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Ca^{2+}-induced gelation of low-methoxy pectin in the presence of oxidised starch. Part 2. Quantitative analysis of moduli

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Abstract

The observed moduli (G'; 10 rad s^{-1}; 0.5% strain; 5°C) of the co-gels formed by mixtures of calcium pectinate (DE 34.2; stoichiometric Ca^{2+}) and oxidised starch (0–40 wt%) have been matched with good precision by an analysis based on the following postulates. (i) Thermodynamic incompatibility between the two polymers causes partial precipitation of pectin chains into aggregated bundles within a supporting calcium pectinate network. (ii) The extent of precipitation is controlled by a solubility product (K), defined as the starch concentration multiplied by the square of the concentration of unprecipitated pectin. (iii) The volume occupied by the precipitated pectin fraction is directly proportional to the amount precipitated (constant of proportionality, k). The system was regarded as comprising a continuous phase of oxidised starch and unprecipitated pectin surrounding a dispersed phase of precipitated pectin, and overall moduli were calculated using the Takayanagi isostress blending law. The root-mean-square difference between observed and calculated values of $\log G'$ was minimised by varying the two adjustable parameters, k and K (which determine phase volumes and partition of pectin between the phases). The moduli of the individual components at their effective concentration within the appropriate phase were determined from standard calibration curves for calcium pectinate and oxidised starch alone. The overall modulus of the continuous phase was estimated by simple addition of the separate contributions from unprecipitated pectin and oxidised starch. The modulus of the dispersed particles of precipitated pectin was effectively infinite in comparison with that of the continuous phase, allowing a simplified form of the isostress equation to be used. Best agreement was obtained with $K = 0.83$ (for concentrations in wt%) and with a value of k corresponding to a pectin concentration of ~16.4 wt% within the precipitated bundles (~5 g water per 1 g of pectin). Discrepancies between observed and fitted values of $\log G'$ were not significantly beyond experimental error, suggesting that the concept of partial precipitation of calcium pectinate in response to segregative interactions with oxidised starch is a realistic interpretation of the reductions in co-gel moduli reported in the preceding paper. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Low-methoxy pectin; Oxidised starch; Thermodynamic incompatability; Rheology of mixed gels

1. Introduction

In the preceding paper (Picout, Richardson, Rolin, Abeysekera & Morris, 2000a) it was shown that the networks formed when pectin is cooled in the presence of Ca^{2+} can be strengthened, weakened, or remain unaffected by progressive incorporation of oxidised starch, depending on the degree of esterification (DE) of the pectin and on the concentration of Ca^{2+}. Strengthening was observed only at comparatively low concentrations of Ca^{2+}, and can be explained by segregative interactions ("thermodynamic incompatibility") between the two polymers promoting conversion of the pectin component from the expanded coil state to the more compact ordered structure that forms the crosslinks of the gel network. For each pectin, however, there was a critical Ca^{2+}-concentration above which the gel strength (storage modulus, G') was decreased, rather than increased, by the presence of oxidised starch. The transition from enhancement to depletion was accompanied by a change in gel structure (as visualised by light microscopy) from homogeneous to grossly heterogeneous, and moved to progressively higher Ca^{2+} concentration with increasing DE.

The reduction in final gel strength (at 5°C) was found to arise from a sharp drop in modulus during cooling, indicating abrupt collapse of the developing network. This collapse occurred at progressively higher temperature as the concentration of starch was increased. Two possible mechanisms were considered: (i) salt-induced phase separation, with the pectin component confined largely to the dispersed phase,

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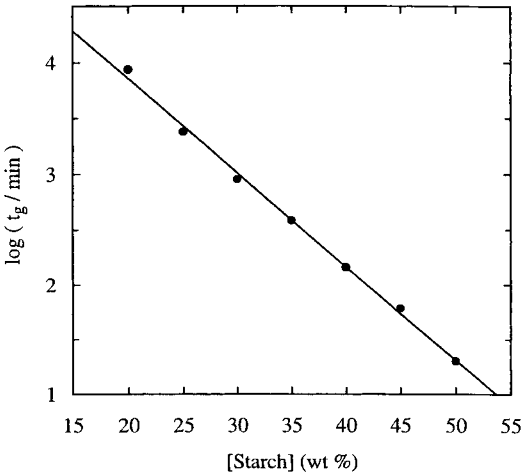

Fig. 1. Concentration-dependence of gel-time (t_g) for oxidised starch at 5°C.

and (ii) association of pectin chains into large aggregated bundles within a supporting calcium–pectinate network (incipient precipitation). It was tentatively concluded that the precipitation mechanism is the more likely, largely on the basis of its consistency with the observed effect of DE. Ester substituents would be expected to inhibit cation-mediated association, and thus displace the onset of aggregation to higher Ca^{2+} concentrations, as observed. Phase separation, by contrast, would be expected to occur at progressively lower salt (calcium chloride) concentrations with increasing DE, due to reduction in the loss of entropy from segregation of counterions with the polyelectrolyte chains.

