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Co-gelation of agarose and waxy maize starch

Z.H. Mohammed, M.W.N. Hember, R.K. Richardson, E.R. Morris*

Cranfield University, Silsoe College, Silsoe, Bedford, MK45 4DT, UK

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Abstract

The rheology of composite gels formed by gelatinization of (uncrosslinked) waxy maize starch (WMS; swelling volume ≈ 12 ml g^{-1}) in agarose solution (0.25 wt%) at 80°C, and gelation of the agarose component by rapid quenching to 5°C, has been examined by small-deformation oscillatory measurements. At low concentrations of starch (up to ~2 wt%) the composite moduli (G'_C) follow those of the agarose phase (isolated by centrifugation in the pre-gel solution state), indicating that the swollen granules are present as a dispersed phase within a continuous biopolymer matrix. At higher concentration of starch (3–5 wt%) there is a sharp reduction in G'_C, and the experimental moduli show close agreement with values calculated for a bicontinuous network. Bicontinuity was confirmed by the presence of a residual agarose network after elimination of the starch component by enzymic hydrolysis. On further increase in WMS concentration to 6 wt% (starch phase-volume ≈ 72%) there is a second sharp reduction in G'_C, towards the modulus of WMS alone, indicating that starch has now become the dominant component. The reduction continues until the starch concentration reaches the reciprocal of the swelling volume (at ~8.3 wt%); at higher concentrations, where swelling is restricted by availability of water, the modulus rises, consistent with a simple increase in the local concentration of the dominant starch network. In the bicontinuous region (3–5 wt% WMS), but not at higher or lower concentrations of starch, the composites show extreme strain-sensitivity, with structural breakdown occurring at ~0.3% strain. From comparison with previous studies of the behaviour of WMS and phosphate-crosslinked waxy maize starch (PCS) in composites with other biopolymers (gelatin and xanthan), it is suggested that the distribution of the two constituent phases is determined by (i) the ease of dissociation of the weak starch network formed on gelatinization, to reduce topological restrictions on formation of a stronger (i.e. more enthalpically favourable) biopolymer matrix, and (ii) the extent to which the biopolymer promotes association of starch granules by depletion flocculation. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Co-gelation; Agarose; Waxy maize starch; Bicontinuous network; Starch gelation

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1. Introduction

The preceding paper (Mohammed et al., 1998b) reports the use of gelatinized granules of phosphate-crosslinked starch (PCS) trapped in an agarose gel matrix as a simple model for phase-separated biopolymer co-gels. The central conclusion was that composite moduli derived by the isostrain and isotress blending laws of Takayanagi et al. (1963) were in good agreement with those observed experimentally.

As a continuation of the same investigation, we also carried out analogous experiments using unmodified starch from the same botanical source (waxy maize), with the expectation of finding qualitatively similar behaviour, but with quantitative changes due to differences in the rigidity of crosslinked and uncrosslinked granules. In practice, however, the results were entirely different, and are reported separately here.

As before, the agarose and starch components are denoted as, respectively, phase X and phase Y throughout.

2. Materials and methods

The same agarose sample was used as in the investigations described in the two preceding papers (Mohammed et al., 1998a,b): Sigma; type I-A; low EEO; lot number 084H0462. Waxy maize starch was kindly supplied by Cerestar and, for brevity, will be denoted as WMS. The sample used is of the same type (SF 04202) as in the studies of starch–gelatin and starch–xanthan systems by Abdulmola et al. (1996a,b), but from a different batch. All solutions were made using distilled deionized water. Agarose was dispersed in water at ~95°C and autoclaved (15 min; 120°C) to ensure complete dissolution. Slurries of

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* To whom correspondence should be addressed.

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ungelatinized starch and solutions of agarose were prepared at twice the required final concentrations and were mixed in equal amounts (w/w) at 45°C, which is well below the gelatinization temperature of WMS (Abdulmola et al., 1996b) and well above the onset temperature for gelation of agarose (Mohammed et al., 1998a).

In most of the experiments, the starch was gelatinized by holding the mixture for 1 h in a water bath at 80°C, with occasional gentle stirring (magnetic stirrer) to keep the granules in suspension. The samples were then split into two portions. One was centrifuged (4000*g*) for 1 h at ~40°C to obtain a clear sample of the agarose phase; the other was loaded on to an oscillatory rheometer at 45°C, quenched to 5°C, and held at 5°C until constant moduli were obtained. The variation of storage modulus (G'), loss modulus (G''), complex dynamic viscosity ($\eta * = (G'^2 + G''^2)^{1/2}/\omega$) and loss tangent ($\tan\delta = G''/G'$) with frequency ($\omega$) at a fixed strain of 0.05% and with strain at a fixed frequency of 10 rad s^{-1} was then recorded. Agarose supernatants from centrifugation and standard agarose solutions for calibration of the concentration-dependence of G' were measured under the same time–temperature regime. The concentration of agarose in the supernatants was then obtained from the calibration curve and used to calculate the phase volumes of agarose and starch. Data analysis was carried out using a standard Microsoft Excel spreadsheet package (Version 5.0).

As described previously (Mohammed et al., 1998b), measurements were made using truncated cone-and-plate geometry (50 mm diameter; 0.05 rad cone angle; 0.5 mm gap) on a sensitive prototype rheometer designed and constructed by one of us (R.K.R.). The periphery of the sample was coated with light silicone oil to minimize evaporation of water. Temperature was controlled by a Haake circulating water bath and measured with a thermocouple attached to the stationary element.

For investigation of network continuity by enzymic hydrolysis of the starch component in the agarose–WMS co-gels, 1 ml of enzyme solution was added to 19 ml of the pre-gel mixture at 45°C, with appropriate adjustment of the initial concentrations of agarose and starch to give the required final concentrations. The enzyme used was α-amylase from *Bacillus licheniformis*, purchased as a commercial preparation (Termamyl 120 L) from Novo Nordisk A/S, 2880 Bagsvaerd, Denmark. Stock solutions were prepared at 1% and 0.01% of the original concentration, giving final dilutions of 2 × 10^3 and 2 × 10^5 after mixing (1:19) with the agarose–starch samples.

3. Results and discussion

In all the experiments reported below, the agarose concentration was held fixed at 0.25 wt%. Sedimentation of gelatinized starch granules on centrifugation was less complete for WMS than for PCS at the same concentration of agarose (Mohammed et al., 1998b), but it proved possible to obtain sufficient clear supernatant for rheological characterization at WMS concentrations up to 5 wt%. The values of G' obtained for the supernatants from mixtures of 0.25 wt% agarose with 1, 2, 3, 4 or 5 wt% WMS, after quenching from 45°C and equilibration at 5°C, are shown in Fig. 1a, in comparison with the corresponding value for 0.25 wt% agarose alone. As anticipated, there is a progressive increase in modulus with increasing concentration of WMS in the mixtures prior to centrifugation, which, as in previous investigations (Abdulmola et al., 1996a,b; Mohammed et al., 1998b), can be attributed to transfer of water into the starch granules during gelatinization, with consequent increase in polymer concentration in the surrounding matrix.

