Phase diagrams of pectin–calcium systems: Influence of pH, ionic strength, and temperature on the gelation of pectins with different degrees of methylation

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ABSTRACT

The effects of ionic strength, pH, and temperature on the gelation of pectin–calcium systems were studied on an apple pectin; the influence of the degree of methylation (dm) was investigated on a family of well-characterised citrus pectins obtained by acid-catalysed deesterification. The reaction led to a decrease in intrinsic viscosities by hydrolysis of glycosidic bonds and in neutral sugar content by elimination of the neutral fraction. Ion-exchange chromatography indicated that the deesterification did not change the charge distribution along the pectic backbone, which was confirmed by potentiometric and conductimetric studies. These methods showed that the charge distribution was statistical in apple and citrus pectins and that no egg-box can occur. Phase diagrams (sol–gel–syneresis), established as calcium concentration versus reduced polymer concentration, showed that gelation was possible before the overlapping of chains because of the strong interactions of calcium with pectins. An increase in the ionic strength, a neutral pH, and a decrease in the setting temperature and in the dm lower the amount of calcium required to give a gel, which can be explained by changes in the probability of crosslinks. Syneresis appeared at relatively low concentrations of calcium.

INTRODUCTION

Pectins are important structural polysaccharides of plant cell walls which are of considerable interest as gelling agents in the food industry[1]. These polymers consist mainly of linearly connected α-$(1 \rightarrow 4)$-D-galacturonic acid residues and their methyl esters, interrupted by (1,2)-linked L-rhamnose residues carrying some neutral sugars (mainly D-galactose and L-arabinose) as side-chains[2]. Industrial pectins are extracted from fruit juice residues such as citrus peels and apple pomaces. They usually have a degree of methylation (molar ratio of methanol to galacturonic acid, dm) of $\sim$ 70%. These high-methoxyl (HM) pectins form gels in the presence of water activity-depressing compounds, such as sucrose, at low pH

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values[3]. Controlled deesterification produces pectins with lower ester contents and the resulting low-methoxyl (LM) pectins, with dm below 50%, can gel in the presence of such divalent ions as calcium, through associations between sequences of charged groups belonging to two different chains.

Gelation of LM pectins, as well as phase separation (syneresis), are markedly affected by both intrinsic and extrinsic parameters, including the dm, the charge distribution along the backbone, the average molecular weight of the sample, the ionic strength, the pH, the temperature, and the presence of cosolutes[4]. The literature has essentially focused on the rheological properties of gels[5–8] for a given set of parameters, but no atttempt has been made to determine the conditions under which gels can be obtained.

For this purpose, an apple pectin of dm = 28% was used to study the effect of the ionic strength (0 and 0.1 M NaCl), the pH (4 and 7), and the quenching temperature (5, 20, and 40°C) on the sol–gel–syneresis phase diagrams as a function of the polymer and calcium concentrations. The influence of the dm on the gelation was also studied on a family of well-characterised citrus pectins having various dm values (between 28 and 48%).

MATERIALS AND METHODS

Pectin samples.—The source materials were commercial apple A30 (batch No. 618F) and citrus C73 (GENU-X-0907) pectins kindly provided by Unipectine (France) and Copenhagen Pectin Factory (Denmark), respectively. Polygalacturonic acid and galacturonic acid were supplied by Sigma Chemical Co.

Pectins with different dm values were obtained by acid deesterification of C73. Acidified EtOH (360 mL; 1.5:2.5:1 EtOH 95%–distilled water–concd HCl) was added to 30 g of C73 in glass-stoppered flasks and held at 30 °C for different times (from 1 day to 2 weeks) with periodical stirring[9]. To stop the reaction, the mixture was cooled to 0°C, diluted with 95% EtOH (3 vol), filtered, washed with 70% EtOH until chloride-free (silver nitrate test), then twice with 95% EtOH and finally dried at 50°C.

The samples were dissolved in distilled water with gentle stirring overnight. The acidic form of the samples (pectinic acids) was obtained by percolating these solutions through a strong H$^+$ exchanger (Amberlite IR-120); their exact neutralisation, followed by conductimetry, with NaOH or Ca(OH)$_2$ gave the sodium or calcium salts, respectively.

Characterisation.—The dm values were determined by titrimetry[10], and the galacturonic acid (GalA) content was measured by the *m*-hydroxybiphenyl method[11]. Individual neutral sugars were analysed by gas chromatography as their alditol acetates[12] after hydrolysis with trifluoroacetic acid (2 M, 120°C, 2.5 h)[13].

