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Application of polymer blending laws to composite gels of agarose and crosslinked waxy maize starch

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Abstract

Composites comprising swollen granules of highly crosslinked waxy maize starch in an agarose gel matrix have been prepared in two ways: (i) by gelatinising the starch in agarose solution at 80°C and then cooling to form the gel network, or (ii) by cooling to gel the agarose and then heating to gelatinise the starch. In the first procedure, phase volumes were derived by using centrifugation to obtain a sample of the agarose phase prior to gelation, and determining the increase in polymer concentration due to swelling of the starch granules. The storage modulus (G') of the agarose phase was obtained by gelling the supernatant from centrifugation, under identical time–temperature conditions to those used for the unseparated composite, and the value of G' for the swollen granules was derived by the 'model independent' method of varying polymer concentration to determine compositions at which both phases (and hence the overall composite) have the same modulus. Measured values of G' for the composite gels agreed, to within experimental error, with values calculated by application of the Takayanagi blending laws, with no adjustable parameters, using the isostress model for systems in which the continuous matrix was weaker than the dispersed particles and the isostrain model for the converse situation (weak filler in strong matrix). For composites formed by the second procedure, the increase in modulus of the continuous phase due to swelling of starch granules within the agarose gel network was calculated by classic swelling/deswelling theory, and the resulting values of G' obtained by application of the polymer blending laws were again in close agreement with those observed experimentally. © 1998 Elsevier Science Ltd. All rights reserved

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1. Introduction

Gels obtained from mixtures of two different biopolymers often have a biphasic structure, induced by segregative interactions (thermodynamic incompatibility) between the constituent polymers (see for example Suchkov et al., 1981; Walkenström and Hermansson, 1994; Picullel et al., 1995; Tolstoguzov, 1995; Morris, 1998). In some systems phase separation occurs in the pre-gel solution state (e.g. Chronakis et al., 1996) or during cooling (e.g. Alevisopoulos et al., 1996); in others, one component gels first, from a single-phase solution, and the other then forms a second (dispersed) phase by gelling within the pores of the original (continuous) network (e.g. Clark et al., 1982; Clark et al., 1983; Kasapis et al., 1993). The ultrastructure of the resulting co-gels can vary widely, depending on the time–temperature course of gelation in relation to the rates of segregation and network formation (e.g. Alevisopoulos et al., 1996), but a common outcome is for one phase to be distributed through the other in approximately spherical inclusions with diameters in the range ~10–100 μm (e.g. Kasapis et al., 1993; Foster et al., 1996), which is comparable to the size of starch granules after gelatinisation (see for example Fannon et al., 1992). The aim of the present work was to generate composites with this type of simple, well-defined, ultrastructure by gelatinisation of starch in a biopolymer matrix, and to compare their overall strength (rigidity modulus) with values calculated using the Takayanagi isostrain and isostress blending laws. To minimise contamination of the matrix by starch polysaccharides, a highly crosslinked waxy maize starch was used, and gelatinisation was carried out at the minimum temperature required to obtain complete disordering of amylopectin (80°C; Abdulmola et al., 1996b).

In the original development of their blending laws, Takayanagi et al. (1963) used sandwich structures with thin layers of two different synthetic polymers arranged either in parallel or in series relative to the direction of deformation. In the parallel arrangement, the deformation of the weaker component is limited by the rigidity of the stronger material, so that both components are deformed to

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the same extent (isostrain conditions). In the series arrangement, the strength of the weaker component limits the force transmitted to the stronger material, so that both are subjected to the same stress (isostress conditions). The resulting relationships between the moduli of the individual constituents and the overall modulus of the composite are strictly valid only for these perfect series and parallel arrangements, but they are expected (Manson and Sperling, 1976) to apply reasonably well to composites with 'filler' particles (phase Y) dispersed through a continuous matrix (phase X). If the matrix is stronger than the filler, the overall resistance (modulus) of the composite (G_C) should approximate to the weighted-average of the individual moduli (G_X and G_Y):

$$G_C = G_X\phi_X + G_Y\phi_Y \quad \text{if } G_X > G_Y \tag{1}$$

where ϕ_X and ϕ_Y denote the volume-fractions (phase volumes) of, respectively, the continuous and discontinuous phases (with $\phi_X + \phi_Y = 1$). If the matrix is weaker than the filler, the overall deformation (compliance) of the composite ($J_C = 1/G_C$) should approximate to the weighted-average of the individual compliances:

$$J_C = J_X\phi_X + J_Y\phi_Y$$

i.e.