For G' values spanning the range shown in Fig. 1a, the variation of log G' with log c for agarose gels formed by

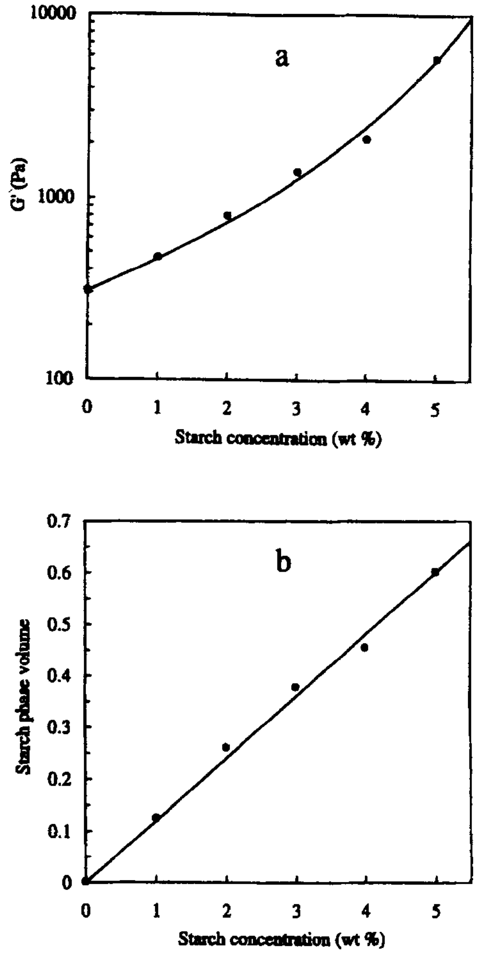

Fig. 1. (a) Variation in G' (10 rad s^{-1}; 0.05% strain; 5°C) with starch concentration for the agarose supernatants obtained by centrifugation of mixtures of WMS with 0.25 wt% agarose after gelatinization of the WMS component at 80°C. (b) Starch phase volumes (ϕ_Y) obtained using the G' values from (a) to determine the increase in agarose concentration on swelling of the starch granules (slope = 0.120, i.e. swelling volume = 12.0 ml g^{-1}); the solid line in (a) shows moduli calculated for the agarose phase by using the swelling volume in conjunction with the concentration-dependence of G' for agarose alone (Eq. (1)).

quenching to 5°C can be described (Mohammed et al., 1998a) to within experimental error by the simple linear relationships:

$$\log G' = 3.204 \log c + 4.414 \quad (1)$$

$$\log c = 0.312 \log G' - 1.377 \quad (2)$$

where concentration (c) is in wt% and G' is in Pa. As before (Mohammed et al., 1998b), Eq. (2) was used to determine the agarose concentrations (c_X) corresponding to the observed moduli of the gelled supernatants (Fig. 1a), and the agarose phase volumes (ϕ_X) were derived (Eq. (3)) by comparison with the initial concentration of agarose (c_O = 0.25 wt%) prior to swelling of the starch granules.

$$\phi_X = c_O / c_X \quad (3)$$

The starch phase volumes (ϕ_Y) were then obtained by difference ($\phi_Y = 1 - \phi_X$).

As shown in Fig. 1b, ϕ_Y increases linearly with WMS concentration, with a slope of 0.120 (corresponding to a swelling volume of 12.0 ml g^{-1}). Thus at the highest concentration of WMS in Fig. 1 (5 wt%), the starch phase occupies ~60% of the total volume, in comparison with ~52% for PCS (Mohammed et al., 1998b). In a previous study of starch–gelatin composites (Abdulmola et al., 1996a), a somewhat lower swelling volume was reported for WMS (9.65 ml g^{-1}), but the difference may be due simply to the use of different batches of material in the two investigations.

In the quantitative analyses of the rheology of WMS–agarose composites described below, the swelling volume of 12.0 ml g^{-1} obtained from Fig. 1b was used in conjunction with Eqs. (1) and (3) to calculate the modulus (G'_X) of the agarose phase as a continuous function of starch concentration. The standard of agreement with the observed values of G' for the gelled supernatants is shown in Fig. 1a.

Fig. 2 shows the mechanical spectra (frequency-dependence of G', G'' and η^*) obtained after quenching from 45°C and equilibration at 5°C for 0.25 wt% of agarose alone (Fig. 2a) and in combination with 5 wt% gelatinized WMS (Fig. 2b). Both have the form typical (e.g. Morris, 1984; Clark and Ross-Murphy, 1987) of a biopolymer gel network ($G' \gg G''$; little frequency-dependence in either modulus; linear decrease in log η^* with increasing log ω, with a slope close to −1). The separation of G' and G'' for the mixed system, however, is smaller than for agarose alone (i.e. higher tan δ) and G' is only slightly greater (~520 Pa at 10 rad s − 1, in comparison with ~305 Pa in the absence of starch).

Since, as discussed above, gelatinized WMS at a concentration of 5 wt% occupies ~60% of the total volume, the limited increase in G' was unexpected, particularly in view of the large enhancements induced by PCS (Mohammed et al., 1998b). Fig. 3 shows the composite moduli (G'_C) observed for 0.25 wt% agarose in combination with PCS or WMS at starch concentrations of 0–5 wt%, in

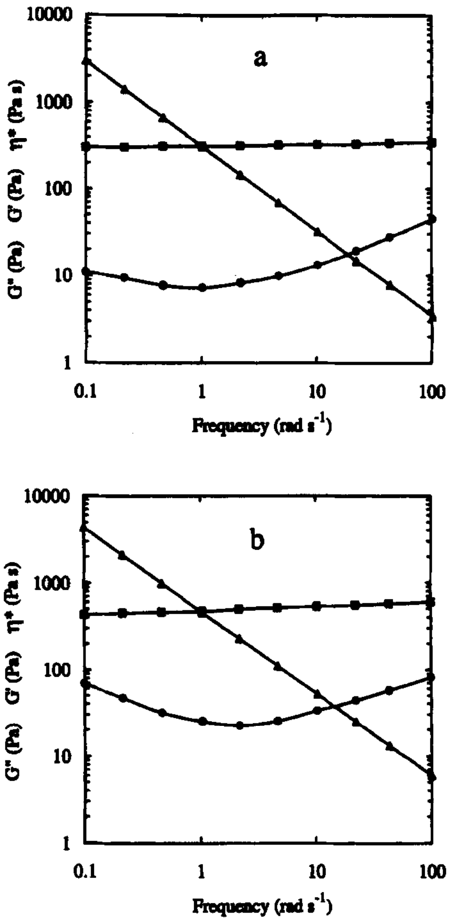

Fig. 2. Mechanical spectra (0.05% strain; 5°C) showing the frequency-dependence of G' (■), G'' (●) and η^* (▲) for 0.25 wt% agarose (a) alone and (b) with 5 wt% gelatinized WMS after quenching from 45°C and holding at 5°C until stable moduli were attained.

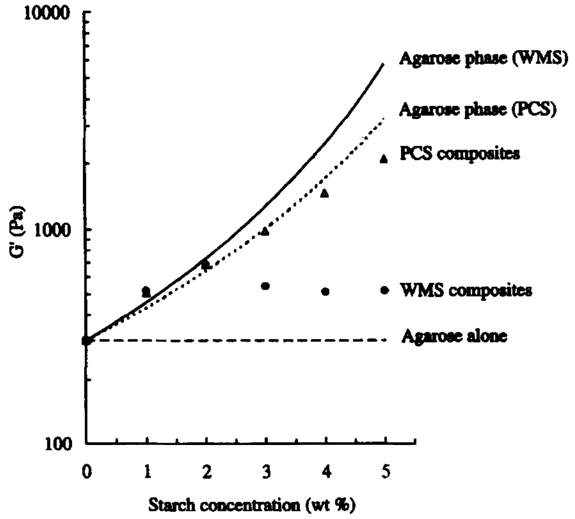

Fig. 3. Observed values of G' (10 rad s^{-1}; 0.05% strain; 5°C) for composites of 0.25 wt% agarose with gelatinized PCS (△) or WMS (●) after rapid quenching from 45°C and equilibration at 5°C, in comparison with corresponding moduli for 0.25 wt% agarose alone (– – –) and for the agarose phase concentrated by swelling of PCS (- - -) or WMS (——).

comparison with G' values for the agarose phase in both systems (derived from swelling volumes by the procedure outlined above), and with the observed value of G' for 0.25 wt% of agarose alone. As described previously (Mohammed et al., 1998b), the modulus determined for individual granules of gelatinized PCS (G'_Y) by a model-independent procedure was ~850 Pa, in good agreement with the value of ~730 Pa estimated by Abdulmola et al. (1996a) from minimization of the root-mean-square difference between observed values of G'_C for PCS–gelatin composites and those calculated by the Takayanagi blending laws. For starch concentrations where the modulus of the agarose phase (G'_X) is lower than that of the swollen PCS granules ($G'_Y \approx 850$ Pa), the presence of the starch "filler" has the effect of raising the composite modulus (G'_C) above that of the (softer) agarose matrix. At higher concentrations of PCS (above ~3 wt%), the agarose matrix becomes the stronger component (because of greater transfer of water to the starch granules during gelatinization), and the dispersed PCS phase then reduces the overall modulus of the composite relative to that of the continuous matrix. The degree of weakening (for $G'_Y < G'_X$) or reinforcement (for $G'_Y > G'_X$) can be matched, with good precision (Abdulmola et al., 1996a; Mohammed et al., 1998b), by application of, respectively, the isostrain (Eq. (4)) or isosotress (Eq. (5)) blending law (Takayanagi et al., 1963).

$$G'_C = G'_X\phi_X + G'_Y\phi_Y \text{ if } G'_Y < G'_X \qquad (4)$$

$$1/G'_C = \phi_X/G'_X + \phi_Y/G'_Y \text{ if } G'_Y > G'_X \qquad (5)$$

It is evident, however, that the dominant effect of PCS in composite gels with agarose (or gelatin) is to increase the polymer concentration in the continuous matrix, with consequent increase in G'_X, and that the direct contribution of the swollen granules causes only comparatively minor deviations from the modulus of the continuous phase.