The structural charge density parameter $\bar{\xi}$, introduced by Lifson and Katchalsky[14], is given by:

$$\bar{\xi} = e^2/bDkT(1 - dm/100) = 1.61(1 - dm/100) \text{ at } 25\,°C$$

where e is the electron charge, kT the Boltzmann term, b the length of the monomeric unit (4.35 Å)[15], and D the dielectric constant of the solvent.

Ion-exchange chromatography was performed on a DEAE-Sepharose CL-6B (Pharmacia) column (20 × 1.6 cm) equilibrated with 0.05 M sodium acetate buffer at pH 4.8. Samples (6 mg) dissolved in water were loaded onto the column and the gel was washed with 65 mL of buffer in order to remove neutral material. The bound material was then eluted by a linear gradient (0.05 → 1 M) of sodium acetate at pH 4.8. The fractions (2.5 mL) were analysed for their GalA and total neutral sugar contents by the m-hydroxybiphenyl and orcinol[16] methods, respectively.

Chromatography on a Sepharose CL-2B column (97.5 × 1.5 cm) and on a Sepharose CL-6B column (52 × 2.5 cm) was carried out with 0.1 M sodium acetate buffer (pH 4) at a flow rate of 25 and 12 mL/h, respectively. Samples (3 mg) dissolved in water were loaded onto the column. Fractions of 2–3 mL were collected and the results were expressed as a function of the partition coefficient K_{av}:

$$K_{av} = (V_e - V_0)/(V_t - V_0)$$

where V_e, V_0, and V_t are the elution volume of the fraction, the void volume, and the total volume of the column, respectively.

The weight-average molecular weights ($\overline{M}_w$) were evaluated using the technique of HPSEC/MALLS. The SEC system consisted of a Waters 6000-A pump on line to a guard column, and three Shodex OHpak columns (30 × 0.8 cm each): a KB-806, a KB-805, and a KB-804 in the order of connection. This system was coupled on line to a Dawn-F multi-angle laser light-scattering photometer and to an Erma Optical differential refractometer. $\overline{M}_w$ values were determined with ASTRA software (v.2.02). A differential index of refraction (dn/dc) of 0.145 mL/g was employed[17]. The eluant (filtered through a 0.22-μm filter) was 0.3 M NaNO$_3$ (pH 6.5) containing 0.2 ppm of sodium azide as bactericide, at a flow rate of 1 mL/min. The samples (concentration such as $C[\eta] \sim 1.7$) were injected through a 0.45-μm filter. The weight-average molecular weight determinations were reproducible with an error less than 10%.

Conductimetric and potentiometric studies.—The conductimetric measurements were carried out at 25.0 ± 0.1°C in dilute salt-free pectinic acid solutions ($C_p < 1$ mequiv/L) with a CDM83 Radiometer conductimeter equipped with a double platinum electrode (Radiometer PP1042). The cell constant was periodically determined with 0.1 M KCl solution. The limiting law for the equivalent conductivity of a polyelectrolyte solution can be written as:

$$\Lambda = f(\lambda_c + \lambda_p)$$

where Λ is the equivalent conductivity (in S · cm^2/equiv) of the salt in solution, λ_p is the equivalent conductivity of the monomeric unit inserted in the polymer, λ_c is the equivalent conductivity of the counterion in pure solvent at infinite dilution at

25°C, and f is the transport parameter, related to the free fraction of the counterion. If the conductivity is obtained for three different ionic forms of the polymer (lithium, potassium, and calcium), the transport parameter f, considered independent of the nature of the monovalent counterion, and the λ_p values can be calculated for monovalent and calcium ions.

The calcium and sodium activity coefficients (γ_{Ca} and γ_{Na}) were determined with selective electrodes (F2112 Ca and G502 Na, Radiometer) on calcium and sodium pectinates, respectively. A saturated calomel electrode was used as reference. Calibration curves were obtained using NaCl and $CaCl_2$ solutions of calculated activities at $25.0 \pm 0.1°C$ without stirring and after stabilisation. The $CaCl_2$ and NaCl concentrations were measured through conductimetric determination with silver nitrate. Values of the activity coefficients given here represent the ratio of the activity of calcium or sodium ions in the presence of pectins to the activity of calcium or sodium in $CaCl_2$ or NaCl solutions at the same ionic concentrations.

Gel formation and phase diagrams. —The pH of pectin solutions was adjusted to 4 or 7 with 0.1 M NaOH. The solutions were filtered through a 0.8-μm filter. Concentrations were calculated by determination of the dry matter.