$$1/G_C = \phi_X/G_X + \phi_Y/G_Y \quad \text{if } G_X < G_Y \tag{2}$$

The first application of the Takayanagi models to biopolymer co-gels was by Dr. A.H. Clark and his colleagues in Unilever Research. A central problem faced was that the values of ϕ_X and ϕ_Y for biphasic aqueous gels are determined by the partition of solvent between the phases, and can no longer be equated to the relative proportions of the polymeric constituents. The approach adopted (Clark et al., 1983; Clark, 1987) was to assume complete segregation of the two components into their respective phases, and to introduce an empirical parameter, p, defined as the ratio of solvent/polymer in phase X divided by the corresponding ratio in phase Y, to characterise their relative 'solvent avidities'. The experimental moduli of the composite gels were then compared with upper and lower bounds calculated by, respectively, the isostrain and isostress equations for a range of trial values of p.

In a later extension of the same basic approach, Morris (1992) suggested using calculated moduli for all possible distributions of solvent (i.e. from $\phi_X = 0$ to $\phi_X = 1$) to determine the value of p required to give best agreement with experimental results, on the assumption that the isostrain (Eq. (1)) and isostress (Eq. (2)) models give precisely the correct overall moduli for composites in which the dispersed phase is, respectively, weaker or stronger than the continuous phase. This procedure was first applied to gelatin–maltodextrin co-gels (Kasapis et al., 1993) and gave good agreement between observed and calculated moduli for two different molecular weights of maltodextrin, and a wide range of polymer concentrations and mixing ratios, using a single value of p. Subsequent applications include characterisation of kappa carrageenan in combination with soy or pea protein concentrates (Ipsen, 1995) and of maltodextrins in combination with milk protein concentrate (Chronakis et al., 1996), sodium caseinate (Manoj et al., 1996) or whey protein concentrate (Manoj et al., 1997).

Use of this method of exploring solvent partition in mixed gels, however, depends on two untested and, at first sight, implausible assumptions: (i) complete segregation of the two polymers, and (ii) strict compliance with the isostrain and isostress blending laws. The first of these assumptions seems to conflict with known behaviour of phase-separated biopolymer solutions (e.g. Morawetz, 1965; Tolstoguzov et al., 1974; Suchkov et al., 1981; Tolstoguzov, 1986; Morris, 1990; Picullel et al., 1995), where each phase includes both polymers in relative proportions defined by a binodal (or 'cloud-point curve'), which represents the optimum balance (i.e. state of minimum free energy) between the enthalpic advantage of further segregation and the entropic advantage of residual mixing. For gelling systems, however, the gelation process may cause major departures from the phase relationships that exist in the pre-gel solution state. In particular, in the early stages of gelation, after the onset of intermolecular association but before development of a continuous network, there will be a massive reduction in the number of species free to move independently, which will reduce the relative importance of entropy of mixing and hence promote further segregation. Thus the assumption of complete de-mixing may not be entirely unrealistic, particularly for systems where the rate of gelation is slow enough to allow redistribution of solvent and polymer between the phases in response to changes in thermodynamic equilibria. The present investigation, however, focusses on the second assumption, of strict applicability of the Takayanagi blending laws.

The idea of using swollen starch granules as a simple analogue for the dispersed phase in biopolymer co-gels was first explored by Abdulmola et al. (1996a). In outline, the procedure adopted was to gelatinise starch in a solution of gelatin, and obtain a clear sample of the gelatin phase by sedimentation of the swollen granules. The gelatin supernatant and the unseparated composite were then allowed to gel under identical time–temperature conditions, and characterised by small deformation oscillatory measurements of storage modulus (G'). The increase in gelatin concentration due to swelling of the starch during gelatinisation was determined from the concentration dependence of G' for gelatin alone, thus yielding the phase volumes of the two components, as well as the modulus of the continuous (gelatin) phase.