At low concentrations of starch (up to ~2 wt%), the observed values of G'_C for the agarose–WMS composites (Fig. 3) also remain close to the modulus of the agarose phase. On further increase in WMS concentration to 3 wt%, however, the composite moduli drop sharply, with little further change as the concentration of starch is raised to 5 wt%. This behaviour cannot be rationalized by blending-law analysis of swollen WMS granules dispersed through a continuous agarose network. For composites where the filler particles are much softer or much harder than the surrounding matrix (by more than about a factor of 10; Abdulmola et al., 1996b), the isostrain and isostress equations reduce to:

$$G'_C \approx G'_X\phi_X \text{ if } G'_Y \ll G'_X \qquad (6)$$

$$1/G'_C \approx \phi_X/G'_X \text{ if } G'_Y \gg G'_X \qquad (7)$$

Thus for 5 wt% WMS, where $\phi_Y = 0.60$ (i.e. $\phi_X = 0.40$), the lowest possible ratio of G'_C/G'_X from isostrain analysis (Eq. (6)) would be 0.4, whereas the observed ratio (Fig. 3) is less than 0.1 ($G'_X \approx 5750$ Pa; $G'_C \approx 520$ Pa). For the corresponding composite of 0.25 wt% agarose with 5 wt% PCS ($\phi_Y = 0.52$; $\phi_X = 0.48$), by contrast, $G'_X \approx 3.2$ kPa and $G'_C \approx 2.1$ kPa, giving $G'_C/G'_X \approx 0.66$, in comparison with the expected minimum ratio of 0.48.

As shown in Fig. 4, it is possible to obtain a reasonable fit to the composite moduli for agarose–WMS by using the isostrain model (Eq. (4)) at 1 and 2 wt% starch and the isostress model (Eq. (5)) at 3, 4 and 5 wt%, with best agreement between the observed and calculated values of G'_C being given by a starch-phase modulus of $G'_Y \approx 295$ Pa. Physically, however, this analysis would imply phase-inversion from a dispersion of starch granules in a continuous agarose network to a starch-continuous system with the agarose phase dispersed as microgel particles, and, as described below, we have found clear evidence that the agarose network remains continuous, by digestion of the starch component with α-amylase.

In the first experiments, the commercial enzyme solution used was diluted by a factor of 100, and 1 ml of the resulting stock solution was added (at 45°C) to 19 ml of a mixture of agarose and gelatinized WMS at the concentrations required to give 0.25 wt% agarose and 5 wt% starch in the final mixture. One sample prepared in this way was loaded onto the rheometer at 45°C and immediately quenched to 5°C; a second sample was held for 10 min at 45°C prior to quenching; control measurements were made using the corresponding mixture with no added enzyme, and 0.25 wt% agarose alone. As shown in Fig. 5, G' for all four samples reached stable values within ~10 min after quenching. The

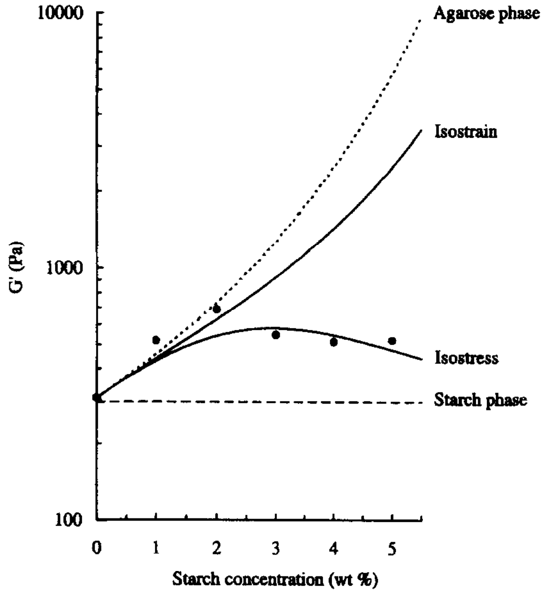

Fig. 4. Comparison of observed values (●) of G' (10 rad s^{-1}; 0.05% strain; 5°C) for composites of WMS gelatinized in 0.25 wt% agarose with calculated values (——) obtained by application of the isostrain and isostress blending laws (Eq. (4) and Eq. (5)), using values of G'_X for the agarose phase (- - -) derived from the swelling volume of 12.0 ml g^{-1} for WMS (Fig. 1b) and the concentration-dependence of G' for agarose alone (Eq. (1)), and a fixed value of $G'_Y = 295$ Pa for the starch phase (– – –).

final modulus for the agarose–WMS mixture held for 10 min before quenching is close to that observed for agarose alone (~350 Pa, in comparison with ~305 Pa), indicating almost total hydrolysis of the starch component during the holding period at 45°C. Surprisingly, the observed modulus for the sample that was quenched immediately is even lower (~190 Pa). The possible significance of this behaviour is discussed later. The absence of any detectable reduction in modulus on holding (for 100 min) at 5°C (Fig. 5), however, suggests that enzymolysis had gone to completion before, or during, quenching. The experiment was therefore repeated with a 100-fold further reduction in enzyme concentration.

After the initial increase in modulus from gelation of agarose (Fig. 6), there was a steady decrease in *G′* for the composite gel of 0.25 wt% agarose with 5 wt% WMS, with the values then levelling out after ~12 h at 5°C. A similar time-course of hydrolysis was observed for WMS gelatinized in water (at a concentration of 10 wt%, chosen to give demouldable plugs in the comparative experiment described below). However, while the *G′* value for starch alone (plotted on the right-hand axis in Fig. 6) dropped to ~5 Pa over the period of the experiment, the final value for the mixed system (plotted on the left-hand axis) was much higher (~210 Pa), indicating that the agarose component is still present as a continuous network in the composite gel.

As an additional test of network continuity, replicate samples were prepared as cylindrical plugs (volume ~10 ml), immersed in enzyme solution (~50 ml; 100-fold dilution) at 45°C, and inspected visually over a period of ~16 h. Under these conditions (i.e. with an enzyme/starch ratio ~10^4 higher than in Fig. 6 and at a temperature closer to the optimum for the enzyme used) the gel of 10 wt% WMS alone disintegrated completely in less than 1 h, leaving a white floc (presumably due to ordering and aggregation of short linear segments released during degradation of amylopectin). Similar flocculated particles were formed in the mixed system (0.25 wt% of agarose; 5 wt% WMS), demonstrating that the enzyme had successfully penetrated the co-gel network, but at the end of the hydrolysis period the sample still retained its original cylindrical shape. Retention of gel structure after extensive hydrolysis of starch demonstrates the presence of a continuous agarose network; the reduction in *G′* shown in Fig. 6, however, indicates that the WMS component also makes a substantial direct contribution to the overall rheology of the composite gel.