The pectin solutions (2–24 g/L) were mixed at 70°C with hot $CaCl_2$ solutions (0.002–0.02 M) for 3 min and then poured into tubes (96 mm × 9 i.d.). Unless otherwise stated, solutions were 0.1 M in NaCl. After standing for 48 h at the setting temperature, the tubes were tilted. When the sample was seen to flow easily, it was said that the system was still a sol. When the meniscus was seen not to deform under its own weight, the system was considered as gelled. The transition is taken at the onset of meniscus deformation when the tube is held horizontal. Syneresis was detected by the presence of water at the gel surface. Determinations of the sol–gel transition and the appearance of syneresis are reproducible within less than 5%.

Viscosity measurements. —Viscosities were measured with a low shear rheometer (LS 40, Contraves) over a shear rate range of 0.01 to 100 s^{-1} on pectin solutions (concentration less than 2 g/L) in 0.1 M NaCl (pH 7) at 25°C. The viscosity of A30 was also measured in 0.1 M NaCl (pH 4) at 20°C, and in 0.1 M NaCl (pH 7) at 40 and 5°C. The intrinsic viscosity [η] of the samples was determined by the double Huggins and Kraemer extrapolation[18].

RESULTS AND DISCUSSION

Sample A30 was used to test the influence of pH, ionic strength, and temperature on the gelation of LM pectin–calcium systems. Acid deesterification of C73 in alcoholic medium was applied in order to obtain samples with a similar distribution of carboxyl groups[19,20] and to have enough material to carry out all the experiments.

Sample characterisation. —The chemical characteristics of the samples used in this study are listed in Table I. Apple pectin A30 was richer in L-rhamnose,

TABLE I

Chemical characteristics of the samples (the composition is in % of dry matter)

Sample	Time of de-esterification (h)	GalA	Rha	Ara	Man	Xyl	Gal	Glc	dm	$[\eta]$ (L/g) 0.1 M NaCl, pH 7, 25°C	$\bar{M}_w$	$\bar{\xi}$ (25°C)
C73		76.3	1.6	2.2	0.2	0.4	5.4	0.4	73	0.562	196 200 ± 6000	0.44
C48	18	82.6	1.3	0.2	0.1	0.1	3.6	0.1	48	0.536	160 300 ± 9700	0.84
C44	42	79.2	1.1	0.2	0.1	0.1	3.1	0.2	44	0.362	114 400 ± 3400	0.90
C40	67.5	80.9	1.2	0.2	0.1	0.2	4.1	0.2	40	0.329	127 000 ± 9500	0.97
C28	324	92.7	0.6	0.1	0.1	0.2	1.1	0.1	28	0.271	70 000 ± 7700	1.16
A30		76.8 [a]	2.2 [a]	1.0 [a]	traces [a]	1.4 [a]	2.6 [a]	3.6 [a]	28 [a]	0.282 [a] / 0.274 [b] / 0.267 [c] / 0.302 [d]	175 740 ± 2700	1.16 [a]

[a] Values taken from Ref. 18. [b] In 0.1 M NaCl, 25°C, pH 4. [c] In 0.1 M NaCl, 40°C, pH 7. [d] In 0.1 M NaCl, 5°C, pH 7.
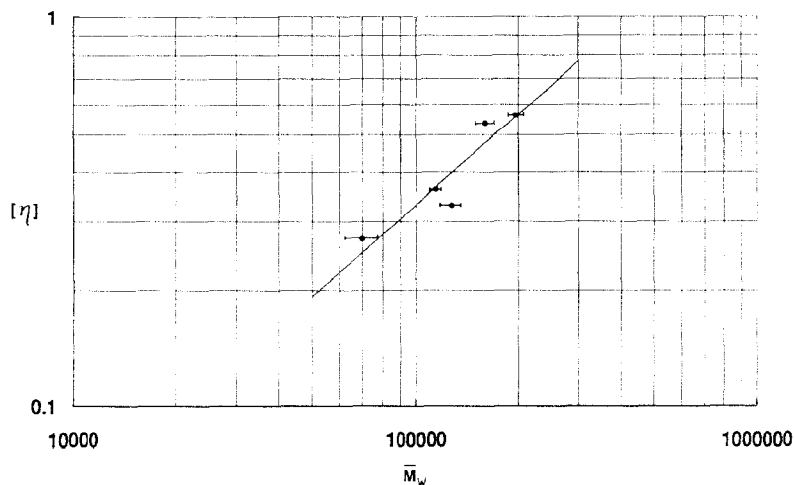
Fig. 1. Intrinsic viscosities of the citrus samples C73, C48, C44, C40, and C28 versus their weight-average molecular weight.