Systematic comparisons of the effect of increasing concentration of oxidised starch were made (Picout et al., 2000a) using 2.0 wt% pectin of DE 34.2, at Ca^{2+} concentrations of 10, 20, 22.5, 25, 30, 50 and 100% of stoichiometric equivalence to the carboxyl groups of the pectin. The transition from enhancement to depletion of network strength came at 25% stoichiometric Ca^{2+} (~7.3 mM), with the extent of depletion then increasing progressively as the Ca^{2+} concentration was raised to 100% stoichiometric. In the present study, we have explored the concept of precipitation of pectin chains within the calcium pectinate network by quantitative analysis of the final moduli at 5°C for mixtures prepared with 100% stoichiometric Ca^{2+}, where the observed reductions in G' with increasing concentration of starch were greatest. Experimental work was confined to adding some further starch concentrations to the existing data set, and to calibration of the concentration-dependence of G' for calcium pectinate and oxidised starch alone, for use in the analysis. The central conclusion is that the observed moduli agree well with calculated values based on a precipitation model.

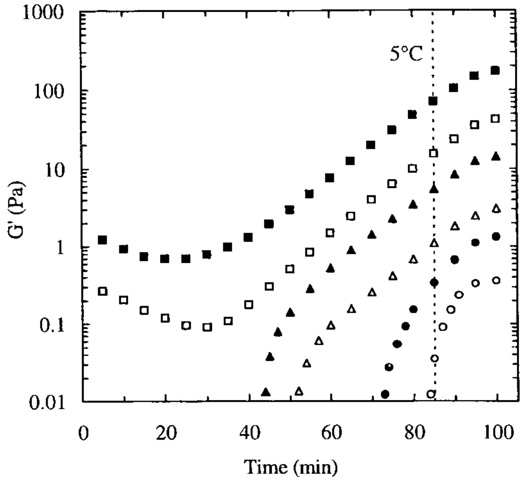

Fig. 2. Variation of G' (10 rad s^{-1}; 0.5% strain) for oxidised starch at concentrations (wt%) of 25 (○), 30 (●), 32.5 (△), 36 (▲), 40 (□) and 45 (■) on cooling from 90 to 5°C at 1°C/min and holding for a further 15 min at 5°C.

2. Materials and methods

The polymer samples used were identical to materials described in the preceding paper (Picout et al., 2000a): oxidised starch from Cerestar (C*Set 06598; batch SH 1338) and low-methoxy pectin from Hercules (batch number 5006; DE 34.2), and the procedure used for preparation of mixed solutions was also identical. The solutions used for construction of modulus–concentration curves for the individual constituents were prepared in exactly the same way, but omitting the second polymer. The procedure used for rheological characterisation was again identical to that described by Picout et al. (2000a): samples were loaded at 90°C and cooled to 5°C at 1°C/min; final measurements (at 10 rad s^{-1} and 0.5% strain) were made after 15 min at 5°C (i.e. after 100 min from the start of the cooling scan). Data analysis was carried out using the in-built curve fitting and minimisation routines on a Microsoft Excel spreadsheet package (Version 5.0).

3. Results

3.1. Gelation of oxidised starch alone

Before embarking on instrumental characterisation of the time–temperature course of gelation for oxidised starch in the absence of pectin, a preliminary study of the effect of concentration on the rate of network formation at fixed temperature was made by simple visual inspection. Solutions (90°C) were prepared at 20, 25, 30, 35, 40, 45 and 50 wt%, and filled into heated tubes (1 cm diameter) to a depth of 3 cm. The tubes were then plunged into a water bath at 5°C, and the reduction in temperature was monitored using a thermocouple immersed in the solution. Timing began when the sample reached 5°C (typically ~9 min after quenching). The time required to form a cohesive network (gel-time, t_g) was estimated by periodically tipping

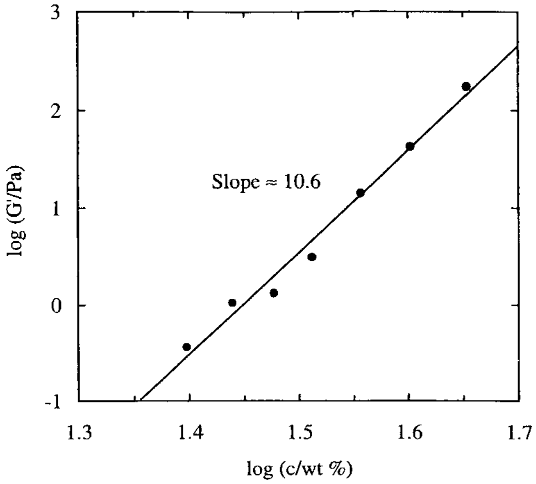

Fig. 3. Concentration-dependence of G' (10 rad s^{-1}; 0.5% strain) for oxidised starch after cooling from 90 to 5°C at 1°C/min and holding for a further 15 min at 5°C.

the tube a few degrees from the vertical, and observing when the surface of the sample first remained stationary (i.e. perpendicular to the walls of the tube) rather than flowing towards the horizontal. As shown in Fig. 1, there is an approximately linear increase in log t_g as the starch concentration is decreased, with gel times ranging from ~20 min for 50 wt% starch (the highest concentration at which solutions could be prepared) to ~6 days for 20 wt%. Small-deformation oscillatory measurements, however, showed substantial development of structure at higher temperatures and shorter times.