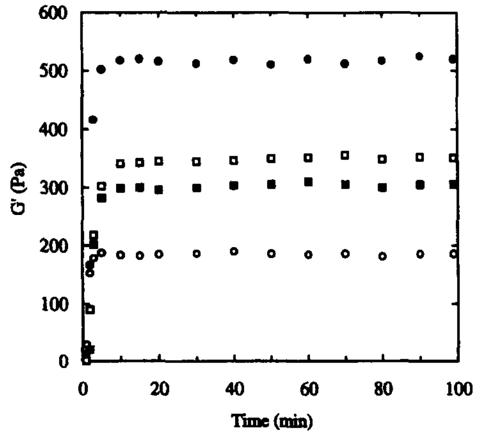

Fig. 5. Effect of α-amylase (2 × 10^3 dilution) on development of *G′* (10 rad s^{-1}; 0.05% strain) by mixtures of 0.25 wt% agarose with 5 wt% gelatinized WMS on rapid quenching from 45 to 5°C and holding at 5°C. Results are shown for a sample quenched immediately after addition of enzyme at 45°C (○) and for one held for 10 min at 45°C prior to quenching (□), in comparison with corresponding values for a mixture with no added enzyme (●) and for 0.25 wt% agarose alone (■).

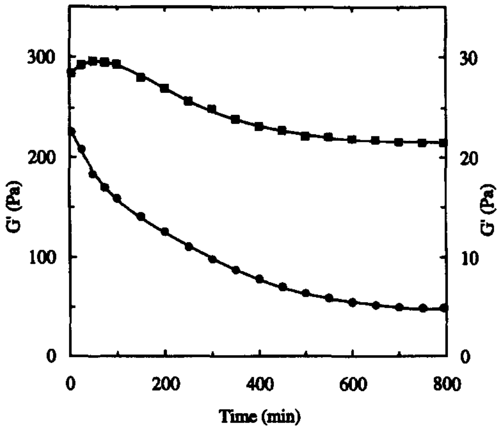

Fig. 6. Time-course of starch hydrolysis (5°C) by α-amylase (2 × 10^5 dilution; i.e. 100-fold lower concentration than in Fig. 5), as monitored by measurement of *G′* at 10 rad s^{-1} and 0.05% strain, for 10 wt% gelatinized WMS (●; right-hand axis) and for a mixture of 5 wt% gelatinized WMS with 0.25 wt% agarose (■; left-hand axis). The enzyme was added at 45°C and the samples were quenched immediately to 5°C.

This conclusion is reinforced by the results of an experiment in which WMS was gelatinized within a pre-formed agarose gel. As discussed previously (Mohammed et al., 1998a, b), the original aim of the experiment was to explore the effect of ''deswelling'' of the agarose network by transfer of water into the starch granules during gelatinization. An unexpected complication, however, was that under the setting conditions required to minimize sedimentation of ungelatinized starch (rapid quenching from the pre-gel solution state) the agarose network was found to melt at a temperature well below the melting point of corresponding gels formed by slow (1°C min^{-1}) cooling (Mohammed et al., 1998a), and close to the temperature required for complete gelatinization of WMS (80°C; Abdulmola et al., 1996b). Nonetheless, the behaviour observed offers some useful insights into the possible nature of the co-gel network.

Gels of agarose (0.25 wt%) alone and in combination with 5 wt% ungelatinsed WMS were formed by quenching from 45°C and equilibration at 5°C; they were then heated to 80°C at 1°C min^{-1} and held there for a further 45 min. Fig. 7 shows the changes in *G′* observed during the heating and holding steps. Results are also shown for 5 wt% ungelatinized WMS in water. As a point of practical detail,

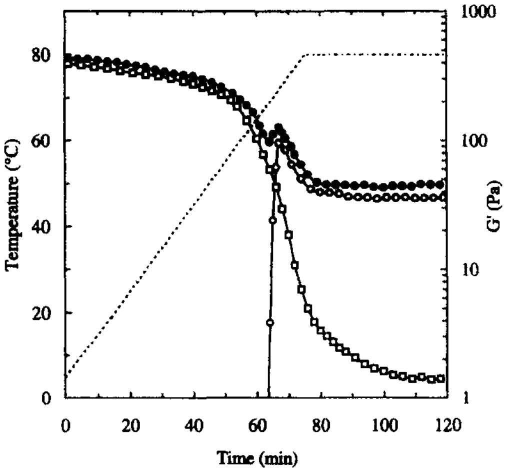

Fig. 7. Variation of G' (10 rad s^{-1}; 0.05% strain) with time and temperature (- - -) during heating from 5 to 80°C at 1°C min^{-1} and holding for 45 min at 80°C for 0.25 wt% agarose gelled by rapid quenching from 45°C and equilibration at 5°C in the presence (●) and absence (☐) of 5 wt% ungelatinized WMS. Measurements are also shown for 5 wt% ungelatinized WMS in water (○); the starch slurry was heated from 45°C, and 40 min has been added to the experimental times to give equivalent temperatures for all three samples.

this sample was heated from 45°C to minimize sedimentation of the granules prior to gelatinization, and the actual elapsed times have been incremented by 40 min to bring the temperatures into coincidence with those of the other samples. The sharp increase in G' marking the onset of gelatinization of WMS alone occurs at ~67°C; the moduli then pass through a maximum at ~72°C, before dropping to a final, stable value. These observations are in close quantitative agreement with results obtained previously by Abdulmola et al. (1996b).

Gelatinization of starch granules in the mixed system can be seen as a sharp increase in G', peaking at the same temperature (72°C) as for WMS alone. Up to this point, the overall modulus of the mixture reflects the behaviour of the agarose component, with G' values running parallel to those observed for 0.25 wt% agarose alone. At higher temperatures and longer times, however, where the modulus of the agarose network decreases steeply, the composite moduli follow the much higher values of G' seen for WMS alone, indicating that the starch component is now present as a continuous network rather than as isolated granules. This behaviour, in conjunction with the evidence of a continuous agarose network from enzymic hydrolysis, suggests that the co-gel network at low temperature may be bicontinuous. This possibility was explored by quantitative analysis of G'_C.

In an investigation of the co-gels formed by mixtures of iota and kappa carrageenan, Piculell et al. (1992) found that G' values calculated by the isostrain (Eq. (4)) and isostress (Eq. (5)) relationships were, respectively, much higher and much lower than those observed experimentally, but obtained excellent agreement using a theoretical relationship (Eq. (8)) derived by Davies (1971) for bicontinuous (i.e. interpenetrating) networks; we therefore used the same equation as a test of bicontinuity for agarose–WMS.

$$(G'_C)^{1/5} = \phi_X (G'_X)^{1/5} + \phi_Y (G'_Y)^{1/5} \tag{8}$$

The first step was to obtain a reliable value for the modulus of the starch phase (G'_Y). It should be noted that the values of G'_Y derived in the previous investigations by Abdulmola et al. (1996a) and Mohammed et al. (1998b) reflect the mechanical properties of individual swollen granules trapped as separate particles in a surrounding gel network. For a bicontinuous system, by contrast, the relevant value is the modulus of the network formed by association between the granules, which can be readily determined by experiment if the starch concentration is known. Since, as shown in Fig. 1b, 1 g of WMS swells to occupy a volume of 12.0 ml, irrespective of the total starch concentration (at least within the range 0–5 wt%), the effective concentration of WMS in the composites remains constant at 1/12 g ml^{-1} ($\approx$ 8.33 wt%).