D-xylose, and D-glucose than the citrus pectins. The proportions of L-arabinose, D-mannose, and D-galactose were higher in the citrus mother pectin than in the apple sample, as already observed for other samples[21].

Four samples having dm between 28 and 48% were obtained by deesterification from C73. The reaction caused a marked decrease in intrinsic viscosity, also reported for grapefruit pectins[9], indicating some hydrolysis of the glycosidic bonds. It must be pointed out that deesterification in alkaline conditions, even at 2°C, produced also a depolymerisation because of β-elimination reactions[22]. Whatever the deesterification method, it appears very difficult to obtain samples of various dm with similar molecular weights. As shown in Fig. 1, the intrinsic viscosity values for the citrus samples varied with $\overline{M}_w$ as $[\eta] = 0.0436 \times 10^{-3} \; \overline{M}_w^{0.78}$, this equation being only valid for this pectin family. The value of the exponent is in good agreement with recent experimental determinations: 0.8 (ref. 22), 0.73 (ref. 23), 0.89 (ref. 24).

During the deesterification, all the neutral sugars, including rhamnose, decreased while galacturonic acid increased. The lower amount of rhamnose in deesterified pectins confirmed that some degradation in the backbone occurred. The rate of disappearance of the neutral sugars depends on their nature. The amount of arabinose decreased by 90% after 24 h of reaction. Xylose, glucose, and mannose decreased by ~50 or 75% during the same time. Galactose and rhamnose decreased more progressively, from 33 to 80% and from 19 to 62%, respectively. These results confirmed that galactose and rhamnose are more resistant to acid hydrolysis than the other neutral sugars[21,25].

The citrus pectins were analysed by ion-exchange and size-exclusion chromatography. The elution patterns of C73 and C44 are shown in Fig. 2. The ion-exchange chromatogram of the initial C73 pectin (Fig. 2a) indicates that ~23% of the
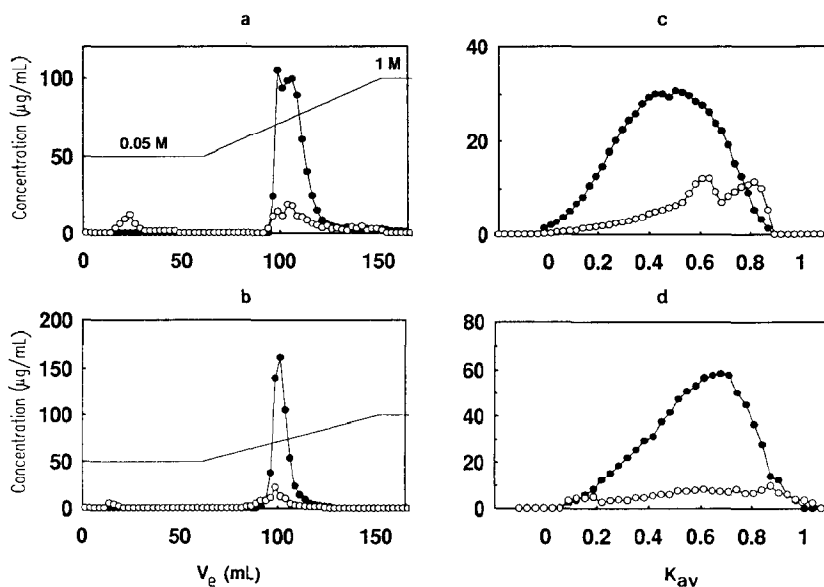
Fig. 2. Ion-exchange chromatography (a,b) on DEAE-Sepharose CL-6B and gel-permeation chromatography (c,d) on Sepharose CL-2B of the C73 (a,c) and C44 (b,d) samples.

neutral sugars were not associated with the pectic backbone. This amount decreased with the deesterification time and reached a value around 2% for C28 (data not shown). Whatever the dm, the elution pattern of the pectic material indicated a narrow intermolecular charge distribution, except for C73 for which the peak was broader and displayed a shoulder that can be ascribed either to inhomogeneity of the charge distribution or to an effect of molecular sieving. As the dm decreased, the ionic strength of the buffer necessary to elute the pectin increased. Fig. 3 shows that the dm can be linearly related to the reciprocal of the square-root of the ionic strength of the buffer, i.e., to the thickness of the ionic atmosphere of the eluant or Debye screening length[26]. This result confirms that the deesterification did not change the charge distribution. This relation was established with the first elution volume of C73. Figs. 2c and 2d indicate that, in size-exclusion chromatography, the samples were eluted within the fractionation range of the column. The width of the peaks decreased with the dm, but remained important in relation to the high $\overline{M}_w$ of these pectin samples. A similar pattern was observed for A30 (data not shown).

