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Enzymatically and chemically de-esterified lime pectins: characterisation, polyelectrolyte behaviour and calcium binding properties

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Abstract

A series of pectins with different levels and patterns of methyl esterification was produced by treatment of a very highly methylated pectin with acid, alkali, plant pectin methyl esterase and fungus pectin methyl esterase. The intrinsic pK values, as well as the free fractions of monovalent and calcium counterions, were determined on pectin salt-free solutions. The variations of pK_a versus the ionisation degree were found to depend on the de-esterification process but a unique value of 2.90 ± 0.15 was estimated for the intrinsic pK value. Calcium binding properties of chemically and enzymatically de-esterified pectins were investigated and experimental results were compared to Manning's theoretical values. A progressive dimerisation process for pectins with a blockwise distribution of carboxyl groups in the presence of calcium ions is hypothesised. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Pectin; Polyelectrolyte; De-esterification; Pectin methyl esterase; Calcium binding

1. Introduction

Pectins are ionic plant polysaccharides that are widely used in food industry for their gelling properties.[1,2] Their main structural features consist of a linear chain of >100 (1→4)-linked α-D-galacturonic acid units.[3] These 'smooth' homogalacturonic regions are interrupted by 'hairy' rhamnogalacturonic regions in which galacturonic acid residues are interspersed with (1→2)-linked α-L-rhamnopyranosyl residues carrying neutral sugars side chains.[4] Pectins also carry nonsugar substituents, essentially methanol, acetic acid, and, in some commercial samples, amide groups.[2] Native pectins are very often highly methylated (HM pectins) and pectins of lower ester content (LM pectins) can be prepared. The latter are able to strongly react with calcium ions and, in defined conditions, to form gels for food applications.[1,2] LM pectins are generally obtained by controlled acid de-esterification but other means, namely alkali, enzymes and ammonia, can be used. Treatment of pectin with acid, alkali, or acidic microbial (*Aspergillus japonicus*, *Aspergillus niger*, *Aspergillus foetidus*) pectin methyl esterases (PME) leads to pectins with a random

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distribution of free carboxyl groups,[5–9] whereas the action of alkaline PMEs from higher plants (tomato, orange, alfalfa, apple) and from fungi (*Trichoderma reesei*) results in a blockwise arrangement of free carboxyl groups in the pectin molecule.[7,8,10] The method using ammonia produces a different type of LM pectins in which some carboxylic groups have been amidated. The resulting amidated LM pectins need less calcium to gel and are less prone to precipitation at high calcium levels than non amidated ones.[2] A blockwise distribution of the amide groups was suggested.[11] The interaction of calcium ions with carboxyl groups has been extensively studied, mainly to elucidate the effect of the methoxyl content of the pectins, expressed as their degree of methylation (DM).[5,8,12–14] The gel forming ability with calcium ions increases with decreasing degree of esterification and it is generally agreed that a transition in calcium affinity towards randomly charged pectins occurs around a DM of 40.[9,12] Below this value, a strong intermolecular interaction between calcium ions and LM pectins has been experimentally demonstrated by various authors.[8,13,15,16] A mechanism of calcium binding similar to the 'egg-box' model proposed by Rees and co-workers for alginates has been proposed. A two-stage process was postulated with initial dimerisation of the molecules and subsequent aggregation of these pre-formed dimers.[17,18] Besides the sole charge density parameter, the distribution pattern of free and esterified carboxyl groups has a profound effect on the strength of calcium binding. LM pectins (DM < 50%) with a blockwise distribution of free carboxyl groups were characterised by low calcium activity coefficients, close to that of calcium pectate.[7,8] It seems that 14–20 consecutive free carboxyl groups are necessary to achieve 'egg-box' structures.[5,17]

Although calcium binding properties of pectins with different levels and patterns of esterification have been studied, none of the previously cited authors investigated and compared the polyelectrolyte behaviour of a complete series (DM 10–80%) of pectins de-esterified by different means (acid, alkali, plant PME and fungus PME). In the present study, we have used plant and fungal PMEs and chemical treatments (acid or base) to generate a series of chemically- and enzymatically-de-esterified pectins. The dissociation of these well-characterised pectin samples was followed by potentiometric titration and the degree of binding of the counterions was determined by conductimetry and spectrophotometry.