Fig. 2 shows the changes in G' observed for solutions of oxidised starch at concentrations in the range 25–45 wt% on cooling from 90 to 5°C at 1°C/min and holding for a further 15 min at 5°C. At the lowest concentration (25 wt%), increase in modulus is confined almost entirely to the holding period at 5°C (i.e. between 85 and 100 min from the start of the cooling scan). As the concentration is increased, however, the onset of structure formation moves to progressively higher temperature. At the highest concentrations (40 and 45 wt%), there is a detectable drop in G' during the first 25–30 min of cooling, before the much larger increase at lower temperatures. A possible explanation is that the initial decrease comes from intramolecular ordering of amylopectin (i.e. association of contiguous strands into double helices), with consequent reduction in molecular dimensions, and that the subsequent increase arises from aggregation of helical sequences on adjacent molecules, to give a crosslinked network.

Table 1
Calibration data for variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with concentration (c) of oxidised starch

c (wt%)	G' (Pa)	log c	log G' observed	log G' fitted[a]
25.0	0.36	1.40	−0.44	−0.54
27.5	1.04	1.44	0.02	−0.10
30.0	1.31	1.48	0.12	0.30
32.5	3.09	1.51	0.49	0.67
36.0	14.2	1.56	1.15	1.13
40.0	42.3	1.60	1.63	1.62
45.0	171.6	1.65	2.23	2.16

[a] Using Eq. (1).

For the purposes of the present investigation, however, the relevant information from Fig. 2 is the effect of starch concentration (c) on the final (100 min) moduli at 5°C. As shown in Fig. 3, there is an essentially linear variation (Eq. (1)) of log G' with log c over the concentration range studied (25–45 wt%):

$$\log G' = 10.56 \log c - 15.30 \qquad (1)$$

where G' is in Pa and c in wt%. The observed moduli and the fitted values from Eq. (1) are shown in Table 1.

The concentration-dependence of G' for oxidised starch is unusual. Plots of log G' versus log c for gelling biopolymers (Clark & Ross-Murphy, 1985) normally have a slope of ~2 (i.e. c^2-dependence) at high concentrations, and then curve to progressively higher slope as the concentration is decreased towards the minimum critical gelling concentration (c_0). A steep, linear variation of log G' with log c has, however, been reported (Kasapis, Morris, Norton & Clark, 1993a) for potato maltodextrin of comparatively low-molecular weight (Paselli SA6), and for agarose gels formed by rapid quenching (Mohammed, Hember, Richardson & Morris, 1998a). This is almost certainly a kinetic effect, reflecting the increasing rate of helix–helix aggregation as concentration is raised, with consequent increase in the degree of crosslinking at equivalent times (before the networks have reached equilibrium). Irrespective of the underlying molecular structures and processes, however, the linear relationship in Eq. (1) allows the modulus of the starch component in the composite gels with calcium pectinate to be calculated as a continuous function of its effective local concentration. The purpose of the work reported in the next section was to obtain the corresponding modulus–concentration relationship for the pectin component.

3.2. Gelation of calcium pectinate alone

Fig. 4 shows the time–temperature course of network formation (as characterised by increase in G') for low-methoxy pectin (in the presence of stoichiometric Ca^{2+}) at the concentration used in the mixed systems with oxidised starch (2.0 wt%) and at other concentrations in the range 0.25–3.0 wt%. As before (Fig. 2), the first 85 min correspond to cooling from 90 to 5°C and the last 15 min to holding at 5°C. The gelling transition becomes broader as the concentration is decreased, but in all cases there is a comparatively sharp increase in G' after ~35 min (i.e. at ~55°C).

Fig. 5 shows the concentration-dependence of the final (100 min) moduli at 5°C. In contrast to the unusual

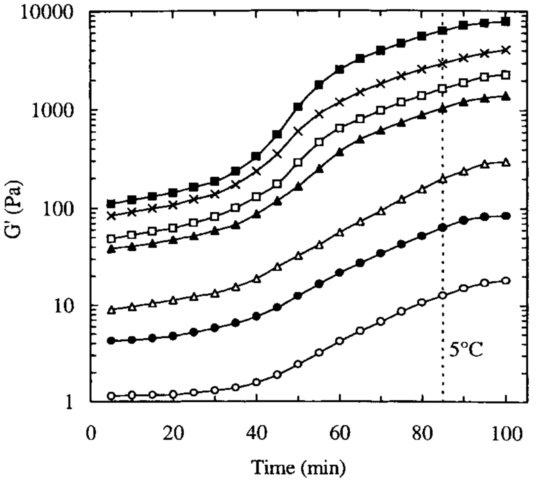

Fig. 4. Variation of G' (10 rad s^{-1}; 0.5% strain) on cooling from 90 to 5°C at 1°C/min and holding for a further 15 min at 5°C for calcium pectinate (DE 34.2; stoichiometric Ca^{2+}) at the concentration of 2.0 wt% used in the mixed systems with oxidised starch (×), and at concentrations (wt%) of 0.25 (○), 0.35 (●), 0.50 (△), 1.0 (▲), 1.5 (□) and 3.0 (■).