The volume of the starch phase (ϕ_Y) was found to increase in direct proportion to the amount of starch present (0–5 wt%), irrespective of gelatin concentration (0.88–1.50 wt%), and in a subsequent investigation (Abdulmola

et al., 1996b) the same swelling volumes were obtained for samples gelatinised in solutions of xanthan (0.25 and 0.50 wt%) and in water. It would therefore appear that the phase volumes in starch–biopolymer composites are dictated solely by the swelling behaviour of the starch component, with no evidence of any significant competition from the 'solvent avidity' of the biopolymer (at least over the range of polymer concentrations studied so far). Also, the close agreement between phase volumes determined using the supernatants from mixtures of different polymer concentration argues against any significant penetration of polymer into the starch granules. Thus complete segregation, invoked as a necessary working assumption in analysis of biopolymer co-gels, appears to be rigorously valid for starch–polymer composites, and the need to characterise phase volumes by a variable parameter (p) is also eliminated by experimental determination of swelling volume, giving a much more reliable starting point for exploring the errors introduced by assuming that the moduli of composite gels with a dispersed phase of spherical particles embedded in a continuous matrix can be calculated exactly by the isostrain and isostress equations.

In the present work, we have used essentially the same experimental procedure as in the previous investigation by Abdulmola et al. (1996a), but the results are analysed in a rather different way. Since the moduli of the composite and polymer matrix (G_C and G_X) and the phase volumes (ϕ_X and ϕ_Y) are determined experimentally, the only unknown parameter in Eq. (1) and Eq. (2) is the modulus of the starch granules (G_Y). The approach adopted by Abdulmola et al. (1996a) was to use a minimisation routine to determine the value of G_Y required to give the best root-mean-square fit between observed and calculated value of log G_C. The method used here was to find combinations of polymer and starch concentrations at which both phases have the same modulus, giving $G_C = G_X = G_Y$ irrespective of the model used, and to use the resulting value of G_Y to compare observed values of G_C with those calculated using the isostrain equation (for $G_X > G_Y$) or isostress equation (for $G_X < G_Y$).

The other distinctive feature of the present work is the use of agarose as matrix biopolymer. The massive thermal hysteresis between the setting and melting temperatures of agar/agarose gels (e.g. Guisley, 1970) has been exploited in previous studies of co-gelation with heat-setting proteins (Clark et al., 1982; McEvoy et al., 1985) to change the order of formation of the constituent networks (i.e. cooling to gel the polysaccharide then heating to gel the protein, or vice versa). In most of our experiments the starch was gelatinised in agarose solution (at 80°C) and the composite gels were formed by cooling. A comparative study, however, was made of the effect of gelatinisation of starch within a preformed agarose gel, to explore the concept of 'network de-swelling' invoked (McEvoy et al., 1985; Kasapis et al., 1993) in previous investigations of biopolymer co-gels in which one component gels first and is then deprived of solvent ('deswollen') by subsequent gelation of the other component to form a second (dispersed) phase within the interstices of the existing network.

2. Materials and methods

The agarose sample used was from Sigma (type I-A; low EEO; lot number 084H0462). Phosphorus oxychloride crosslinked hydroxypropylated waxy maize starch was kindly supplied by Cerestar and, for brevity, will be denoted as PCS (phosphate-crosslinked starch). The sample used is of the same type (C* Cream 06716) as in the investigation of starch–gelatin composites by Abdulmola et al. (1996a), but from a different batch. The reagent levels used in preparation of PCS, expressed relative to the initial dry weight of starch, are ~0.05% $POCl_3$ and ~6.5% propylene oxide (Dr. A.A. Procter, Cerestar, personal communication) which, for full conversion, would correspond to ~1 crosslink per 1900 sugar residues and ~1 hydroxypropyl substituent per 5.5 residues (i.e. D.S. ≈ 0.18). All solutions were made using distilled deionised water. Agarose was dispersed in water at ~95°C and autoclaved (15 min; 120°C) to ensure complete dissolution.

Slurries of ungelatinised 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 gelatinisation temperature of PCS and well above the onset temperature for gelation of agarose). In most of the experiments, the starch was gelatinised 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. Selected 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 onto 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 0.5% 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.

For investigation of network deswelling, a slurry of ungelatinised PCS in agarose solution was loaded onto the rheometer at 45°C, cooled to 5°C at 1°C min^{-1}, held for 35 min to allow complete development of the agarose network, heated to 80°C at 1°C min^{-1}, held for 35 min to ensure complete gelatinisation, re-cooled to 5°C at 1°C min^{-1}, and held for a further period of 35 min, with measurement of G' (10 rad s^{-1}; 0.5% strain) throughout.