2. Results and discussion

Three series of model pectins (P16–P76, F11–F76, B15–B71) were generated by separately treating the same mother pectin (GRINDSTED™ Pectin URS 1200; E81) with plant-PME, fungus-PME and base, respectively.[19] Some acid-de-esterified samples (A61–A75) were also generated from E81. Two amidated commercial samples (Am29–20 and Am36–16) derived from lime pectin were also studied.

The relationships between pK_a and the degree of dissociation α of pectins from the B-, F- and P-series are presented in Fig. 1 together with the Lifson and Katchalsky[20] theoretical curves $\Delta pK = f(\alpha)$. A pK_0 value of ~ 2.80 could be extrapolated for polygalacturonic acid, in agreement with previously published data.[11] For pectins from B- or F-series (Fig. 1), pK_a curves revealed a concave curvature for high DMs, a monotonic quasi-linear curve for intermediate DMs and a convex curvature for lower DMs. For a given degree of dissociation, the pK_a decreased with an increase of DM in agreement with previously reported data.[11] The agreement between experimental and theoretical curves is mediocre, except for intermediate DMs ($45\% < DM < 70\%$) for which experimental points were fitted satisfactorily by the theoretical curves. For high degrees of dissociation, highly methoxylated pectins ($DM > 70\%$) exhibited higher pK_a values than expected from the Lifson and Katchalsky theory. Rinaudo and Milas[21] have suggested that for low-charge density, the uniformly charged-rod model is incorrect as long as the distance between two sites is larger than about 0.7 nm. Lowly methoxylated pectins ($DM < 45\%$) showed the same global behaviour than polygalacturonic acid, the exper-

imental values being lower than the theoretical ones for high degrees of dissociation. Furthermore, polygalacturonic acid, B15, F19 and F11 (F11 curve not shown) pK_a experimental curves perfectly superimposed. Condensation is known to occur for a structural density value above 1 (i.e. DM < 38%) but probably takes place progressively, as suggested by Truong et al.[22] In agreement with previously reported data,[11,23] a pK_0 value of 2.90 ± 0.15 was determined for all pectic samples from the B- and F-series, confirming that pK_0 is independent of the DM for pectins with randomly distributed charges.[13,21] In order to standardise the experimental curves, pK_a values were also plotted versus the effective charge density parameter (Fig. 2), together with the theoretical curve (pK_0 2.9). For pectins from the B- and F-series, experimental points were fitted satisfactorily by the theoretical curve for effective charge density values up to ~ 0.7. Condensation phenomenon was shown for higher effective charge density values as discussed above. Pectins from the A- and Am-series exhibited (data not shown) the same behaviours as pectins from the B- and F-series and pK_0 values of 2.9 ± 0.1 were also extrapolated.

