.9	2.5
20	2.1	11.4	6.5	5.3	6.8

Table 3
Pectin dissolution as a function of phosphate concentration for a DE of 30%.

Time (h)	Phosphate concentration (mM)				
	0	5	15	25	50
	Pectin dissolved (%)				
1	0	0.6	0	4.9	20.5
2	0.25	11.6	4.9	7.2	23.3
3	0.37	15.6	7.2	9.4	26.5
4	0.38	18.7	9.4	10.9	28.1
5	0.5	21.8	10.9	12.6	30.56
20	1.75	37.4	12.6	21.8	41.9

It was found that increasing the phosphate concentration from 0 mM to 50 mM, caused a dramatic elevation in pectin dissolution. This was true for both pectin types but was more pronounced for a DE of 30%. At 20 h, the difference in dissolution between 5 mM phosphate and 50 mM phosphate was not significant indicating that the amount of phosphate is not a limiting factor for the pectin dissolution process.

In general, films composed of the pectin with a lower DE were more stable. This feature is consistent with the fact that a lower DE means a greater Ca ion content and thus more cross-links, yielding a more rigid and stable structure. The CaP with a DE of 16% was essentially stable to phosphate buffer for 20 h. This film always lost less than 10% of its pectin at all phosphate concentrations tested. In contrast, films with a DE of 30% were only partially stable over the 20 h study and lost up to 40% of their pectin into solution. However, these films still appeared intact at the end of the experiment. Another result was that CaP films made from apple pectin with a DE of 30% dissolved totally in SIF (PBS of 50 mM) (data not shown).

It is noteworthy that no dissolution of pectin from CaP films of either DE of 30% or DE of 16% occurred when these films were exposed to acetate buffer at pH 7.5. This result taken together with Table 3 data strongly suggest that the dissolution of pectin from CaP films is associated with the instability of CaP film in PBS specifically and not the dissolution of free (uncross-linked) pectin from CaP films. In particular, we hypothesize that some calcium ions bind to the phosphate ions in solution. Consequently, at the sites of calcium ion release, the film surface becomes non-cross-linked and this facilitates free pectin dissolution from the CaP film. We believe this mechanism suggests that the CaP films undergo surface degradation rather than bulk degradation. This can be supported by the observation seen during the exposure of the film in phosphate buffer which showed that the films generally keep their integrity during the degradation process. Furthermore, such a mechanism may allow a controlled degradation process to occur, depending upon the calcium ion concentrations at the film surface. Accordingly, the calcium carboxylate bond is somewhat labile. At any given time, there is a very small concentration of free calcium ions in solution which can be precipitated by phosphate at pH 7 or above. This drives the equilibrium toward the calcium phosphate precipitate and pectin in solution. It can be concluded that while CaP films are insoluble in both water and many buffers, they are decomposed by phosphate buffer. It seems that the lower degree of cross-linking exhibited by a pectin with higher DE makes the calcium carboxylate bonds more accessible to phosphate ion attacks and thus more pectin dissolution eventually occurs.

By comparing the data presented in Table 2 (MW of pectin = 90 000 Da) with the data shown in Fig. 1E (MW of pectin = 122 000 Da), it is possible to determine the effect of pectin molecular weight on pectin dissolution from CaP films. Thus, it seems that pectin molecular weight has no effect on pectin dissolution.

Table 4
Mechanical analysis data for CaP films.

DE (%)	MW (Da)	Water swelling (%)	Strain (%)	Stress (MPa)	Young's Modulus
15.0	122 000	73	15	11.7	163
15.8	107 000	67	13	16.3	270
16.1	90 000	61	8	22.5	689
27.0[a]	85 100	81	45	5.3	40
30.6	114 700	104	15	5.4	65
52.8	110 000	489	52	0.5	14

[a] An apple pectin.

3.3. Mechanical properties of CaP film

Table 4 lists the results of the mechanical analysis undertaken on CaP films. It can be seen that the Young's Modulus values were quite high while the values of % strain values were low. This means that the CaP films were generally quite brittle despite the fact that they were tested in the wetted state. Such properties probably arise as a consequence of the inflexibility of the pectin chains as well as their high level of cross-linking.

The influence of molecular weight on mechanical properties can be determined by comparing the three CaP films exhibiting DE values ranging between 15 and 16.1% *i.e.* the first three entries in Table 4. It can be seen that the lower the molecular weight the stronger the film (higher stress value) and the more rigid it is (higher Young's Modulus value).