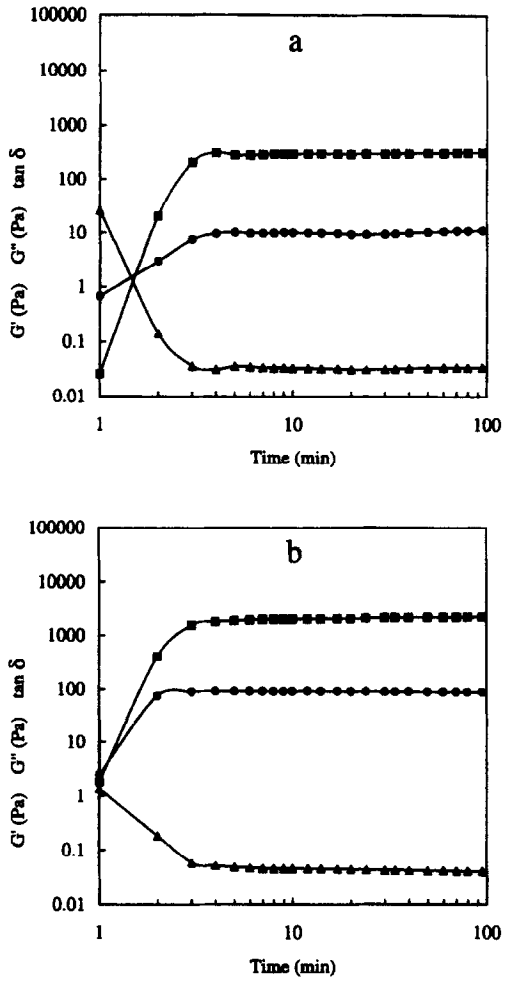

Fig. 1. Time course of network formation at 5°C after rapid quenching from 45°C for 0.25 wt% agarose (*a*) alone and (*b*) with 5 wt% gelatinised PCS, as monitored by measurement of G' (■), G'' (●) and tan δ (▲) at 10 rad s^{-1} and 0.5% strain

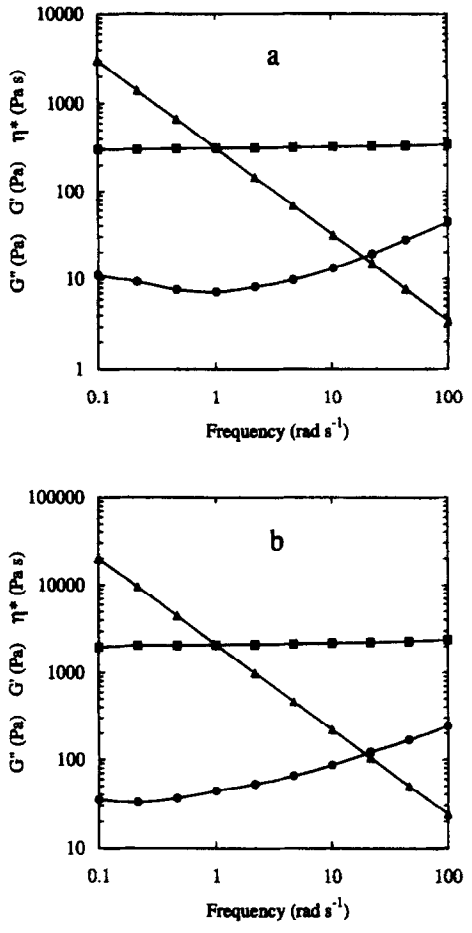

Fig. 2. Mechanical spectra (0.5% strain; 5°C) showing the frequency dependence of G' (■), G'' (●) and η^* (▲) for 0.25 wt% agarose (*a*) alone and (*b*) with 5 wt% gelatinised PCS, at the end of the holding period shown in Fig. 1 (after rapid quenching from 45°C)

Measurements were made using cone-and-plate geometry (50 mm diameter; 0.05 rad cone angle) on a sensitive prototype rheometer designed and constructed by one of us (R.K.R.). To circumvent problems of thermal expansion/contraction during heating and cooling, the cone was truncated over 45% of its diameter, giving a gap of 0.5 mm between the flat surfaces of the two elements, but keeping strain constant at a fixed, maximum, value across the outer portion (which constitutes 80% of the total area). The periphery of the sample was coated with light silicone oil to minimise evaporation of water. Temperature was controlled by a Haake circulating water bath and measured with a thermocouple attached to the stationary element. Data analysis was carried out using a standard Microsoft Excel spreadsheet package (Version 5.0).

3. Results

In the first series of experiments, agarose concentration was held constant at 0.25 wt% and PCS concentration was varied from 0 to 5 wt% in increments of 1 wt%. As described in Section 2, the mixtures were heated to 80°C to gelatinise the starch. The reason for choosing 5 wt% as the highest starch concentration was that at 6 wt% and above it proved impossible to obtain a useable quantity of clear supernatant on centrifugation of the gelatinised suspensions.