The conductimetric data reported in Table II show a decrease in the λ_p values (from 49.9 for the polygalacturonic acid to 27.2 for C73), and an increase of the transport parameter of monovalent counterions (from 0.52 to 0.85, respectively) with an increasing dm. The values of the transport parameter of divalent ions also decreased with increasing charge parameter and reached a value of ~ 0.2 for the samples of dm = 28%. The value obtained for polygalacturonic acid was very low (0.07).
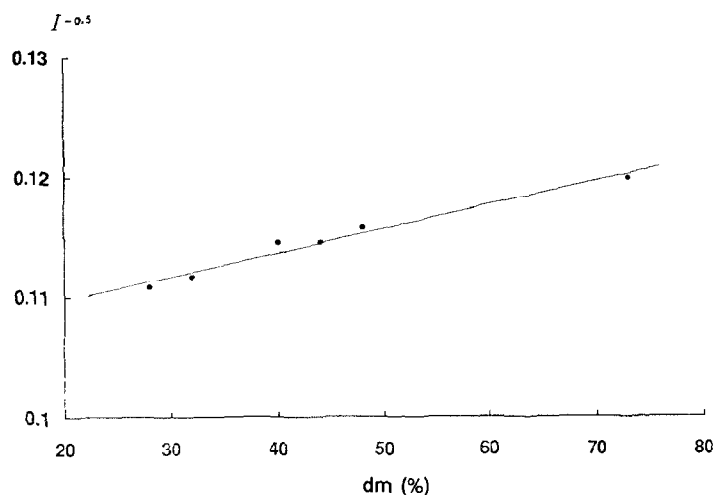
$I^{-0.5}$

Fig. 3. Ionic strength at the elution volume of the ion-exchange chromatograms versus the degree of methylation (dm).

Similar results were observed for calcium activity coefficients; γ_{Ca} decreased regularly with the dm, from 0.46 for C73 to 0.10 for polygalacturonic acid.

The results of calcium binding of polygalacturonic acid are in agreement with previous results[27,28]. The very low values indicated a very strong binding of calcium ions which can be similar to the egg-box model proposed for alginates[29]. The values obtained for pectins were at least twice as high, indicating that an egg-box cannot be present. The values, though a little higher, were close to those obtained for pectins with similar dm and a rather statistical distribution of the carboxyl groups[28].

It can be concluded that the deesterification method used in this work produced citrus samples with an unchanged distribution of the carboxylic groups and that a similar charge distribution occurred in the apple pectin.

TABLE II

Potentiometric and conductimetric data

Samples	λ_p	f_{K^+, Li^+}	$f_{Ca^{2+}}$	$\gamma_{Ca^{2+}}$
Galacturonic acid	21.5	0.91	0.89	0.90
C73	27.2	0.85	0.50	0.46
C48	35.4	0.85	0.35	0.28
C44	43.6	0.75	0.27	0.27
C40	44.2	0.74	0.26	0.24
C28	46.5	0.62	0.18	0.21
A30	47.8	0.74	0.22	0.23
Polygalacturonic acid	49.9	0.52	0.07	0.10

Phase diagrams.—The influence of both intrinsic and extrinsic parameters on the gelation of these well-characterised pectins in the presence of calcium ions has been investigated. The different phases (sol, gel, and syneresis) were observed for the citrus samples in order to study the effect of the charge density on gelation. The behaviour of the A30 sample was tested in different conditions of pH, ionic strength, and temperature.

In order to take into account differences in the molecular weight of the different samples and thus to be able to compare their gelation properties, the results of the visual observation (phase diagram) were reported as calcium concentration in the medium versus the reduced polymer concentration $C[\eta]$ instead of the polymer concentration. Indeed, $C[\eta]$ corresponds to the volume filled in solution by the polymer. If the phase diagram is established in a salt-free solution of pectin (cf. A30 at pH 7), one must take care that the intrinsic viscosity varies with the ionic strength brought by the polymer as[30]:

$$[\eta]_I = [\eta]_\infty + SI^{-0.5}$$

where $[\eta]_\infty$ is the intrinsic viscosity at infinite ionic strength, and S is the slope of the straight line. In this relation, the ionic strength is given[31] by:

$$I = \alpha\gamma C$$

where α is the fraction of the ionized groups ($\alpha = 1$ at pH 7), γ is the monovalent counterion activity coefficient, and C is the concentration in polymer in equiv/L. The values of all the parameters have been previously determined for the A30 sample ($[\eta]_\infty = 0.229$ L/g, $S = 0.0162$, $\gamma_{Na} = 0.507$)[18].