It is also possible to examine the data in a manner that reveals the role of DE on mechanical properties. This involves comparing the 1st, 5th and 6th entries in Table 4. These films have similar molecular weights but differing DE values. The films with a higher DE are less rigid and much weaker. The stress needed to break the film goes down as does the Young's Modulus. The % strain increases with increasing DE. A less rigid film absorbs more water and swells much more. This is explainable by the fact that films with a higher DE have less molecular sites available for cross-linking the pectin.

It is also interesting to compare the mechanical properties of the tested apple pectin film with the citrus pectin (Genu pectin series) films. It is apparent that the apple pectin-based CaP is less rigid. This is explainable by the fact that the molecular structure of apple pectin is less linear than that of citrus pectin (De Vries, Rombouts, Vorgan, & Pilnik, 1982; Englyst, Hay, & Macfarlane, 1987; Powell, Morris, Gidley, & Rees, 1982) and therefore it has less cross-linking junctions.

4. Conclusions

This study showed for the first time that it is possible to synthesize an insoluble calcium pectinate (CaP) film by *in situ* cross-linking of pectin film in a calcium chloride ($CaCl_2$) solution. The parameters; immersion time, $CaCl_2$ concentration and solution temperature all influenced the final properties of the CaP film. Such effects were mediated by changes in both bound calcium ion content as well as the dispersion of the cross-linking bonds. Another finding was that a calcium ion content of 2% (w/w) in CaP films was sufficient to hinder the dissolution of free pectin (not cross-linked or poorly cross-linked pectin chains) from cross-linked films. However, further increases in cross-linking affected both SIF uptake as well as swelling at equilibrium. Hence, by selecting the optimal cross-linking parameters (*i.e.* $CaCl_2$ concentration, immersion time and temperature) it should be possible to modulate the films' SIF uptake and swelling behavior. This study also demonstrated that other CaP film characteristics such as the final mechanical properties, and stability in buffers of different ionic strengths, depend on the degree of cross-linking. For instance, less rigid CaP films could be obtained by using pectin with a higher DE which contains fewer calcium ion cross-links.

CaP films exhibit aqueous insolubility together with susceptibility to colon microflora-specific enzymatic degradation. This rare combination of properties makes these polymers highly suitable for colonic drug delivery and targeting applications. For such purposes, CaP films are often used to coat solid dosage forms. Such coats must have the capacity to swell upon exposure to intestinal fluid, with the extent of swelling being compatible with that undergone by the dosage form core. Hence, a good CaP film must exhibit the mechanical properties, especially flexibility/rigidity and strength, to tolerate the stress exerted by the swelling core. The extent of swelling may also affect the rate and duration of enzymatic degradation in the colon. We believe that the cross-linking paradigms revealed in this paper can be applied in order to optimize coating process conditions. This should facilitate the synthesis of a stable but less rigid CaP film that readily swells to form a mechanically firm coating film. Future research could focus on another crucial aspect which is the nature of cross-linking distribution and uniformity through the film thickness, the effect of pectin DE and molecular weight on the enzymatic stability of CaP films.

References

Adkin, D. A., Kenyon, C. J., Lerner, E. I., Landau, I., Strauss, E., Caron, D., Penhasi, A., Rubinstein, A., & Wilding, I. R. (1997). The use of scintigraphy to provide proof of concept for novel polysaccharide preparations designed for colonic drug delivery. *Pharmaceutical Research*, *14*, 103–107.

Aspinall, G. O., Craig, J. W. T., & Whyte, J. L. (1968). Lemon-peel pectin. *Carbohydrate Research*, *7*, 442–452.

Colquhoun, I. J., De Ruiter, G. A., Schols, H. A., & Vorgan, A. G. J. (1990). Identification by n.m.r. spectroscopy of oligosaccharides obtained by treatment of the hairy regions of apple pectin with rhamnogalacturonase. *Carbohydrate Research*, *206*, 131–144.

Darvill, A., McNeil, M., Albersheim, P., & Delmer, D. P. (1980). The primary cell walls of flowering plants. In N. E. Tolber (Ed.), *Biochemistry of plants* (pp. 91–162). New York: Academic Press.

Das, S., Chaudhury, A., & Ng, K. Y. (2011). Polyethyleneimine-modified pectin beads for colon-specific drug delivery: In vitro and in vivo implications. *International Journal of Pharmaceutics*, *406*, 11–20.