Fig. 1 shows the time course of structure development at 5°C after quenching from 45°C for the two extremes of this series, 0.25 wt% agarose alone, and 0.25 wt% agarose plus 5 wt% gelatinised PCS. In both cases, stable values of G' and G'' are attained within ~10 min. Fig. 2 shows the mechanical spectra (frequency dependence of G', G'' and η^*) recorded at the end of the holding period at 5°C, using a fixed strain of 0.5%. Both samples show typical gel-like response (see for example Morris, 1984; Clark and Ross-Murphy, 1987) and have similar values of tan δ (i.e. similar separation of log G' and log G''), but the moduli of the composite are ~7 times higher than those of agarose alone. As shown in Fig. 3, G' and G'' for both samples (measured at 10 rad s^{-1}) remain independent of the amplitude of oscillation up to the highest values attainable on the

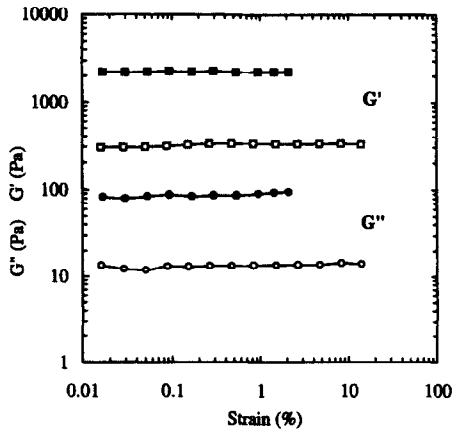

Fig. 3. Strain dependence of G' (squares) and G'' (circles) at 10 rad s^{-1} for 0.25 wt% agarose alone (open symbols) and with 5 wt% gelatinised PCS (filled symbols), recorded immediately after the mechanical spectra shown in Fig. 2

instrument used (~15% strain for 0.25 wt% agarose alone, and ~2% for the stronger, composite, material), demonstrating that the strain of 0.5% used to record the mechanical spectra in Fig. 2, and the setting curves in Fig. 1, is well within the linear viscoelastic region. Similar results were obtained for the other starch concentrations used (1, 2, 3 and 4 wt%).

The agarose supernatants obtained by centrifugation were gelled under identical conditions to the unseparated composites and, as shown in Fig. 4*a*, the final values of G' recorded at 5°C increased by about a factor of 10 as the starch concentration was raised from 0 to 5 wt% PCS. As reported elsewhere (Mohammed et al., 1998a), the variation of log G' with log c for agarose gels formed by quenching to 5°C can be fitted with good precision (for G' values spanning the range shown in Fig. 4*a*) by the simple linear relationships:

$$\log G' = 3.204 \log c + 4.414 \quad (3)$$

$$\log c = 0.312 \log G' - 1.377 \quad (4)$$

where concentration (c) is in wt% and G' is in Pa. Eq. (4) was used to determine the agarose concentrations (c_X) corresponding to the observed moduli of the gelled supernatants (Fig. 4*a*), and the agarose phase volumes (ϕ_X) were then obtained from the ratio of the initial and final polymer concentrations:

$$\phi_X = c_O/c_X \quad (5)$$

where c_O is the initial concentration of agarose prior to swelling of the starch granules.

As found in the previous investigations of starch–polymer composites by Abdulmola et al. (1996a,b), the starch phase volume ($\phi_Y = 1 - \phi_X$) increases linearly (Fig. 4*b*) with total concentration of starch present. The slope is slightly higher than the value reported for PCS by Abdulmola et al. (0.104 in comparison with 0.090), but the difference is probably due to the use of a different sample of PCS. In both cases, however, the starch phase at the highest concentration