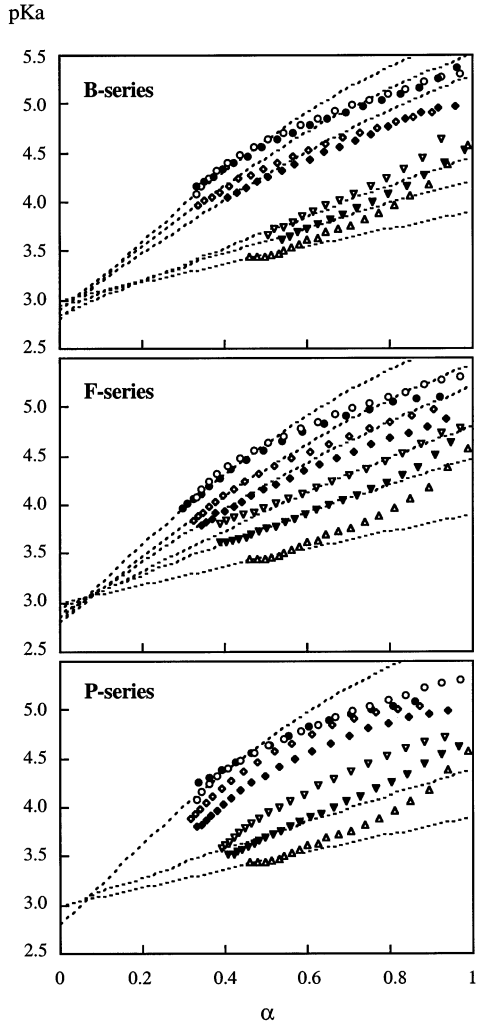

Fig. 1. Variations in pK_a with the degree of dissociation. The lines are the corresponding theoretical $\Delta pK_a = f(\alpha)$ functions. B-series: alkali-de-esterified pectins. (○) Polygalacturonic acid; (●) B15; (◇) B34; (◆) B43; (▽) B64; (▼) B71; (△) E81. F-series: fungus-pectin methyl esterase de-esterified pectins. (○) polygalacturonic acid; (●) F19; (◇) F31; (◆) F43; (▽) F58; (▼) F69; (△) E81. P-series: plant-pectin methyl esterase de-esterified pectins. (○) polygalacturonic acid; (●) P16; (◇) P32; (◆) P41; (▽) P60; (▼) P70; (△) E81.

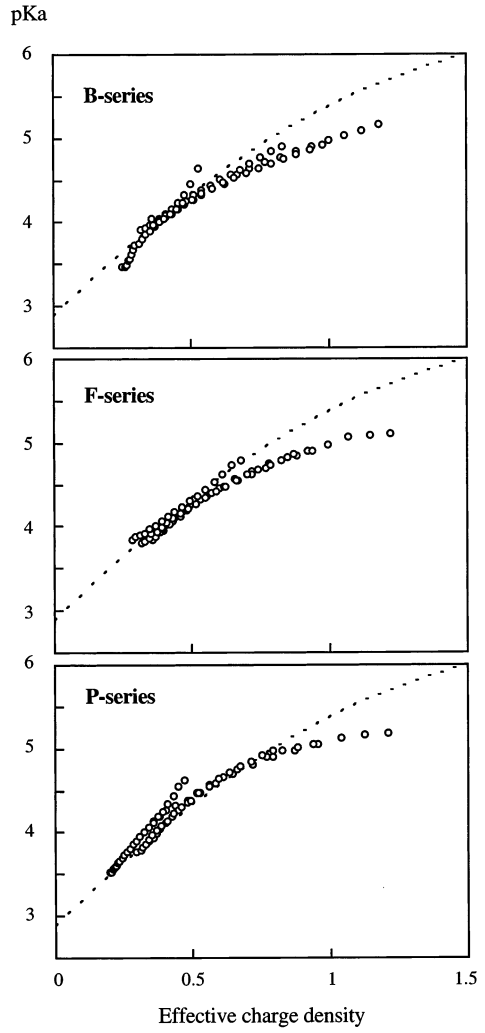

Fig. 2. Variations in pK_a with the effective charge density. The lines are the corresponding theoretical $\Delta pK_a = f(\xi)$ functions for p$K_0 = 2.9$. B-series, alkali-de-esterified pectins; F-series, fungus-pectin methyl esterase de-esterified pectins; P-series, plant-pectin methyl esterase de-esterified pectins.