Fig. 8a shows the mechanical spectrum obtained for a sample of WMS at this concentration, after treatment identical to that used for the mixed systems (dispersed at 45°C; gelatinized for 1 h at 80°C; loaded at 45°C; quenched,

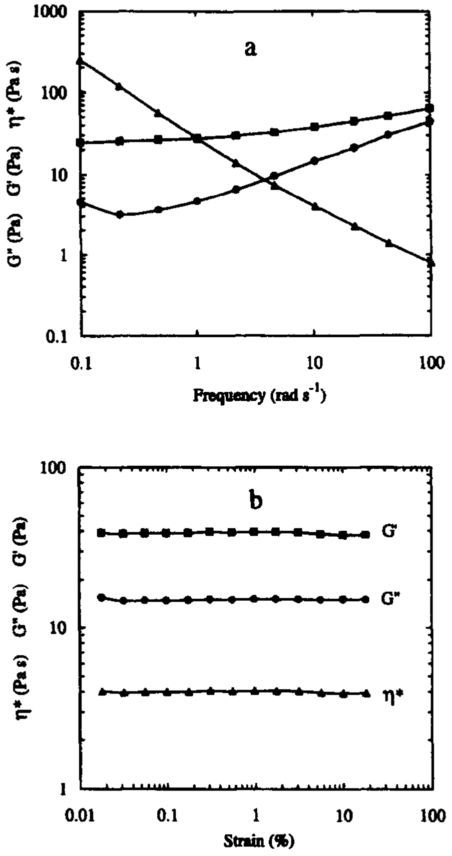

Fig. 8. Variation in G' (■), G'' (●) and η^* (▲) with (a) frequency at a fixed strain of 0.05% and (b) strain at a fixed frequency of 10 rad s^{-1} for 8.33 wt% WMS gelatinized (in water) for 45 min at 80°C, loaded onto the rheometer at 45°C, quenched to 5°C, and held at 5°C until stable moduli were attained.

and equilibrated at 5°C). The spectrum is predominantly gel-like, but with smaller separation of G' and G'' (i.e. higher tan δ) and greater frequency-dependence of both moduli than for typical biopolymer networks. As show in Fig. 8b, there is no detectable strain-dependence over the range accessible on the instrument used (~0.02% to ~20%). The observed value of G' at $10\,\mathrm{rad\,s^{-1}}$ and 0.05% strain (i.e. the conditions used in characterization of the agarose–WMS composites) is 38 Pa, which was therefore taken as the relevant value of G'_Y for the bicontinuous-network analysis.

The other parameters were calculated, as before, from the swelling volume of $12.0\,\mathrm{ml\,g^{-1}}$ for WMS (giving ϕ_X and ϕ_Y) and the concentration-dependence of G' for agarose alone (giving G'_X). The resulting values of G'_C from Eq. (8) are shown in Fig. 9, in comparison with the corresponding values obtained (also using $G'_Y = 38$ Pa) from the isostrain and isostress models (Eqs. (4) and (5)) and with the observed moduli for the agarose–WMS composites. As discussed previously, the composite moduli at low concentrations of starch (1 and 2 wt% WMS) follow the increase of G' of the agarose phase, consistent with dispersion of individual granules within a continuous agarose matrix. At higher starch concentrations, however, the observed moduli drop below those calculated by the isostrain model (Eq. (4)), and at 4 and 5 wt% WMS fall into almost perfect agreement with the values calculated for a bicontinuous network by application of Eq. (8). It must be emphasized that this fit does not involve the use of any adjustable parameters: ϕ_X, ϕ_Y, G'_X and G'_Y are all derived experimentally.

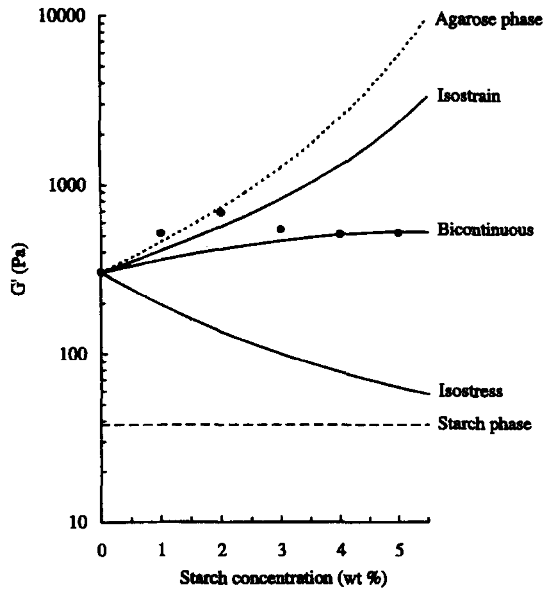

Fig. 9. Observed values (●) of G' ($10\,\mathrm{rad\,s^{-1}}$; 0.05% strain; 5°C) for composites of WMS gelatinized in 0.25 wt% agarose, in comparison with calculated values (——) obtained by application of the isostrain and isostress blending laws (Eq. (4) and Eq. (5)), and of the relationship for a bicontinuous network (Eq. (8)), using values of G'_X for the agarose phase (- - -) derived from the swelling volume of $12.0\,\mathrm{ml\,g^{-1}}$ for WMS and the concentration-dependence of G' for agarose alone (Eq. (1)), and a fixed value of $G'_Y = 38$ Pa (– – –) determined experimentally (Fig. 8) for WMS at the concentration of the starch phase in the composite gels (8.33 wt%).

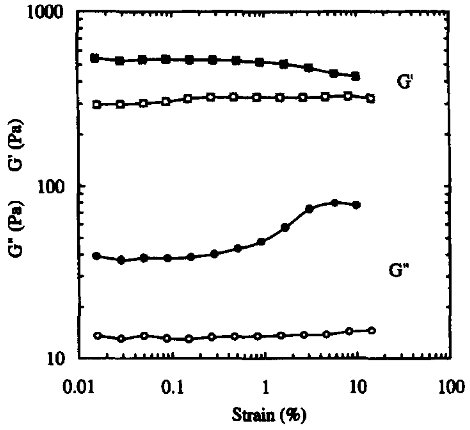

Fig. 10. Strain-dependence of G' (squares) and G'' (circles), measured at $10\,\mathrm{rad\,s^{-1}}$ and 5°C, for 0.25 wt% agarose alone (open symbols) and in combination with 5 wt% gelatinized WMS (filled symbols).

The remaining results concern the rheological consequences of bicontinuity and the range of WMS concentrations over which it occurs. Fig. 10 shows the strain-dependence of G' and G'' for 0.25 wt% agarose quenched from 45°C and equilibrated at 5°C in the presence and absence of 5 wt% gelatinized WMS. As found previously (Mohammed et al., 1998b), there is no systematic change in moduli for agarose alone over the 3 decades of strain accessible on the rheometer used. With 5 wt% WMS present, however, the moduli depart from linearity at ~0.3% strain, with G' decreasing steadily and G'' rising sharply and then passing through a shallow maximum, as expected for progressive disruption of network structure. This pronounced strain-dependence for agarose–WMS is in marked contrast to the behaviour of the corresponding agarose–PCS composites, where the moduli remained constant throughout the accessible strain-range (Mohammed et al., 1998b).

Fig. 11 shows the strain-dependence observed for 0.25 wt% agarose in the presence of other concentrations

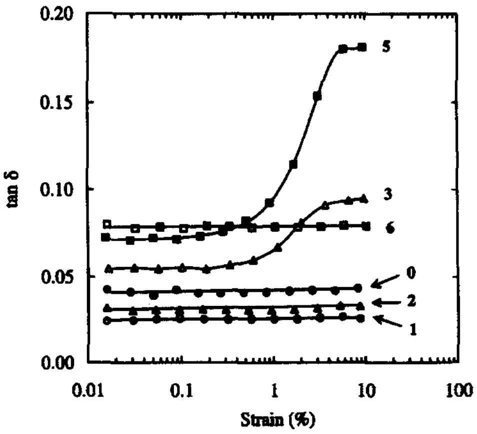

Fig. 11. Strain-dependence of tan δ ($10\,\mathrm{rad\,s^{-1}}$; 5°C) for 0.25 wt% agarose alone (●) and in combination with gelatinized WMS at concentrations of 1 (○), 2 (▲), 3 (△), 5 (■) and 6 (□) wt%.