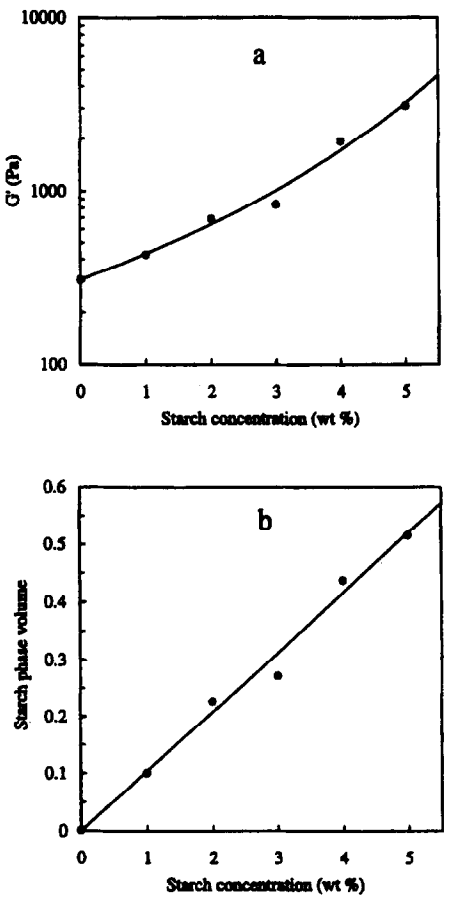

Fig. 4. (*a*) Variation of G' (10 rad s^{-1}; 0.5% strain; 5°C) with starch concentration for the agarose supernatants obtained by centrifugation of mixtures of PCS with 0.25 wt% agarose after gelatinisation of the PCS component at 80°C. (*b*) Starch phase volumes (ϕ_Y) obtained using the G' values from (*a*) to determine the increase in agarose concentration on swelling of the starch granules (slope = 0.104, i.e. swelling volume = 10.4 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. (3))

of PCS used (5 wt%) occupies approximately half the total volume of the system. Further increase in PCS content to 6 wt% would bring the volume of the starch phase almost to the point of close-packing, thus explaining why, at this starch concentration, and above, it was no longer possible to sample the agarose phase by sedimentation of the swollen granules.

In subsequent analyses of the rheology of the starch–agarose composites, the swelling volume of 10.4 ml g^{-1} obtained from Fig. 4*b* was used, in conjunction with Eq. (3) and Eq. (5), 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' is shown in Fig. 4*a*.

As discussed previously, the modulus of the swollen starch granules (G'_Y) was determined by finding combinations of agarose and PCS concentrations at which both phases have the same strength, which is therefore also the strength of the composite, giving $G'_X = G'_Y = G'_C$. Starch concentration was held constant at either 4 wt% or 5 wt%

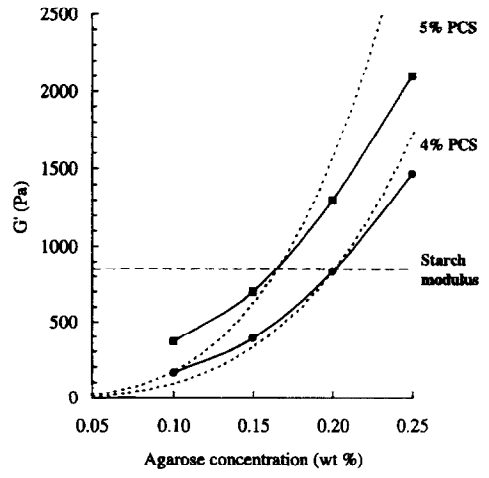

Fig. 5. Direct determination of the modulus (G'_Y) of gelatinised PCS granules in composite gels with agarose. The solid lines are interpolated empirically through the observed moduli (G'_C; 10 rad s^{-1}; 0.5% strain; 5°C) of composites containing 4 wt.% (●) or 5 wt.% (■) PCS gelatinised in agarose solutions of different concentrations, the dotted lines show the modulus (G'_X) of the agarose phase, calculated using the swelling volume of 10.4 ml g^{-1} for PCS and the concentration dependence of G' for agarose alone (Eq. (3)); the modulus of the starch phase (- - -) is given by the points of intersection, where $G'_X = G'_Y = G'_C$

(chosen to give phase volumes well below the onset of close packing, but high enough to allow the dispersed phase to make a significant contribution to the overall strength of the composite), and agarose concentration was varied between 0.10 and 0.25 wt%. Fig. 5 shows the experimental values of G'_C in comparison with calculated values of G'_X obtained by the procedure described above. For both concentrations of PCS, the curves cross at $G'_C = G'_X = \sim 850$ Pa, which can therefore be regarded as a 'model independent' value of the modulus of the swollen granules (G'_Y).