As for pectins from the B- and F-series, for a given degree of dissociation, the pK_a values of pectins from the P-series decreased with an increase of DM (Fig. 1). Concave, quasi-linear and convex curvatures were observed for pK_a curves but the convex curvatures appeared for much higher DMs (60–65%) than those found for pectins from the B- and F-series. The initial slope of the pK_a curves remained roughly constant at ~ 3.3 from P16 to P66. Such high values were observed for polygalacturonic acid and low DM samples from the B- and F-series (F11, F19, F31, B15). pK_0 values of 2.9 ± 0.1 could be extrapolated for very highly methoxylated samples (up to P70) and for very lowly methoxylated ones (P16, P24). No pK_0 values could be extrapolated for intermediate DM samples as experimental points could not fit satisfactorily with the theoretical curves due to large discrepancies in slopes. The excess of condensation observed for pectins from the P-series is likely to be due to the blockwise arrangement of free carboxyl groups, even for high DM samples. Such an excess of condensation was observed on acrylamide–acrylic acid copolymers presenting acrylate sequences.[22] pK_a values were also plotted versus the effective charge-density parameter (Fig. 2), together with the theoretical curve (pK_0 2.9). Although the experimental points curvature was higher than predicted by the theoretical approach, pK_0 values of 2.9 ± 0.1 could be extrapolated for pectins from the P-series.

It was concluded that pK_a curves vary with the DM and the distribution of free carboxyl groups along the molecule but a unique pK_0 value (~ 2.9) could be extrapolated whatever the DM and the substitution pattern.

Transport parameters and activity coefficients were compared to theoretical values calculated from Manning's theory[24–26] (Table 1). The equivalent conductivity of the polyion (λ_p) increased with decreasing DM and no differences were revealed between the different series. The transport parameter of monovalent counterions decreased regularly with decreasing DM (Fig. 3) in agreement with previously reported data.[8,11] No clear difference could be observed between the different series, contrary to the findings of Thibault and Rinaudo[8] who observed slightly lower values for samples with a blockwise distribution of carboxyl groups. Experimental values were in fairly good agreement with Manning's theoretical ones, showing that the interactions between monovalent ions and chemically or enzymatically de-esterified pectins are close to a classical electrostatic binding.

Calcium binding properties of chemically and enzymatically de-esterified pectins were determined as calcium transport parameters and calcium activity coefficients by conductimetry and by a metallochromic indicator method, respectively. Calcium activity coefficient values were similar or slightly higher than calcium transport parameter ones, in agreement with previous findings.[8,11] Calcium transport parameter and activity coefficient values decreased with decreasing DM down to DM $\sim 20\%$ for pectic samples of the F-, B- and P-series (Fig. 3). Below DM $\sim 20\%$, values remained roughly stable at 0.13 and 0.11 for calcium transport parameter and calcium activity coefficient, respectively. These values are about half that predicted by the Manning's theory indicating the occurrence of pectic dimers.[8] In the range $20\% < DM < 75\%$, pectins from the P-series exhibited significantly lower calcium transport parameter and calcium activity coefficient values than pectins from the B-, A-, and F-series, indicating a stronger binding of calcium ions. A slight but significant difference between chemically de-esterified samples (A- and B-series) and samples from the F-series was observed in the range $35\% < DM < 70\%$, pectins from the F-series being able to bind calcium more tightly. This confirms a more 'ordered' random-de-esterification process hypothesised for samples from the F-series.[19] Amidated samples exhibited an intermediate behaviour between that of pectins from the F- and P-series. Contrary to previously reported data,[5] no clear transition in the calcium transport parameter nor calcium activity coefficient values was observed around DM 40% for none of the studied series. Nevertheless, if the ratio of experimental to theoretical values of the calcium transport parameter (or the calcium activity coefficient) is plotted versus the degree of esterification (Fig. 4), a clear transition can

be seen for pectins from the B- and F- series for DM around 35%. Due to the different calculation means between calcium transport parameter and calcium activity coefficient in the Manning's theory, a much better agreement was found between experimental and theoretical values for calcium activity coefficients. In the range $35\% < DM < 80\%$, experimental to theoretical ratio remained roughly constant for B-, A- and F-series around 0.65 and 1 for calcium transport parameter and for calcium activity coefficient, respectively. The

Table 1
Conductimetric and spectrophotometric data: experimental and theoretical values