The phase diagrams of A30 at 20°C and pH 7 in 0.1 M NaCl and in salt-free solutions are presented in Fig. 4. Such diagrams evidenced three states. In region 1, below a critical concentration of calcium, the system was a homogeneous sol. In region 2, above a given polymer concentration (C_0) and calcium concentration, gelation took place. Region 3, at high concentrations of calcium, was a large zone in which continuous gels with syneresis were obtained for high concentrations of polymer while, at lower concentrations of polymer, precipitation and microgel formation occurred. The delimitations between the two demixing states, and between homogeneous sol and the onset of precipitation, were very difficult to determine just by visual observation and were not further examined.

The sol–gel transition curves show that the minimal reduced polymer concentration able to promote gelation, $C_0[\eta]$, and therefore the corresponding minimal polymer concentration C_0, depended on the solvent conditions. In the presence of an excess of monovalent salt, gelation was possible before the overlap threshold concentration, C^*, defined as $C^*[\eta] = 0.77$ (ref. 32). In this dilute regime, the amount of calcium required to obtain a gel decreased when $C[\eta]$ increased while, for values of $C[\eta]$ larger than 0.8, it became independent of the polymer concentration. In salt-free solutions, C_0 was very near C^* ($C[\eta] = 0.8$), and the transition line raised with the polymer concentration. This difference may be ascribed to the
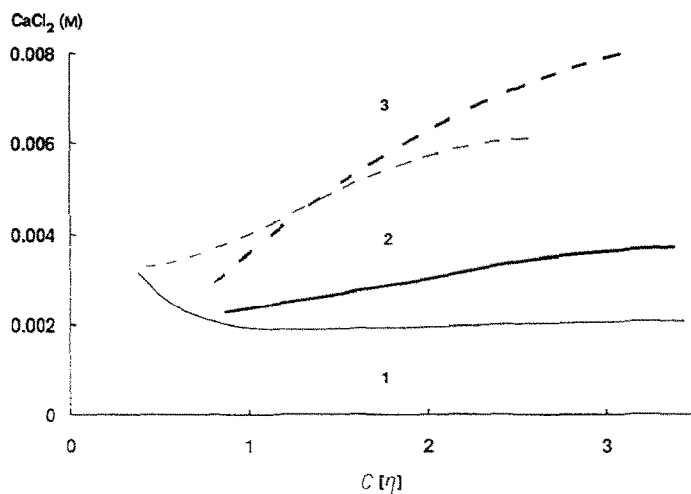
CaCl$_2$ (M)

Fig. 4. Influence of the ionic strength on the gelling behaviour of the A30 sample at 20 °C, pH 7: full line, sol–gel transition; dotted line, gel–syneresis transitions; thin lines, 0.1 M NaCl; thick lines, salt-free solution.

fact that in salt-free solution, electrostatic repulsions do not allow the chains to be close enough to induce gelation.

The two upper curves indicated that the amount of calcium at the syneresis transition was in the same range as the number of carboxylate groups, which confirmed the strong binding of calcium by pectins[33]. The presence of 0.1 M NaCl caused a lower dependence of the critical demixing concentration of calcium on the polymer concentration, leading to a slight increase in stability at polymer concentrations lower than $C[\eta] = 1.3$.

The influence of pH was also tested on the same sample in 0.1 M NaCl at 20°C (Fig. 5). C_0 was higher at pH 4 than at pH 7, because of a decrease in the number of carboxylate groups and, therefore, a decrease in the possibility of ionic crosslinks. In contrast, the formation of junctions was promoted by an increase of the polymer concentration, and the critical calcium concentration required to form a gel became independent of α when $C[\eta] > 2$. For a constant polymer concentration, when the pH was lowered, i.e., the number of carboxylate groups was reduced, it was expected that the calcium concentration at the onset of precipitation would decrease with α. This phenomenon was not very marked even at high concentrations of polymer and can be ascribed to a strong ion-binding[33].