The value of G'_Y derived in this way for the starch phase was then used in conjunction with the values of G'_X for the agarose phase (Fig. 4a) and phase volumes (ϕ_X and ϕ_Y)

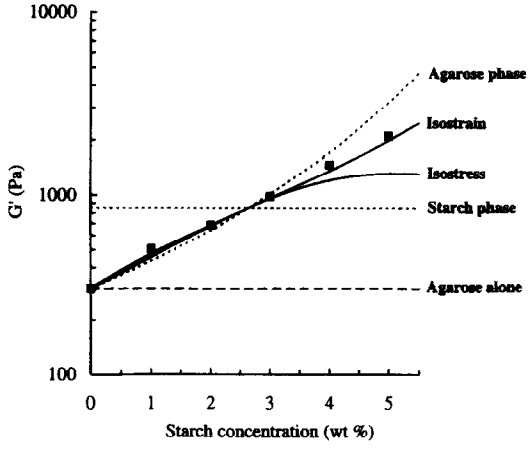

Fig. 6. Comparison of observed values (■) of G' (10 rad s^{-1}; 0.5% strain; 5°C) for composites of PCS gelatinised in 0.25 wt.% agarose with calculated values (——) obtained from the moduli of the constituent phases (- - -) by application of the isostrain and isostress blending laws (Eqs. (1) and (2))

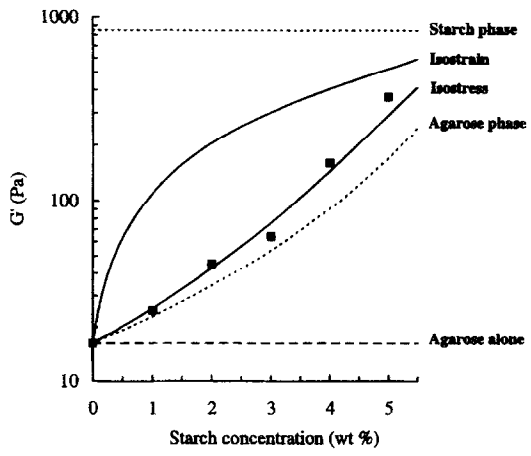

Fig. 7. Comparison of observed values (■) of G' (10 rad s^{-1}; 0.5% strain; 5°C) for composites of PCS gelatinised in 0.10 wt.% agarose with calculated values (—) obtained from the moduli of the constituent phases (- - -) by application of the isostrain and isostress blending laws (Eqs. (1) and (2))

from Fig. 4b to calculate upper (isostrain) and lower (isostress) bounds for the overall moduli of the composite gels of gelatinised PCS (1–5 wt%) in 0.25 wt% agarose. At starch concentrations up to 3 wt%, the isostrain model (Eq. (1)) and isostress model (Eq. (2)) give closely similar values (Fig. 6), with little deviation from the modulus (G'_X) of the agarose matrix, and the observed moduli of the composite gels (G'_C) are in good agreement with both. At 4 and 5 wt% PCS, however, where the modulus of the agarose phase is substantially higher than that of the starch granules, the bounds diverge, with the observed values of G'_C following the isostrain curve, as anticipated for a composite with weak filler particles dispersed in a strong, continuous matrix.

To explore the converse situation of a strong filler in a weaker matrix, the experiment was repeated using a lower concentration of agarose (0.10 wt%, in place of 0.25 wt%). As shown in Fig. 7, the modulus of the agarose phase (G'_X) is now substantially lower than that of the gelatinised granules (G'_Y) throughout the range of PCS concentrations studied (1–5 wt%), and there is a very substantial divergence between the isostrain and isostress curves, with the observed moduli of the composite gels (G'_C) remaining close to the lower values derived by the isostress model (Eq. (2)), as anticipated. Thus the central conclusion from the results presented so far is that for 'filled' gels obtained by cooling gelatinised PCS in solutions of agarose, the observed moduli agree, to within the experimental scatter of the measurements (Figs. 6 and 7), with values calculated by the Takayanagi blending laws, using the isostrain model (Eq. (1)) when the modulus of the gelled agarose matrix (G'_X) is higher than the (model independent) value of $G'_Y \approx 850$ Pa derived for the swollen granules and the isostress model (Eq. (2)) when G'_X is less than G'_Y. The experimental moduli of the quench-cooled composites and agarose supernatants are listed in Table 1, and an overview of the relationship between the composite moduli (G'_C) and those of the constituent phases (G'_X and G'_Y) is given in Fig. 8.

Table 1
Moduli (G'; 10 rad s^{-1}; 0.5% strain; 5°C) for quench-cooled composites

Agarose (wt%)	Starch (wt%)	Composite, G'_C (Pa)	Agarose phase, G'_X (Pa)	
			Calculated[a]	Observed
0.25	0	305	305	305
0.25	1	508	434	427
0.25	2	688	644	692
0.25	3	985	1011	840
0.25	4	1466	1709	1902
0.25	5	2103	3205	3092
0.20	4	833	836	
0.20	5	1298	1568	
0.15	4	390	333	
0.15	5	704	624	
0.10	0	16	16	
0.10	1	25	23	
0.10	2	45	34	
0.10	3	64	54	
0.10	4	160	91	
0.10	5	368	170	

[a]Calculated values of G'_X were derived using the swelling volume for PCS from Fig. 4*b* and the concentration-dependence of G' for agarose alone from Eq. (3).