	DM (DAm)	ξ	λ_p	Experimental values			Theoretical values		
				f_{Li-K}	f_{Ca}	γ_{Ca}	f_{Li-K}	f_{Ca}	γ_{Ca}
Mother pectin GRINSTED™ Pectin URS 1200									
E81	81.1	0.305	28.0	0.905	0.615	0.715	0.985	0.945	0.737
Samples from de-esterification with f-PME									
F76	75.7	0.391	28.7	0.855	0.580	0.675	0.976	0.914	0.676
F69	66.5	0.539	36.3	0.797	0.468	0.555	0.957	0.807	0.562
F58	56.6	0.699	35.3	0.793	0.400	0.430	0.930	0.623	0.434
F43	42.3	0.929	47.3	0.713	0.280	0.365	0.883	0.468	0.326
F31	31.6	1.101	43.7	0.723	0.247	0.285	0.790	0.395	0.275
F19	17.6	1.327	51.9	0.660	0.145	0.155	0.656	0.328	0.229
F11	10.0	1.449	53.0	0.623	0.135	0.143	0.600	0.300	0.209
Samples from de-esterification with p-PME									
P76	75.6	0.394	37.8	0.835	0.535	0.600	0.976	0.913	0.675
P73	72.8	0.438	33.8	0.765	0.510	0.595	0.971	0.895	0.645
P70	69.9	0.485	35.4	0.805	0.475	0.565	0.964	0.874	0.616
P66	66.0	0.547	35.3	0.835	0.440	0.455	0.955	0.795	0.554
P60	59.4	0.654	39.8	0.720	0.340	0.370	0.938	0.665	0.464
P53	52.7	0.762	40.5	0.737	0.300	0.340	0.918	0.571	0.398
P46	46.1	0.869	42.5	0.720	0.255	0.280	0.897	0.501	0.349
P41	39.8	0.969	40.3	0.710	0.210	0.220	0.874	0.449	0.313
P32	32.9	1.080	40.8	0.695	0.175	0.210	0.805	0.403	0.281
P24	20.8	1.275	45.1	0.650	0.145	0.155	0.682	0.341	0.238
P16	14.2	1.381	43.0	0.670	0.125	0.125	0.630	0.315	0.220
Samples from alkali de-esterification									
B71	72.7	0.440	31.6	0.860	0.575	0.700	0.970	0.894	0.644
B64	64.6	0.570	39.3	0.825	0.485	0.625	0.952	0.763	0.532
B43	41.2	0.947	47.8	0.760	0.315	0.450	0.879	0.460	0.320
B34	32.4	1.088	51.8	0.705	0.240	0.320	0.799	0.400	0.279
B15	15.6	1.359	58.2	0.705	0.130	0.110	0.640	0.320	0.223
Samples from acid de-esterification									
A75	73.4	0.428	31.9	0.860	0.575	0.690	0.972	0.899	0.652
A72	69.0	0.499	33.8	0.860	0.535	0.660	0.962	0.868	0.608
A61	57.7	0.681	39.0	0.835	0.427	0.565	0.933	0.639	0.445
Amidated samples									
Am29-20	28.7 (20.2) [a]	0.823	46.7	0.767	0.300	0.405	0.906	0.530	0.369
Am36-16	35.9 (15.9) [a]	0.776	47.3	0.760	0.327	0.420	0.916	0.563	0.392
Polygalacturonic acid									
PGA	2.0	1.578	61.0	0.615	0.107	0.098	0.551	0.276	0.192
Galacturonic acid									
GalA	0	1.610	22.0	1.005	1.015	1.000	1.000	1.000	1.000

[a] Danisco-Cultor data.