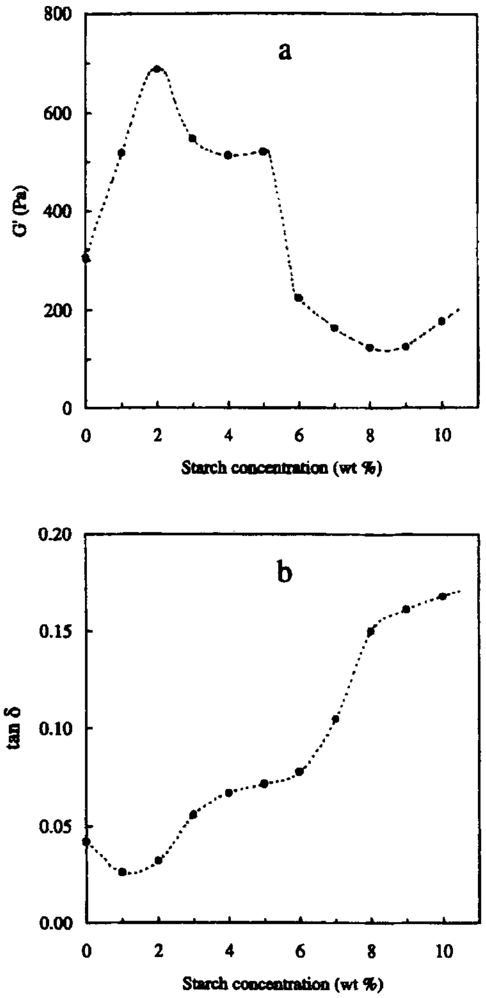

Fig. 12. Variation in (a) G' and (b) tan δ (10 rad s^{-1}; 0.05% strain; 5°C) with starch concentration in composite gels of 0.25 wt% agarose with gelatinized WMS.

Table 1
Rheological parameters (10 rad s^{-1}; 0.05% strain; 5°C) for composites of gelatinized WMS with 0.25 wt% agarose

[WMS] (wt%)	G' (Pa)	tan δ
0	305	0.042
1	518	0.026
2	688	0.032
3	546	0.056
4	512	0.067
5	520	0.072
6	223	0.078
7	163	0.105
8	123	0.150
9	124	0.161
10	177	0.168

of gelatinized WMS, using tan δ (G''/G') as a convenient single index of changes in G' and G''. At WMS concentrations up to 2 wt%, tan δ shows no detectable strain-dependence; at 3–5 wt% there is obvious evidence of structural breakdown at strains above ~0.3%; on further increase in WMS concentration to 6 wt%, however, the response again becomes independent of strain.

In the final stage of the investigation, the range of WMS concentrations studied was extended to 10 wt%. Fig. 12 shows the observed values (Table 1) of G' and tan δ (measured at 0.05% strain; i.e. in the low-strain linear viscoelastic region in Fig. 11) for composites with 0.25 wt% agarose across this range of starch concentrations. The initial effect of incorporation of small amounts of WMS is to increase G' and decrease tan δ (i.e. the overall structure becomes stronger and more gel-like), which can be explained by the increase in agarose concentration due to transfer of water to the starch granules. There is then a sharp reduction in G', between 2 and 3 wt% WMS (Fig. 12a), with an accompanying increase in tan δ (Fig. 12b) and, as shown in Fig. 11, development of pronounced strain-dependence (i.e. the composites become weaker, less gel-like, and more fragile). As discussed above, these changes appear to be associated with a transition from a dispersion of individual gelatinized granules in a continuous agarose matrix to a bicontinuous composite, with the starch granules linking together into a second continuous network.

When the WMS concentration is increased above ~5 wt%, there is a second sharp reduction in G', which continues to ~8.3 wt%, with an accompanying increase in tan δ. The beginning and end of this range correspond, roughly, to the point at which the granules will become close-packed ($\phi_Y \approx 0.72$ at 6 wt% WMS) and the point at which their combined volume (for full swelling; Fig. 1b) will become equal to the total volume of the system (at $c_Y = 8.33$ wt%). The onset of close-packing appears to have the effect of bringing the composite rheology towards that of the starch component. At WMS concentrations of 6 wt% and above, the strain-sensitivity seen in the biphasic region (from ~3–5 wt%) is lost, with the moduli again becoming constant at all accessible strains, as observed (Fig. 8b) for WMS alone at its effective concentration within the composites (8.33 wt%), and G' and tan δ approach (but do not reach) the corresponding values for 8.33 wt% alone ($G' = 38$ Pa; tan $\delta = 0.38$). The appearance of the gels also changes within this range: at 5 wt% WMS the composites have the glossy appearance and smooth surface typical of agarose alone; by 7 wt% (and above) they have the creamy colour and sticky feel found for gels of WMS alone. Finally, at starch concentrations above ~8.33 wt%, where the degree of swelling will be restricted by availability of water, there is a sharp up-turn in G' (Fig. 12a), consistent with a simple increase in the effective concentration of WMS.

However, although it is evident that the overall rheology of the composite gels becomes progressively dominated by the starch component as WMS concentration is increased across the range of 6–10 wt%, enzymic-digestion experiments of the type described previously (immersion of gel plugs in α-amylose solution at 100-fold dilution and 45°C) showed clear evidence of a residual agarose network after

Table 2
Overview of rheological properties of biopolymer–starch composites

Biopolymer	Starch type	Dominant component	Starch concentration range (wt%)	Reference
Gelatin	PCS	Biopolymer	1–5	Abdulmola et al., 1996a
	WMS	Biopolymer	1–5	
Xanthan	PCS	Neither (bicontinuous)	1–5	Abdulmola et al., 1996b
	WMS	Starch	1–5	
Agarose	PCS	Biopolymer	1–5	Mohammed et al., 1998b
Agarose	WMS	Biopolymer	1–2	This work
		Neither (bicontinuous)	3–5	
		Starch	6–10	

extensive hydrolysis of starch. In contrast to the behaviour observed at 5 wt% WMS, where the sample retained its original cylindrical shape, however, the remnants at higher starch concentration were tenuous and fragile, and disintegrated into fragments with a flake-like appearance on gentle agitation of the container.

4. General discussion and conclusions

This is the fourth in a series of related publications on the rheology of the composites formed by gelatinization of crosslinked and uncrosslinked waxy maize starch in a biopolyer matrix. The results of the present study, and of the three preceding investigations (Abdulmola et al., 1996a,b; Mohammed et al., 1998b) are outlined in Table 2.

The overall experimental strategy was based on the generally accepted conclusion that interactions between swollen starch granules become significant only when the system approaches close-packing (e.g. Evans and Haisman, 1979; Wong and Lelievre, 1981; Eliasson, 1986; Steeneken, 1989; Evans and Lips, 1992). It was therefore assumed that if samples of lower concentration were gelatinized in a biopolymer solution, which was then gelled, the individual granules would remain as separate particles dispersed through the surrounding gel matrix, thus providing simple model systems that could be used to test procedures adopted in analysis of the rheology of phase-separated biopolymer co-gels.

This expectation was fulfilled in the first investigation in the series (Abdulmola et al., 1996a), where gelatin was used as matrix biopolymer, in combination with both PCS and WMS, and in the study of agarose–PCS composites reported in the paper immediately preceding this one (Mohammed et al., 1998b). For all three systems, the overall moduli of the composites remained close to the modulus of the biopolymer phase (isolated by centrifugation in the pre-gel solution state), and the small differences in modulus between matrix and composite could be matched with good precision by application of the isostrain and isostress blending laws (Eqs. (4) and (5)).

When xanthan was used as matrix biopolymer (Abdulmola et al., 1996b), however, the composite moduli could not be rationalized in the same way; the observed ratios of G'_C/G'_X were much larger than the maximum values anticipated (Eq. (7)) for hard 'filler' particles in a weaker continuous matrix. Another surprising finding from the same investigation was that both starch samples (after gelatinization in water at 80°C) gave gel-like mechanical spectra at concentrations well below the expected onset of close-packing (at $\phi_Y < 0.2$), with the tendency to network formation being particularly pronounced for WMS ($c_0 \approx 1$ wt%; $\phi_Y \approx 0.1$). It was therefore tentatively suggested that the swollen granules are capable of associative interactions, and that these are promoted by the presence of xanthan, possibly through a depletion–flocculation mechanism. However, in view of the apparent success of the bicontinuous-network model (Eq. (8)) in rationalizing the behaviour of agarose–WMS composites at intermediate starch concentrations (~3–5 wt%; Fig. 9), we have explored the applicability of the same model to the xanthan–starch composites studied by Abdulmola et al. (1996b), using the swelling volumes and modulus-concentration relationships determined in the original investigation.