behaviour shown in Fig. 3 for oxidised starch, the variation of $\log G'$ with $\log c$ for calcium pectinate has the form typical (Clark & Ross-Murphy, 1985) of a normal biopolymer gel, with progressive reduction in slope towards a limiting value of ~2 as the polymer concentration is increased. For quantitative use in analysis of G' for the mixed systems, the relationship between $\log G'$ and $\log c$ was fitted to a third-order polynomial function of the form:

$$B = p(3)A^3 + p(2)A^2 + p(1)A + p(0) \qquad (2)$$

where $A = \log(c/\text{wt\%})$ and $B = \log(G'/\text{Pa})$. The polynomial parameters used were: $p(3) = 1.7455$; $p(2) = -1.7866$; $p(1) = 1.716$; and $p(0) = 3.19$. The observed moduli and the fitted values from Eq. (2) are listed in Table 2.

Table 2
Calibration data for variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with concentration (c) of calcium pectinate (DE 34.2; stoichiometric Ca^{2+})

c (wt%)	G' (Pa)	$\log c$	$\log G'$ observed	$\log G'$ fitted[a]
0.25	18.1	−0.60	1.26	1.24
0.35	83.8	−0.46	1.92	1.92
0.50	297	−0.30	2.47	2.42
1.0	1398	0.00	3.15	3.11
1.5	2264	0.18	3.35	3.43
2.0	4100	0.30	3.61	3.62
3.0	7878	0.48	3.90	3.87

[a] Using Eq. (2).

For reasons that will become apparent later, when the method of analysis is described, it was also necessary to express the concentration-dependence of G' for calcium pectinate in reciprocal form (i.e. with $\log c$ as a continuous function as $\log G'$). This was again done using a third-order polynomial:

$$A = p'(3)B^3 + p'(2)B^2 + p'(1)B + p'(0) \qquad (3)$$

where $p'(3) = 0.01294$; $p'(2) = 0.00980$; $p'(1) = 0.08345$; and $p'(0) = -0.7446$.

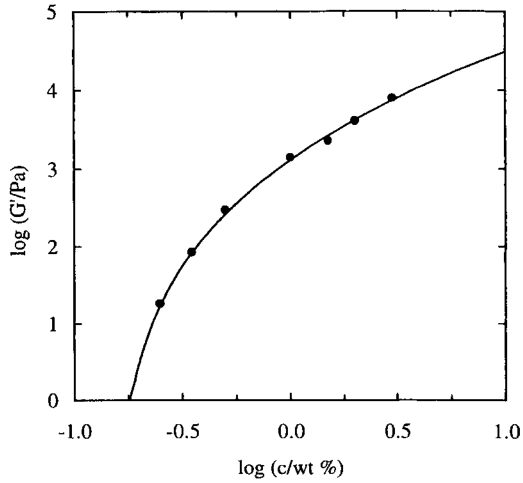

Fig. 5. Concentration-dependence of G' (10 rad s^{-1}; 0.5% strain) for calcium pectinate (DE 34.2; stoichiometric Ca^{2+}) after cooling from 90 to 5°C at 1°C/min and holding for a further 15 min at 5°C.

3.3. Composition-dependence of co-gel moduli

The overall effect of progressive incorporation of oxidised starch on the final moduli (G' after 15 min at 5°C) of calcium pectinate gels (2.0 wt%; DE 34.2; stoichiometric Ca^{2+}) is shown in Figs. 5 and 6 of the preceding paper (Picout et al., 2000a). To give a more rigorous check on the validity of the present analysis, however, some additional measurements were made at starch concentrations in critical regions of the curve. The combined data set is listed in Table 3, together with fitted values from the analysis described below.

Fig. 6 shows the variation of $\log G'$ with increasing

Table 3
Co-gel moduli (G'; 10 rad s^{-1}; 0.5% strain; 5°C) for 2.0 wt% calcium pectinate with varying concentrations of oxidised starch

[Starch] (wt%)	G' (Pa)	$\log G'$ observed	$\log G'$ fitted[a]
0.0	4100	3.61	3.59
1.5	2072	3.32	3.05
3.0	877	2.94	2.70
5.0	176.3	2.25	2.36
7.0	89.0	1.95	2.09
10.0	51.9	1.72	1.75
12.0	33.0	1.52	1.56
15.0	20.3	1.31	1.31
17.0	13.1	1.12	1.16
20.0	9.10	0.96	0.96
25.0	7.84	0.89	0.74
30.0	10.5	1.02	1.01
35.0	33.9	1.53	1.62
40.0	198.7	2.30	2.22

[a] By analysis described in the text.