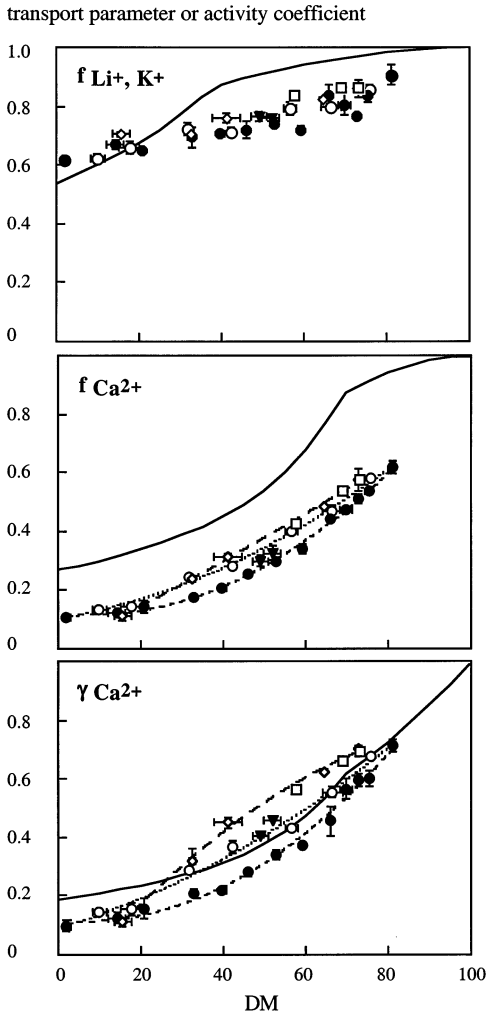

Fig. 3. Variations of values of monovalent ion transport parameter (f_{Li^+,K^+}), calcium transport parameter ($f_{Ca^{2+}}$) and calcium activity coefficient ($\gamma_{Ca^{2+}}$) with the degree of methylation. (◇) Alkali-de-esterified pectins; (□) acid-de-esterified pectins; (○) fungus-pectin methyl esterase de-esterified pectins; (●) plant-pectin methyl esterase de-esterified pectins; (▼) amidated pectins. Lines are Manning's theoretical values.

sudden drop in the ratio of experimental over theoretical values around DM 35%, in agreement with previous findings on alkali-de-esterified pectins,[5,8] can be explained by an intermolecular binding of the calcium ions to carboxyl groups of two molecules leading to the formation of dimers.[8,13] Pectins from the P-series exhibited a radically different behaviour. A roughly continuous decrease in the ratio of experimental over theoretical calcium transport parameter values with decreasing DM (20–80%), that was confirmed by calcium activity coefficient data, was observed (Fig. 4). The de-esterification by plant PME is known to lead to a blockwise arrangement of carboxyl groups and segments long enough to form 'egg-boxes' might be rapidly generated during the de-esterification kinetics. This is in agreement with the excess of condensation revealed by potentiometry for DM < 60–65%. Manning's theoretical values calculated for pectins from the P-series must however be considered cautiously as this theory uses a model of uniform charge density along the polyion. Furthermore, pectins are known to be highly heterogeneous with respect to sugar composition, molar mass and charge density.[2,27] The use of both size-exclusion and ion-exchange chromatography could permit the recovery of more homogeneous pectins and help to establish unambiguous relations between structure and physico-chemical properties.

3. Experimental

Material.—Polygalacturonic acid (PGA) and tetramethylmurexide (TMMX) are from Sigma, LiOH from Prolabo and NaOH, KOH and $Ca(OH)_2$ from E. Merck.

Synthesis of model pectins.—A commercial pectin (L72) from Mexican lime peel (*Citrus aurantifolia*), with a DM of 72% was esterified in acid–MeOH medium to give a pectin (E81) of a DM of 81%. A series of pectins with defined DM were prepared by enzymatic or

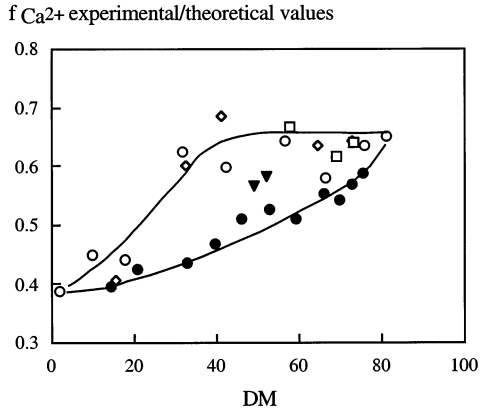

Fig. 4. Variations of the ratio of experimental to theoretical values of calcium transport parameter with the degree of methylation of (◇) alkali-de-esterified pectins; (□) acid-de-esterified pectins; (○) fungus-pectin methyl esterase de-esterified pectins; (●) plant-pectin methyl esterase de-esterified pectins; (▼) amidated pectins.

chemical treatment of E81. The synthesis of these model pectins has been performed by Danisco Biotechnology (Denmark) and a detailed description of the reaction conditions has been previously published.[19] Two commercial amidated pectins from Danisco, GRINDSTED™ Pectin LA 410 (Am2920) and GRINDSTED™ Pectin LA 110 (Am3616) derived from lime pectin were also studied.