The effect of the setting temperature on gelation is shown in Fig. 6. The sol–gel transition occurred for higher $c[\eta]$ and calcium concentration when the setting temperature was increased from 5 to 40°C. Two mechanisms, probably associated, can be considered. The binding of calcium ions becomes less stable at high temperature and, therefore, more ions are required to form an elastically active junction zone. The distribution in length of the junction zones, in relation to the
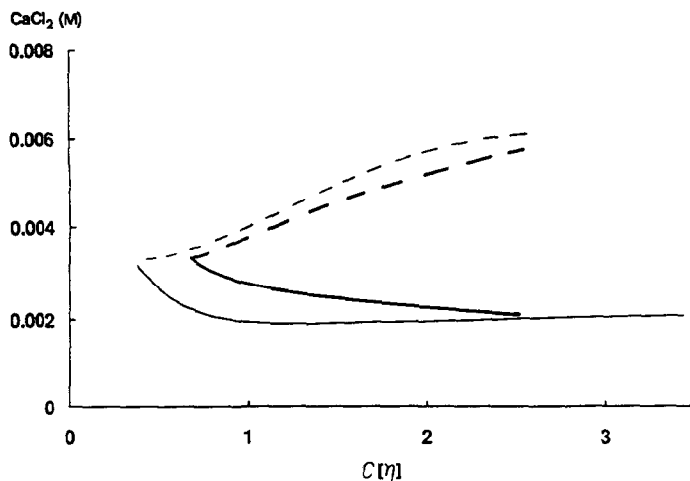
CaCl$_2$ (M)

Fig. 5. Influence of the pH on the phase diagrams of the A30 sample in 0.1 M NaCl, 20°C: thin lines, pH 7; thick lines, pH 4.

dm, may then play an important role in this phenomenon. But as the probability of finding long sequences of dissociated carboxylate groups is very low, only a few junction zones of this type can be envisaged. On the other hand, the probability of forming short junction zones is higher but the crosslinks are not stable at 40 °C, the ionic equilibrium (bonding ⇄ unbonding) being shifted towards the free state. The quantity of junctions zones then has to balance their poor stability. The amount of

CaCl$_2$ (M)
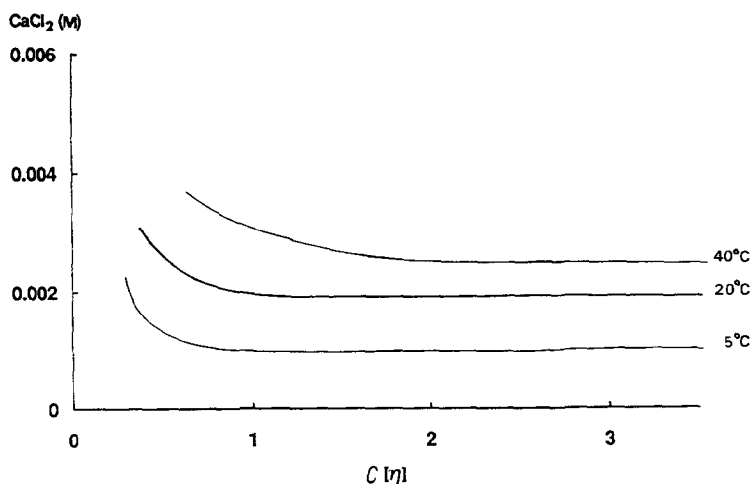
Fig. 6. Influence of the setting temperature on the sol–gel transition of the A30 sample, 0.1 M NaCl, pH 7.
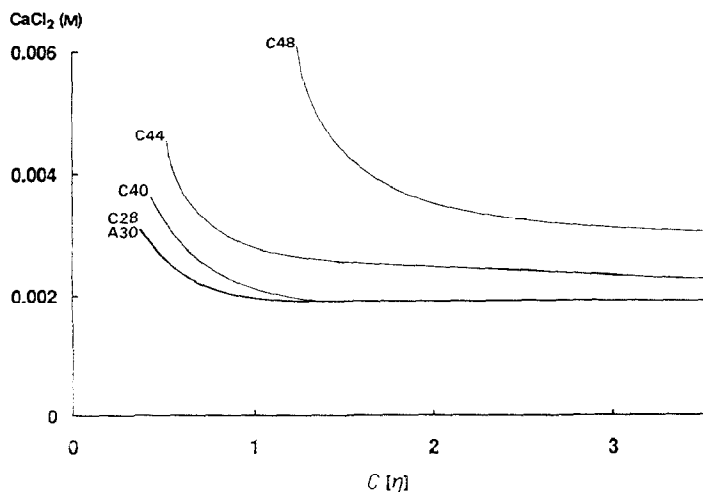
CaCl$_2$ (M)

Fig. 7. Sol–gel transition curves of the different deesterified pectins, pH 7, 0.1 M NaCl, 20°C.

calcium at the gel–syneresis boundary as a function of the temperature was not determined.