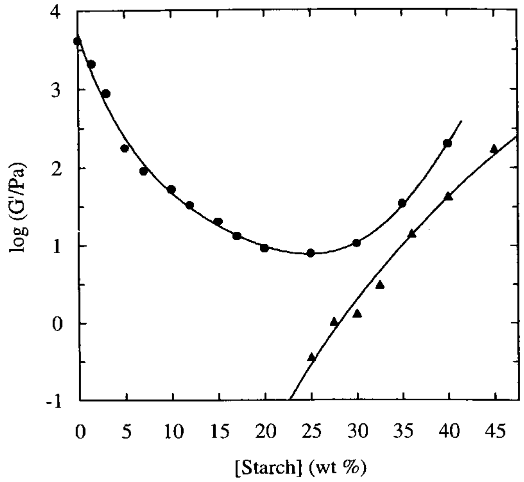

Fig. 6. Concentration-dependence of G' (10 rad s^{-1}; 0.5% strain) after cooling from 90 to 5°C at 1°C/min and holding for a further 15 min at 5°C for oxidised starch alone (▲) and in mixtures with 2.0 wt% pectin in the presence of stoichiometric Ca^{2+} (●).

concentration of oxidised starch in the mixtures with calcium pectinate, in direct comparison with the corresponding values for starch alone (Table 1). The modulus for the pectin component alone is indicated by the first point on the curve for the mixed systems (at zero starch concentration). The upturn in co-gel moduli at high concentrations of starch is obviously due to the onset of starch gelation. As discussed in greater detail later, however, the separation between the two curves indicates that the local concentration of the starch component in the mixed gels is higher than the nominal concentration across the whole system. The dominant feature in Fig. 6, however, is the massive reduction in modulus at lower starch concentrations. The likely origin of this steep decrease in G' with increasing concentration of starch (between 0 and ~25 wt%) is explored in the following section.

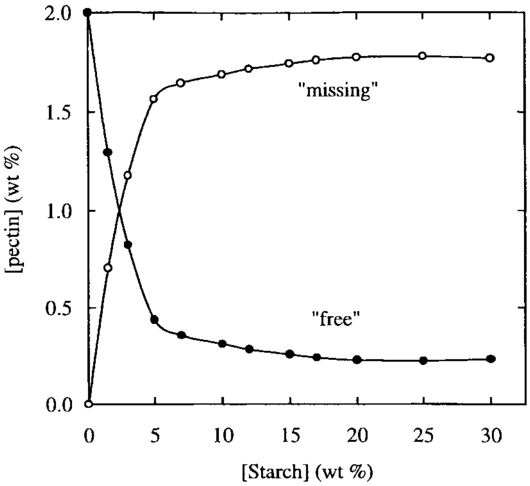

Fig. 7. Effect of starch concentration on the proportions of precipitated ("missing") and unprecipitated ("free") pectin calculated from experimental values of G' for mixtures of 2.0 wt% calcium pectinate with oxidised starch.

3.4. Precipitation analysis

As discussed in Section 1, the tentative conclusion from the preceding paper (Picout et al., 2000a) was that the reduction in modulus comes from "wastage" of pectin chains by precipitation into large aggregated bundles which make little contribution to the connectivity of the calcium pectinate network. As a starting point for quantitative analysis, we will assume for the moment that this interpretation is correct, and consider the total pectin content of the mixed systems to be split into two fractions: precipitated chains, which we will call "missing" pectin, and unprecipitated material, which we will call "free" pectin. We will further assume that, at starch concentrations below ~25 wt% (i.e. below the onset of gelation), the final moduli of the mixed systems come solely from gelation of "free" pectin. The concentration of free pectin in each mixture can then be calculated directly from the relationship between $\log G'$ and $\log c$ for calcium pectinate alone (Eq. (3)), with the concentration of missing pectin being given by difference from the starting concentration of 2.0 wt%. The values obtained are shown in Fig. 7.

At starch concentrations up to ~5 wt%, there is a steep, and almost linear, reduction in the concentration of free pectin, with a corresponding increase in the missing fraction. An analogous linear relationship between the concentration of one component in a single-phase mixture and the extent of precipitation of the second component has been observed previously for maltodextrin in combination with globular proteins (Manoj, Kasapis, Hember, Richardson & Morris, 2000) or gelatin (Kasapis, Morris, Norton & Gidley, 1993c). At higher concentrations of oxidised starch, however, the change in the relative proportions of the two pectin fractions is much more gradual, suggesting the possibility that the extent of precipitation is controlled by a solubility product (K). Since precipitation will occur by association of free pectin into larger assemblies, in response to thermodynamic incompatibility with oxidised starch, it would seem reasonable to expect a relationship of the form:

$$[\text{starch}][\text{free}]^2 = K \tag{4}$$

which should then yield a straight line of slope $1/K$ when $1/[\text{free}]^2$ is plotted against starch concentration. As shown in Fig. 8, this plot does indeed remain acceptably linear up to ~20 wt% oxidised starch, before showing the downturn expected from the onset of starch gelation (which will introduce an additional contribution to G', and thus give spuriously high values for the calculated concentration of free pectin). In the remaining analyses, we will therefore assume that the concept of partial precipitation of pectin within a supporting calcium pectinate network is essentially correct, and that the concentration of free pectin is determined by the relationship given in Eq. (4).