Chemical composition and molar mass determination.—All values were calculated on a moisture free basis. Galacturonic acid was quantified colorimetrically, after saponification of the pectin samples (0.05 M NaOH, 30 min, rt) and neutralisation (0.05 M HCl), by the automated *m*-phenylphenol method.[28] For neutral sugars identification, pectins were hydrolysed in 2 M trifluoroacetic acid (2 h, 121 °C). The individual sugars were reduced, acetylated, and analysed by GLC.[29] For DM determination, pectins were recovered in their acidic form (see Potentiometric measurements section). Free carboxylic functions were then quantified at the neutralisation point by conductimetric titrations with three bases (KOH, LiOH, $Ca(OH)_2$) of known molarity (see Conductimetric measurements section) and total carboxylic functions by colorimetry on the same solutions after saponification. DM was calculated by:

$$\begin{aligned}\mathrm{DM} = 100 &\times (\text{total carboxylic functions} \\ &- \text{free carboxylic functions}) \\ &/\text{total carboxylic functions}\end{aligned}$$

On-line intrinsic viscosity and molar mass determinations were performed using a differential viscosimeter (T-50A, Viscotek) and a differential refractometer (ERC 7517 A) ($dn/dc = 0.146$ mL/g) as already described.[27]

Sugar composition and molecular parameter values are given in Table 2.

Potentiometric measurements.—Pectin samples were extensively washed with 65% aq EtOH in order to eliminate salt traces. Pectins were dissolved in ultra-pure water at ~7 meq/L under magnetic stirring overnight at rt. Percolating the sample through a strong H^+-exchanger (Rohm & Hass Amberlite IR 120) allowed the recovery of pectin samples in the acidic form at a concentration (C_p) of ~1 meq/L. Polygalacturonic acid and pectins of low DM (P16, F11, F19, B15) were left in EtOH–HCl under magnetic stirring for 1 h before being extensively washed with 65% aq EtOH.

pH measurements were performed at 25.0 ± 0.2 °C with a pH-meter LPH 430 T (Radiometer Analytical S.A.) fitted out with a combined pH-electrode Ingold (type U 402-S7/120) and a temperature probe (XT 130, Radiometer). The titrations were performed on pectin samples in the acidic form (C_p ~1 meq/L) with freshly prepared 10 meq/L NaOH solution. From the degree of neutralisation α' and the pH value for each neutralisation step, the degree of dissociation α and the apparent pK (pK_a) of the polyelectrolyte were calculated.

The treatment of experimental data was deduced from the model of Lifson and Katchalsky.[20] The structural charge density ($\bar{\xi}$) was calculated from Eq. (1):

$$\bar{\xi} = \frac{\varepsilon^2}{bDkT} \times \frac{100 - DS}{100} = 1.61 \times \frac{100 - DS}{100} \quad (1)$$

where ε is the electron charge, kT the Boltzmann term, b the length of the monomeric unit (0.435 nm[30]), D the dielectric constant of the solvent and DS the degree of substitution ($DM + DA$m). The effective charge density (ξ) can be calculated ($\xi = \alpha\bar{\xi}$) and compared to the critical value (ξ_0) given by Eq. (2):

$$\xi_0 = \frac{\ln\left(\frac{R}{a}\right)}{1 + \ln\left(\frac{R}{a}\right)} \quad (2)$$

where R, the radius of the cylindrical subvolume, is calculated from C_p, and a, the minimum distance of approach, taken as 0.6 nm.[21]