De Vries, J., Rombouts, F. M., Vorgan, A. G. J., & Pilnik, W. (1982). Enzymatic degradation of apple pectins. *Carbohydrate Polymers*, *2*, 25–33.

Durand, D., Bertrand, C., Clark, A. H., & Lips, A. (1990). Calcium-induced gelation of low methoxy pectin solutions—Thermodynamic and rheological considerations. *International Journal of Biological Macromolecules*, *12*, 14–18.

Englyst, H. N., Hay, S., & Macfarlane, G. T. (1987). Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiology Ecology*, *95*, 163–171.

Garnier, C., Axelo, 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.

Garnier, C., Axelo, M. A., & Thibault, V. J. F. (1994). Selectivity and cooperativity in the binding of calcium ions by pectins. *Carbohydrate Research*, *256*, 71–81.

Illum, L. (2012). Nasal drug delivery – Recent developments and future prospects. *Journal of Controlled Release*, *161*, 254–263.

Juq, M., Kosalec, I., Maestrelli, F., & Mura, P. (2012). Development of low methoxy amidated pectin-based mucoadhesive patches for buccal delivery of triclosan: Effect of cyclodextrin complexation. *Carbohydrate Polymers*, *90*, 1794–1803.

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

Liu, L., Fishman, M. L., Kost, J., & Hicks, K. B. (2003). Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials*, *24*, 3333–3343.

Morris, E. R., Powell, D. A., Gidley, M. J., & Rees, D. A. (1982). Polymorphism between gel and solid states of calcium polygalacturonate. *Journal of Molecular Biology*, *155*, 507–516.

Naggar, V. F., El-Khawas, M., Ismail, F. A., & Boraie, N. A. (1992). Pectin, a possible matrix for oral sustained release preparations of water-soluble drugs. *STP Pharma Sciences*, *2*, 227–234.

Oakenfull, D. G. (1991). The chemistry of high methoxy pectins. In R. H. Walter (Ed.), *The chemistry and technology of pectin* (pp. 87–108). New York: Academic Press.

Pedersen, J. K. (1980). Pectin. In R. L. Davidson (Ed.), *Handbook of water-soluble gums and resins*. New York: McGraw Hill.

Powell, D. A., Morris, E. R., Gidley, M. J., & Rees, D. A. (1982). Conformations and interactions of pectins. I: Polymorphism between gel and solid states of calcium polygalacturonate. *Journal of Molecular Biology*, *15*, 517–531.

Reitsma, J. C. E., Thibault, J. F., & Pilnik, W. (1986). Properties of amidated pectins. I: Preparation and characterization of amidated pectic acids. *Food Hydrocolloids*, *1*, 121–127.

Rolin, C. (1993). Pectin. In R. L. Whistle, & J. N. Bemiller (Eds.), *Industrial gums: Polysaccharides and their derivatives* (pp. 257–293). New York: Academic Press.

Rubinstein, A., Radai, R., Ezra, M., Pathak, S., & Rokem, J. S. (1993). In vitro evaluation of calcium pectinate: A potential colon-specific drug delivery carrier. *Pharmaceutical Research*, *10*, 258–263.

Sande, S. A. (2005). Pectin-based oral drug delivery to the colon. *Expert Opinion on Drug Delivery*, *2*, 441–450.

Sriamornsak, P. (2011). Application of pectin in oral drug delivery. *Expert Opinion on Drug Delivery*, *8*, 1009–1023.

Sungthongjeen, S., Pitaksuteepong, T., Somsiri, A., & Sriamornsak, P. (1999). Studies on pectins as potential hydrogel matrices for controlled-release drug delivery. *Drug Development and Industrial Pharmacy*, *25*, 1271–1276.

Thibault, J. F., & Rinaudo, M. (1985). Interactions of mono- and divalent counterions with alkali- and enzyme-deesterified pectins in salt-free solutions. *Biopolymer*, *24*, 2131–2143.

Wong, T. W., Colombo, G., & Sonvico, F. (2011). Pectin matrix as oral drug delivery vehicle for colon cancer treatment. *AAPS PharmSciTech*, *12*, 201–214.

Yu, C. Y., Cao, H., Zhang, X. C., Zhou, F. Z., Cheng, S. X., Zhang, X. Z., et al. (2009). Hybrid nanospheres and vesicles based on pectin as drug carriers. *Langmuir*, *25*, 11720–11726.