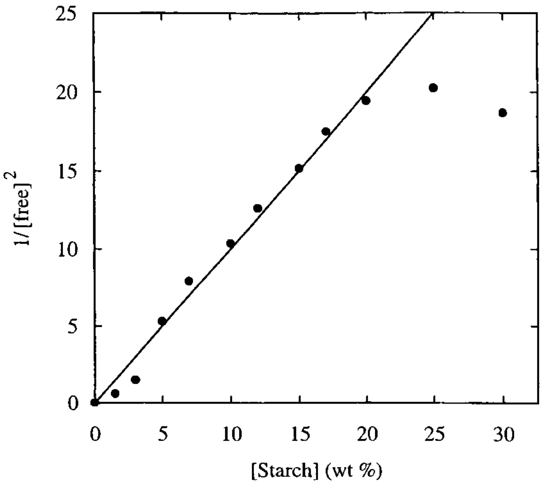

Fig. 8. Analysis of calculated concentrations of free pectin in mixtures with oxidised starch, in terms of a solubility product (Eq. (4)).

3.5. Calculation of co-gel moduli

In the analysis described in the previous section, the precipitated pectin was, in effect, regarded as having vanished from the system (as implied by describing it as "missing"). In reality, of course, it will still be present, in the form of hydrated particles or bundles which will occupy a finite fraction of the total volume, and therefore raise the effective concentration of the other constituents ("free" pectin and oxidised starch). As a working hypothesis, we will assume that the volume occupied by the precipitated material is directly proportional to its concentration across the whole system (i.e. that the density of the precipitated bundles is independent of the concentration of the other constituents). The mixtures can then be regarded as comprising two phases: a continuous matrix (phase X), containing free pectin and oxidised starch, and dispersed particles of precipitated calcium pectinate (phase Y).

The first stage in obtaining calculated values of co-gel moduli was to use Eq. (4) to determine the concentrations of free and missing pectin across the whole system:

$$[\text{free}] = (K/[\text{starch}])^{1/2} \tag{5}$$

$$[\text{missing}] = [\text{pectin}] - [\text{free}] \tag{6}$$

where [pectin] denotes the overall starting concentration of 2.0 wt%. It should be noted that the solubility product, K, cannot be taken directly from the slope of the linear region in Fig. 8 since, as described above, the local concentrations of free pectin giving rise to the experimental moduli on which the plot is based will have been raised slightly by the precipitated chains making part of the total volume unavailable to the free component. Instead, K was regarded as an adjustable parameter.

The other adjustable parameter used was the constant of proportionality (k) between the overall concentration of "missing" pectin and the phase volume of the dispersed particles (Eq. (7)), with the phase volume of the continuous matrix then being obtained by difference (Eq. (8))

$$\phi_{\text{Y}} = k[\text{missing}] \tag{7}$$

$$\phi_{\text{X}} = 1 - \phi_{\text{Y}}. \tag{8}$$

The local concentrations of the three components (oxidised starch, free pectin and missing pectin) within their respective phases can then be obtained by dividing the nominal concentrations by the appropriate phase volume:

$$[\text{starch}]_{\text{X}} = [\text{starch}]/\phi_{\text{X}} \tag{9}$$

$$[\text{free}]_{\text{X}} = [\text{free}]/\phi_{\text{X}} \tag{10}$$

$$[\text{missing}]_{\text{Y}} = [\text{missing}]/\phi_{\text{Y}} \tag{11}$$

where the subscripts correspond to the phase to which each component is confined. Combining Eqs. (7) and (11) gives:

$$[\text{missing}]_{\text{Y}} = 1/k \tag{12}$$

Thus k is the reciprocal of the local concentration of pectin within the precipitated bundles.

The next step was to determine the moduli of the individual phases. The contribution of the starch component to the modulus of the continuous phase can be obtained directly from its local concentration (Eq. (9)) and the linear relationship between log G' and log c shown in Eq. (1). The contribution of the free pectin component can be derived in the same way, from Eqs. (10) and (2). The overall modulus of the continuous matrix was then estimated by simple addition of the individual values for free pectin and oxidised starch. This approximation assumes, in effect, that the two components form interpenetrating networks, with neither influencing the other. In practice, addition of constituent moduli had no significant effect outside a very narrow range of initial starch concentrations (from ~25 to 30 wt%): at lower concentrations the starch component remains ungelled; at higher concentrations the contribution of free pectin to the calculated moduli was negligible.

It should be noted that the lowest value of co-gel modulus (Fig. 6) is ~8 Pa (log $G' \approx 0.89$), which is only slightly below the experimental range of the pectin calibration curve in Fig. 5. Thus in the range of starch concentrations where free pectin makes any significant contribution to calculated values of co-gel moduli, the empirical polynomial relationship in Eq. (2) is used almost solely for interpolation. Similarly, the modulus of the co-gel formed at the highest starch concentration used (40 wt%) is only marginally above the range of the starch calibration curve in Fig. 3, so that the linear relationship in Eq. (1) is again used almost solely for interpolation.