The intrinsic dissociation constant (pK_0) can be obtained by superimposing the experimental curves $pK_a = f(\alpha)$ on the theoretical ones $\Delta pK = f(\alpha)$, as described by Rinaudo and Milas.[21]

Conductimetric measurements.—Transport parameters were determined using conductimetric measurements as already described.[8,11]

Table 2
Sugar composition (mg/g) [a] and HPSEC/Viscotek molecular parameter values of the pectin samples

Samples	GalA	Rha	Ara	Gal	[η] (mL/g)	M_w (mol/g)	I	R_{gw} (nm)
Mother pectin GRINSTED™ Pectin URS 1200								
E81	877	14	3	44	608	124,400	2.2	27.8
Samples from de-esterification with f-PME								
F76	902	15	3	31	555	120,600	2.1	26.3
F69	914	12	3	26	538	116,500	2.1	26.4
F58	883	12	3	30	533	116,800	2.1	26.0
F43	892	13	3	35	473	109,200	2.2	23.2
F31	858	11	3	35	427	101,400	1.9	23.0
F19	877	12	3	36	543	109,800	1.9	25.7
F11	839	11	3	36	395	89,300	1.6	21.6
Samples from de-esterification with p-PME								
P76	855	13	3	36	535	101,900	2.1	24.9
P73	904	12	3	32	493	101,300	2.2	23.9
P70	882	11	3	31	432	96,300	2.2	22.4
P66	893	11	3	31	417	96,100	2.2	22.2
P60	865	13	3	36	400	94,000	2.1	21.6
P53	897	14	3	35	373	95,200	2.1	21.3
P46	864	12	3	33	374	92,900	2.1	21.2
P41	856	13	3	36	368	88,500	1.9	20.8
P32	819	13	4	37	584	112,200	2.1	26.5
P24	819	14	3	39	533	115,400	2.0	26.0
P16	803	13	3	39	539	115,900	2.0	26.2
Samples from alkali de-esterification								
B71	892	15	3	36	614	93,200	2.1	25.3
B64	901	12	3	30	568	93,700	2.0	24.8
B43	913	10	2	22	231	66,800	1.6	15.9
B34	920	9	2	17	471	106,100	1.8	24.3
B15	928	9	2	17	171	75,600	2.0	14.8
Samples from acid de-esterification								
A75	893	9	2	24	645	104,800	2.2	26.8
A72	887	10	2	28	645	113,500	2.3	27.4
A61	924	11	2	22	552	109,100	2.1	25.7
Amidated samples								
Am29-20 [b]	738	13	5	35	543	103,900	2.1	25.0
Am36-16 [b]	776	12	7	24	622	115,800	2.2	27.7

[a] Traces (<2 mg/g) of Fuc, Xyl and Glc were detected.
[b] Estimation.

All conductimetric measurements were carried out in triplicate (standard error < 5%) at 25.0 ± 0.2 °C with a CDM 83 conductimeter (Radiometer Analytical S.A.) equipped with a double platinum electrode CDC 241U (Radiometer Analytical S.A.). The cell constant was determined with 0.05% (w/w) NaCl before each set of measurements. The titrations were performed with freshly prepared 10 meq/L solutions of KOH, LiOH and $Ca(OH)_2$.

The calcium activity coefficients at the neutralisation point ($\gamma_{Ca^{2+}}$) were determined in triplicate (standard error < 8%) by means of a dual-wavelength spectrophotometric method using TMMX as an activity probe for calcium ions.[31]

The transport parameter values and activity coefficients were compared with theoretical predictions from Manning's model:[24–26]

$$z\bar{\xi} < 1 \qquad f = 1 - \frac{0.55(|z|\bar{\xi})^2}{|z|\bar{\xi} + \pi} \tag{3}$$

$$\gamma = e^{-\frac{|z|\bar{\xi}}{2}} \tag{4}$$

$$z\bar{\xi} \geq 1 \qquad f = \frac{0.87}{|z|\bar{\xi}} \tag{5}$$

$$\gamma = \frac{e^{-1/2}}{|z|\bar{\xi}} \tag{6}$$

where z is the charge of the counterion.

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