The influence of the dm on the sol–gel transition for the different samples in 0.1 M NaCl at pH 7 and 20°C is shown in Fig. 7. It can be noted that, from C28 to C44, the $C_0[\eta]$ values were close to each other, whereas the value obtained for C48 was much higher. No gel was obtained for C48 if $C[\eta] < 1.1$. This fact stresses the critical role of the dm in the gelation phenomenon. Samples C28, A30, and C40 needed similar amounts of calcium to pass the transition above $C[\eta] \approx 0.72$, which corresponds to the beginning of the semi-dilute regime. Below $C[\eta] \approx 0.72$, the transition lines of A30 and C28 were superposed, but the C40 sample required more calcium to gel. The transition curves of C44 and C48 samples were similar to the others, although they were shifted towards higher calcium and reduced polymer concentrations. The ability to obtain a gel was a direct consequence of the probability of having along the chains enough dissociated carboxylate groups to allow the formation of junction zones. This probability varied significantly with the dm of the samples and the entanglement of the chains, which can explain the results. Moreover, it was observed that the transition curve of C48 was still a decreasing function of the polymer concentration at $C[\eta] = 4$ and tended towards the other ones (data not shown). Thus, at high concentrations of polymer, the calcium required at the transition seemed to become less dependent on the dm. On the other hand, the similar results obtained for the A30 and C28 samples indicated that the sol–gel transition was not affected by the molecular weight when dm, charge distribution, and affinity parameters were identical. For reasons of clarity, the syneresis transition lines are not represented on this figure.

The stoichiometric ratio $R = 2[Ca^{2+}]/[COO^-]$ is often used in the study of phase transitions[7,34] because it is considered as describing the junction density. Fig.
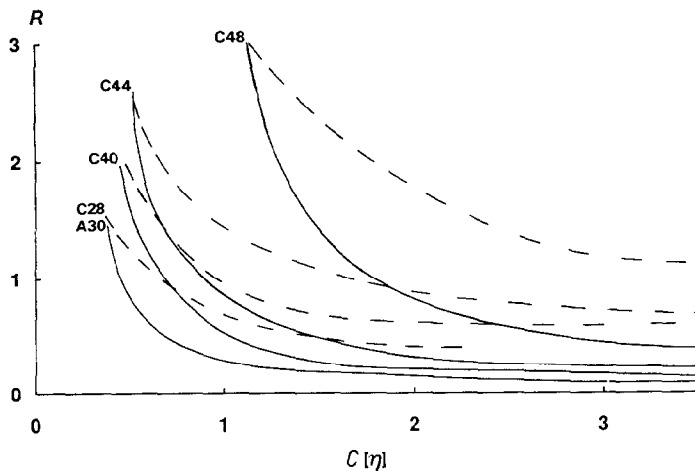
Fig. 8. Influence of the degree of methylation on the extension of the homogeneous gel domain, pH 7, 0.1 M NaCl, 20°C.

8, where R versus $C[\eta]$ is presented, allows one to visualize clearly the relative position of the homogeneous gel domain. This domain became larger when the charge density decreased. At high dm, the charged groups were rather sparse, the network had a loose structure, and syneresis occurred for higher R and $C[\eta]$ values. Moreover, both gelation and syneresis can occur at $R < 1$ for all the samples at different reduced polymer concentrations, which showed that all the carboxylate groups were not involved in these phenomena.

CONCLUSION

This study of the gelation conditions of LM pectin–calcium systems led to a better insight into the effects of extrinsic and intrinsic parameters. It has been shown that an increase in the ionic strength, as well as a neutral pH, and a decrease in the setting temperature and in the dm lower the amount of calcium chloride required to obtain the sol–gel transition. Furthermore, the gelation of such systems is generally possible before the overlapping of polymer chains, because of the high affinity of calcium ions towards pectic chains. Phase separation in these polyelectrolytes was found to occur at relatively low calcium concentration as already found for synthetic polyelectrolytes[35].

The phase diagrams allow prediction of the polymer and calcium concentrations required for the gelation. These results have now to be related (*i*) to the amount of calcium ions effectively bound to the polymer and (*ii*) to the determination of the shear modulus in order to quantify the number of junction zones.

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