The modulus of the dispersed phase of precipitated calcium pectinate was calculated in two different ways. As described in the preceding paper (Picout et al., 2000a), gelation of calcium pectinate in the presence of oxidised starch is accompanied by a sharp reduction in G' during cooling,

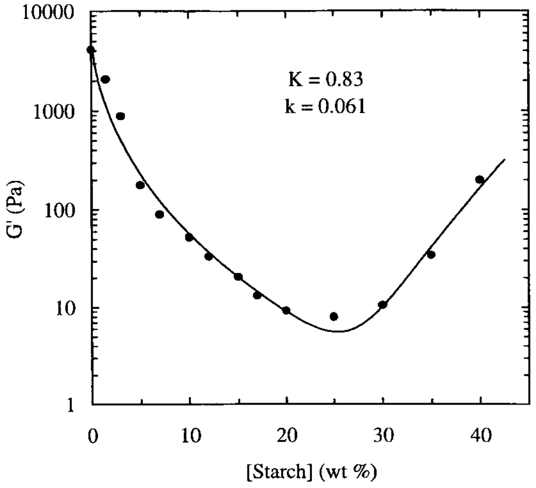

Fig. 9. Comparison of observed moduli for calcium pectinate—oxidised starch co-gels (●) with calculated values (—) from the analysis described in the text.

suggesting collapse of the developing network to a compressed (or "deswollen") state. One approach was therefore to use classic swelling/deswelling theory (Flory, 1953) to relate the final modulus of the deswollen network (G_f) to the corresponding value for the uncollapsed gel (initial modulus, G_i)

$$G_f/G_i = (c_f/c_i)^{2/3} \tag{13}$$

where c_i and c_f are the local polymer concentrations before and after deswelling, with c_i = [missing] and c_f = [missing]$_Y$. The second approach was to use the final concentration (Eq. (11)) in conjunction with the modulus–concentration relationship from Eq. (2), in the same way as for the free pectin component. Since the calibration curve in Fig. 5 has a minimum slope of ~2, the moduli calculated by this method were much higher than those obtained by deswelling theory, which corresponds (Eq. (13)) to an invariant slope of ~0.67. In practice, however, the choice of method proved to be irrelevant, since, as described below, even the lower values obtained by the deswelling approach were so much higher than the moduli derived for the continuous phase that they could be regarded as effectively infinite.

The final stage of the analysis was to relate the individual moduli and volume fractions of the constituent phases to the overall modulus of the composite gel by application of the isostrain (Eq. (14)) and isostress (Eq. (15)) blending laws of Takayanagi, Harima and Iwata (1963):

$$G = \phi_X G_X + \phi_Y G_Y \tag{14}$$

$$1/G = \phi_X/G_X + \phi_Y/G_Y \tag{15}$$

These relationships were developed originally for laminated composites of condensed, synthetic polymers, with layers arranged in parallel (isostrain model) or in series (isostress model) to the direction of the imposed deformation. In a recent series of experiments in this laboratory (Abdulmola, Hember, Richardson & Morris, 1996; Mohammed, Hember, Richardson & Morris, 1998b), however, it has been shown that, as anticipated for blends of condensed polymers (e.g. Manson & Sperling, 1976), they give essentially perfect agreement with experimental moduli for hydrated biopolymer composites of dispersed "filler" particles in a continuous matrix, if the isostrain model is used when the filler particles are weaker than the matrix and the isostress model is applied to the converse situation. Since, by any realistic interpretation, the concentrated particles of precipitated pectin will have higher modulus than the surrounding matrix of free pectin and oxidised starch, only the isostress model (Eq. (15)) is relevant.

In systems where the dispersed particles are much stronger than the continuous matrix ($G_Y \gg G_X$), Eq. (15) reduces to:

$$1/G = \phi_X/G_X \tag{16}$$

i.e.

$$G = G_X/\phi_X \qquad \text{if } G_Y \gg G_X \tag{17}$$

The error introduced by this approximation becomes negligible (Abdulmola et al., 1996) when G_Y exceeds G_X more than a factor of ~10. In preliminary fits using deswelling theory (Eq. (13)) or calibration data (Eq. (2)) to estimate the modulus of the dispersed phase, the ratio of G'_Y/G'_X was invariably greater than 10.

In summary, therefore, calculated values of co-gel moduli were obtained by: (i) using Eqs. (7) and (8) to give ϕ_X; (ii) calculating the effective local concentrations of free pectin and oxidised starch in the continuous phase by Eqs. (9) and (10); (iii) using the modulus–concentration relationships from Eqs. (1) and (2) to obtain the individual moduli of starch and pectin in the continuous phase; (iv) combining these by simple addition to give an approximate value for the overall modulus of the continuous matrix; and (v) obtaining the calculated value of co-gel modulus by application of the isostress relationship in the limiting form for $G_Y \gg G_X$ (Eq. (17)). The final step was to use the Excel "Solver" routine to minimise the root-mean-square difference between observed and calculated values of log G' by varying K and k. The standard of agreement obtained is shown in Fig. 9.