The swelling volumes reported for the starch samples used by Abdulmola et al. (1996b) were 9.0 ml g^{-1} for PCS and 9.65 ml g^{-1} for WMS, giving effective starch concentrations of ~11.1 and ~10.4 wt%, respectively. The values of starch modulus (G'_Y) were then obtained from the linear relationships reported between log G' and log c (Eqs. (9) and (10)) for PCS and WMS at concentrations of ~1 wt% and above (giving ~3.3 kPa for PCS and ~61 Pa for WMS), and the modulus of the xanthan phase (G'X) was derived from the swelling volumes and the reported linear relationship (Eq. (11)) between log G' and log c for xanthan.

$$\text{PCS} \quad : \log G' = 4.15 \log c - 0.82 \tag{9}$$

$$\text{WMS} \quad : \log G' = 1.15 \log c + 0.62 \tag{10}$$

$$\text{XANTHAN} : \log G' = 2.258 \log c + 1.612 \tag{11}$$

The resulting values of ϕ_X, ϕ_Y, G'_X and G'_Y were then used to obtain calculated values of the composite modulus (G'_C)

from the isostrain, isostress and bicontinuous models (Eqs. (4), (5) and (8), respectively).

As shown in Fig. 13, the bicontinuous-network model gives a convincing fit to the observed moduli of the xanthan–PCS composites at both concentrations of xanthan studied by Abdulmola et al. (1996b): 0.25 wt% (Fig. 13a) and 0.50 wt% (Fig. 13b). It should again be noted that, as in the case of the agarose–WMS data shown in Fig. 9, the agreement between observed and calculated values of G'_C comes entirely from experiment, with no adjustable parameters. The observed moduli for the xanthan–WMS composites, by contrast, are consistently higher (Fig. 14) than the values calculated for a bicontinuous network, and at high concentrations of starch they converge on the modulus of WMS alone at its effective local concentration in the mixed systems, indicating that, as proposed originally (Abdulmola et al., 1996b), the overall rheology is dominated by the starch component (Table 2).

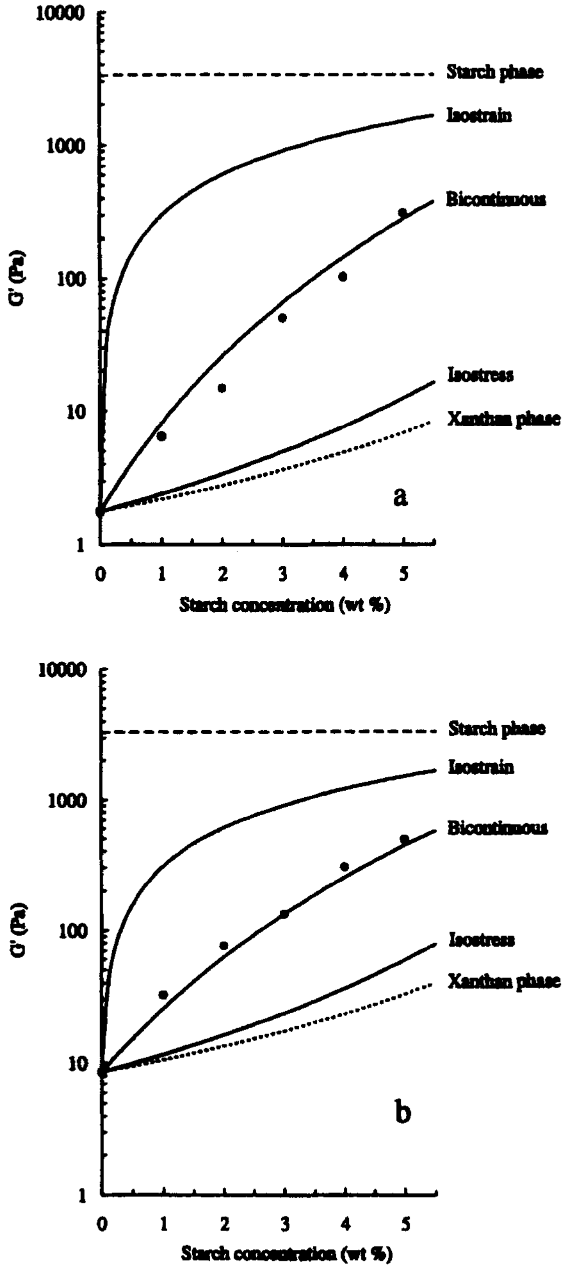

Fig. 13. Observed values (●) of G'_C for composites of gelatinized PCS with (a) 0.25 wt% and (b) 0.50 wt% xanthan, in comparison with calculated values (—) obtained from the individual moduli of the starch (– – –) and xanthan (- - -) phases by application of the isostrain, isostress and bicontinuous-network relationships (Eqs. (4), (5) and (8), respectively). The data are taken from Abdulmola et al. (1996b) and were analysed in the same way as the results for agarose–WMS from the present work (Fig. 9).

As discussed previously, the agarose–WMS composites studied in the present work appear to show all three types of behaviour seen for the other starch–biopolymer systems. At low concentrations of WMS (1–2 wt%) the overall moduli are dominated by the biopolymer component, as found for gelatin–PCS, gelatin–WMS and agarose–PCS. At intermediate concentrations (3–5 wt% WMS) the composite moduli can be fitted by the bicontinuous-network model (Eq. (8)), as found (Fig. 13) for xanthan–PCS. At higher concentrations (~6 wt% and above) starch becomes the dominant component, as indicated in Fig. 14 for xanthan–WMS.

With hindsight, the surprising feature of the results summarized in Table 2 is not that some of the systems show departures from the initial expectation of individual granules dispersed in a biopolymer matrix, but that this topology occurs at all, except at very low concentrations of starch. In the investigation by Abdulmola et al. (1996b), gelatinization of PCS and WMS was studied by small-deformation oscillatory measurements. The heating curve shown for starch alone in Fig. 7 is typical of the behaviour observed for WMS; for PCS the reduction in modulus after the initial sharp increase was virtually undetectable, consistent with reinforcement of the granule structure by chemical crosslinking. In both cases, however, the moduli remained essentially constant on cooling from 80 to 5°C. Thus the starch networks characterized at low

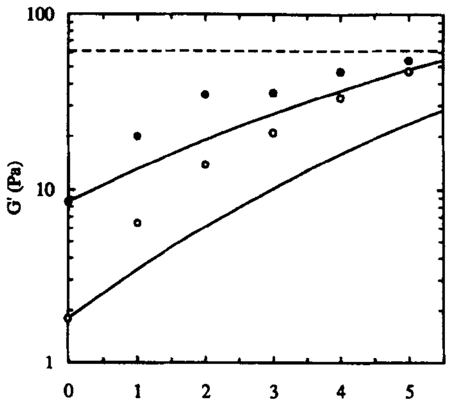

Fig. 14. Observed values (Abdulmola et al., 1996b) of G'_C for composites of gelatinized WMS with 0.25 (○) and 0.50 (●) wt% xanthan, in comparison with calculated values (——) derived for a bicontinuous network by application of Eq. (8), and with the modulus of WMS alone (– – –) at its effective concentration in the mixed systems.

temperature are already present at high temperature, immediately after gelatinization, and at that stage the interstices of the gel are presumably filled by the dissolved biopolymer. It might therefore be expected that gelation of the biopolymer component on cooling would give a bicontinuous co-gel, by formation of a second network permeating the original starch gel.

In practice, however, bicontinuity appears to occur only for a narrow range of starch concentrations in the agarose–WMS mixtures studied in the present work, and for the xanthan–PCS systems characterized by Abdulmola et al. (1996b), where, under the ionic conditions used, the xanthan 'weak gel' network (Ross-Murphy et al., 1983) was already present at 80°C. Thus in the systems where the gelled biopolymer becomes the sole continuous phase, the starch network present at high temperature must be dissociated during the gelation process. Conversely, emergence of starch as the dominant component implies inhibition of network formation by the biopolymer. Mutual inhibition of network formation by the two constituents (presumably due to topological constraints) is indicated by the strain-sensitivity of the agarose–WMS composites formed in the bicontinuous region (Fig. 11), and by the fragile nature of the agarose networks remaining after after enzymic hydrolysis of the starch component at higher concentrations of WMS.

We suggest, tentatively, that the different outcomes of co-gelation (Table 2) may be the result of two competing trends. First, there may be an enthalpic advantage from dissociation of comparatively tenuous associations between swollen granules (Fig. 8a) to allow more complete formation of a biopolymer network crosslinked by stronger junctions. As suggested by Abdulmola et al. (1996b), however, the presence of the biopolymer may promote starch–starch interactions by depletion flocculation. If this interpretation is correct, the balance between the two opposing effects would depend on the ease of dissociation of the starch network present initially (i.e. the strength of interaction between the constituent granules), and on the effectiveness of the biopolymer in promoting flocculation.

At starch concentrations above ~3 wt%, the values of G' observed (Abdulmola et al., 1996b) for PCS (gelatinized in water at 80°C) rise steeply above the corresponding values for WMS, consistent with greater rigidity of the constituent granules (due to the internal crosslinking introduced by chemical modification). At lower concentrations, however, the moduli for WMS exceed those for PCS, indicating that the unmodified granules have a greater tendency to self-association. This may explain why, in systems where the two materials show qualitative differences in behaviour, the changes observed (Table 2) on going from PCS to WMS are in the direction of a greater contribution from the starch component to the overall rheology of the composites (from bicontinuous to starch-dominated in composites with xanthan, and from biopolymer-dominated to bicontinuous or starch-dominated in composites with agarose).

The differences in behaviour observed on changing the biopolymer component while holding starch-type constant are consistent with the idea of enhancement of granule–granule association by depletion flocculation. The relative effectiveness of different biopolymers in inducing flocculation will depend (e.g. Walstra, 1993) on the volume swept out by the individual chains (or assemblies of chains), and this in turn will depend on molecular weight and stiffness. Gelatin (used in the studies by Abdulmola et al., 1996a) has a comparatively low molecular weight ($\sim 10^5$ for individual α-chains) and, like other denatured proteins, a relatively flexible (and hence compact) chain geometry in solution. Xanthan (Abdulmola et al., 1996b) has a high molecular weight (typically $\sim 2 \times 10^6$) and, in its ordered conformation, has an extremely high persistence length. Agarose (used by Mohammed et al., 1998b, and in the present work) is intermediate in both flexibility and molecular weight (Stanley, 1995). We would therefore expect their effectiveness in promoting association of gelatinized starch granules to increase in the order: gelatin < agarose < ordered xanthan.

As shown in Table 2, the observed behaviour of the mixed systems follows the same progression. With PCS as the starch component, the composites formed with gelatin and agarose are biopolymer-continuous, but with xanthan they become bicontinuous (Fig. 13), indicating survival of the starch–starch associations formed on gelatinization. With the (evidently more adhesive) unmodified WMS as starch component, there is a progressive change from biopolymer-dominated to starch-dominated rheology on increasing the expected effectiveness of the biopolymer component in inducing flocculation of starch, with the agarose–WMS composites occupying the intermediate position, as expected, and showing all three types of topology at different ranges of starch concentration. Although this interpretation is qualitative and speculative, it does appear to offer a unified framework for interpretation of the overall rheology of starch–biopolymer composites.

Finally, we propose a tentative explanation for the results shown in Fig. 5, where it was found that if a mixture of agarose (0.25 wt%), gelatinized WMS (5 wt%) and α-amylase (at the higher of the two concentrations used) was held for 10 min at 45°C prior to quenching, it gave a higher modulus than the same preparation quenched to 5°C immediately after mixing. We suggest that, as discussed above, the mixture is initially bicontinuous; on immediate quenching the bicontinuous structure is locked in by rapid gelation of agarose, but the modulus of the starch component (G'_Y) is severely reduced by rapid hydrolysis of amylopectin at the very high concentration of enzyme used (quantitatively, the value of G'_Y required to give exact agreement with the observed composite modulus of $G'_C = 190$ Pa is ~1 Pa); on holding for 10 min at 45°C, however, the starch component is reduced to short oligomers, leaving a single-phase solution which, as observed (Fig. 5), then forms a gel with a modulus close to that of agarose alone.

Acknowledgements

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References

Abdulmola, N.A., Hember, M.W.N., Richardson, R.K., & Morris, E.R. (1996a). Application of polymer blending laws to starch–gelatin composites. *Carbohydr. Polym.*, *31*, 53–63.

Abdulmola, N.A., Hember, M.W.N., Richardson, R.K., & Morris, E.R. (1996b). Effect of xanthan on the small-deformation rheology of crosslinked and uncrosslinked waxy maize starch. *Carbohydr. Polym.*, *31*, 65–78.

Clark, A.H., & Ross-Murphy, S.B. (1987). Structural and mechanical properties of biopolymer gels. *Advan. Polym. Sci.*, *83*, 57–192.

Davies, W.E.A. (1971). The theory of elastic composite materials. *J. Phys. D. Appl. Phys.*, *4*, 1325–1339.

Eliasson, A.-C. (1986). Viscoelastic behaviour during the gelatinization of starch. *J. Text. Stud.*, *17*, 253–265.

Evans, I.D., & Haisman, D.R. (1979). Rheology of gelatinised starch suspensions. *J. Text. Stud.*, *10*, 347–370.

Evans, I.D., & Lips, A. (1992). Viscoelasticity of gelatinized starch dispersions. *J. Text. Stud.*, *23*, 69–86.

Mohammed, Z.H., Hember, M.W.N., Richardson, R.K., & Morris, E.R. (1998a). Kinetic and equilibrium processes in the formation and melting of agarose gels. *Carbohydr. Polym.*, *36*, 15–26.

Mohammed, Z.H., Hember, M.W.N., Richardson, R.K., & Morris, E.R. (1998b). Application of polymer blending laws to composite gels of agarose and crosslinked waxy maize starch. *Carbohydr. Polym.*, *36*, 27–36.

Morris, E. R. (1984). Rheology of hydrocolloids. In G.O. Phillips, D.J. Wedlock and P.A. Williams (Eds), *Gums and Stabilisers for the Food Industry 2* (pp. 57–78). Oxford: Pergamon Press.

Piculell, L., Nilsson, S., & Muhrbeck, P. (1992). Effect of small amounts of kappa-carrageenan on the rheology of aqueous iota-carrageenan. *Carbohydr. Polym.*, *18*, 199–208.

Ross-Murphy, S.B., Morris, V.J., & Morris, E.R. (1983). Molecular viscoelasticity of xanthan polysaccharide . *Faraday Symp. Chem. Soc.*, *18*, 115–129.

Stanley, N.F. (1995). Agars. In A. M. Stephen. (Ed.), *Food Polysaccharides and their Applications* (pp. 187–204). New York: Marcel Dekker.

Steeneken, P.A.M. (1989). Rheological properties of aqueous suspensions of swollen starch granules. *Carbohydr. Polym.*, *11*, 23–42.

Takayanagi, M., Harima, H., & Iwata, Y. (1963). Viscoelastic behaviour of polymer blends and its comparison with model experiments. *Mem. Fac. Eng. Kyusha Univ.*, *23*, 1–13.

Walstra, P. (1993). Introduction to aggregation phenomena in food colloids. In E. Dickinson (Ed.), *Food Colloids and Polymers: Stability and Mechanical Properties*, Special Publication No. 113 (pp. 3–15). Cambridge, UK: The Royal Society of Chemistry.

Wong, R.B.K., & Lelievre, J. (1981). Viscoelastic behaviour of wheat starch pastes. *Rheol. Acta*, *20*, 299–307.