The value of K required to achieve the best fit was ~0.83 (with concentrations expressed in wt%). As would be expected, this is close to the value of ~1.0 obtained from the slope of the linear region in Fig. 8; as mentioned above, the difference comes from the local concentration of free pectin in the continuous phase being higher than the nominal concentration across the whole system. The best-fitting value of k was ~0.061, which corresponds (Eq. (12)) to a pectin concentration of ~16.4 wt% within the precipitated bundles (i.e. with the particles containing ~5 g of water for each 1 g of pectin).

4. Discussion

The above analysis is based on three interlocking assumptions: (i) that the effect of segregative interactions between calcium pectinate and oxidised starch is to cause partial precipitation of the calcium pectinate component; (ii) that the extent of precipitation is controlled by a solubility product (Eq. (4)); and (iii) that the volume of the precipitated particles is directly proportional to their pectin content (Eq. (7)). As discussed below, the validity of the third assumption and, by implication, the first, seems reasonably certain; the second is less firmly established.

Although the fit shown in Fig. 9 was obtained by the "black box" approach of least-squares minimisation, the effect of the individual variables (K and k) could be readily explored by changing them manually and observing the resulting changes in calculated values of G'. Both parameters do, of course, have some influence on all parts of the curve, but the initial decrease in G' at starch concentrations up to ~20 wt% is largely dictated by the solubility product, K, whereas the subsequent increase at higher concentrations of starch is dominated by k. Because of the extremely steep concentration-dependence of G' for oxidised starch (Fig. 3), small changes in k (which controls the phase volume of the dispersed particles, and hence the polymer concentrations in the continuous phase) have a massive effect on the calculated moduli at starch concentrations above the onset of gelation. Thus the fitted value ($k = 0.061$) is very tightly defined. More significantly, the standard of fit (Fig. 9) at high starch concentrations suggests that the form of the relationship used (i.e. direct proportionality between mass and volume) is realistic.

The validity of this relationship (Eq. (7)) is demonstrated more explicitly in the investigation of calcium pectinate–maltodextrin co-gels reported in the two following papers (Picout, Richardson & Morris, 2000b,c), where it is compared with the concept of "relative solvent avidity" (the p-factor approach; Clark, 1987), which has been found to apply well to biphasic networks formed by conventional phase separation (e.g. Clark, Richardson, Robinson, Ross-Murphy & Weaver, 1982; Clark, Richardson, Ross-Murphy & Stubbs, 1983; Chronakis, Kasapis & Richardson, 1996; Gilsenan, Richardson & Morris, 2000; Ipsen, 1995; Kasapis, Morris, Norton & Clark, 1993b). For mixtures where the maltodextrin concentration was held constant and the concentration of calcium pectinate was varied over a wide range, the changes in moduli arising from the progressive increase in local concentration of maltodextrin with increasing space-occupancy by calcium pectinate could not be rationalised by pro-rata distribution of solvent between the two components (i.e. by a constant p-factor), but complied closely with the concept of network collapse to a fixed concentration of calcium pectinate within precipitated bundles (Picout et al., 2000c). The value of k required to give best agreement between observed and calculated values of $\log G'$ was virtually identical to the corresponding value from the present analysis.

Thus the central conclusion from both studies, that reduction in modulus arises from precipitation of pectin chains within a supporting calcium pectinate network, rather than from a conventional phase-separation mechanism, appears to offer a physically realistic explanation of the experimental results.

The principles governing the extent of precipitation, however, are less clear. Use of a solubility product (Eq. (4)) implies that the precipitated chains have no influence on the concentration of residual "free" pectin. An alternative possibility, however, would be a dynamic exchange of material between the free and precipitated states, with the relative proportions determined by an equilibrium constant. Neither seems entirely satisfactory. There is no realistic mechanism by which chains embedded in the interior of large aggregated bundles could participate in a dynamic equilibrium with the exterior phase. Conversely, however, it seems unlikely that there would be no exchange of material between the surface of the precipitated particles and the surrounding matrix. It might, therefore, be more reasonable to expect some form of progression from a dynamic equilibrium to a solubility–product relationship as the precipitated particles increase in size (i.e. as the surface-to-volume ratio decreases). As can be seen in Figs. 8 and 9, there does indeed seem to be a systematic tendency for the extent of precipitation of pectin at low concentrations of oxidised starch (up to ~5 wt%) to be overestimated by the solubility product derived by extrapolation from higher starch concentrations. It would, of course, be possible to modify the analysis to take account of this effect, but at the expense of introducing a third adjustable parameter, which seems an unwarranted elaboration.

The discrepancies between the observed moduli and the values calculated on the basis of the working assumptions used in the present study, however, are almost within the experimental scatter of the observed results (Fig. 9). The same assumptions were therefore adopted in analysis (Picout et al., 2000c) of the experimental results from the investigation of calcium pectinate–maltodextrin co-gels reported in the following paper (Picout et al., 2000b).

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