In the final part of the investigation, the sequence of formation of the constituent phases in the overall composite was changed, by gelatinising PCS granules within a pre-existing agarose gel. As described in Section 2, slurries of ungelatinised PCS (5 wt%) in agarose solution (0.25 wt%) were loaded onto the rheometer at 45°C. The original intention was then to use the quenching procedure illustrated in Fig. 1 to induce rapid gelation and thus trap the ungelatinised granules in homogeneous dispersion throughout the agarose gel. Trial runs using composites prepared in this way, however, showed almost complete loss of network structure at the minimum temperature required for complete gelatinisation of PCS (80°C), whereas preliminary studies of the temperature dependence of modulus for agarose alone on cooling and heating at 1°C min^{-1} had shown the gel–sol transition to be centred well above 80°C. In a subsequent detailed investigation (Mohammed et al., 1998a) it was found that the difference in melting behaviour was due to the difference in gelation procedure (quenching in comparison with controlled cooling), rather than to the presence of starch. The initial composites of agarose with ungelatinised PCS were therefore prepared by cooling from 45 to 5°C at 1°C min^{-1} and holding for 35 min to ensure complete gelation.

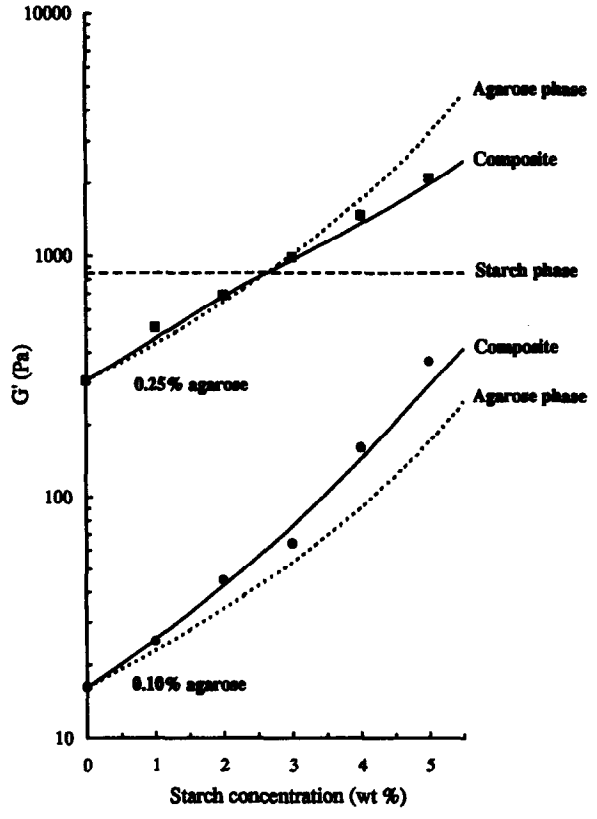

Fig. 8. Overview of the observed values of composite modulus (G'_C) for gelatinised PCS in combination with 0.10 wt.% (●) and 0.25 wt.% (■) agarose, in relation to the modulus (G'_Y) of the starch phase (– – –), the moduli (G'_X) of the agarose phase (- - -), and the calculated values of G'_C (————) from the isostrain model (for $G'_X > G'_Y$) or isostress model (for $G'_X < G'_Y$)

Although necessary to achieve the aim of observing the effect of gelatinisation of PCS within an essentially intact agarose network, this procedure is inherently less satisfactory than the intended method of rapid quenching, since the longer setting time introduces the possibility of significant sedimentation, and perhaps flocculation (Abdulmola et al., 1996b; Mohammed et al., 1998b), of the starch granules before they become trapped in the agarose gel. Complications of this type were evident in erratic and irreproducible results from replicate experiments. In particular, the values of G' recorded at the end of the cooling and holding period at 5°C were often much (up to ~2 ×) higher than the equilibrium value of ~550 Pa obtained (Mohammed et al., 1998a) for 0.25% agarose alone at 5°C, although the presence of 5 wt% ungelatinised PCS would be expected (Eq. (2)) to

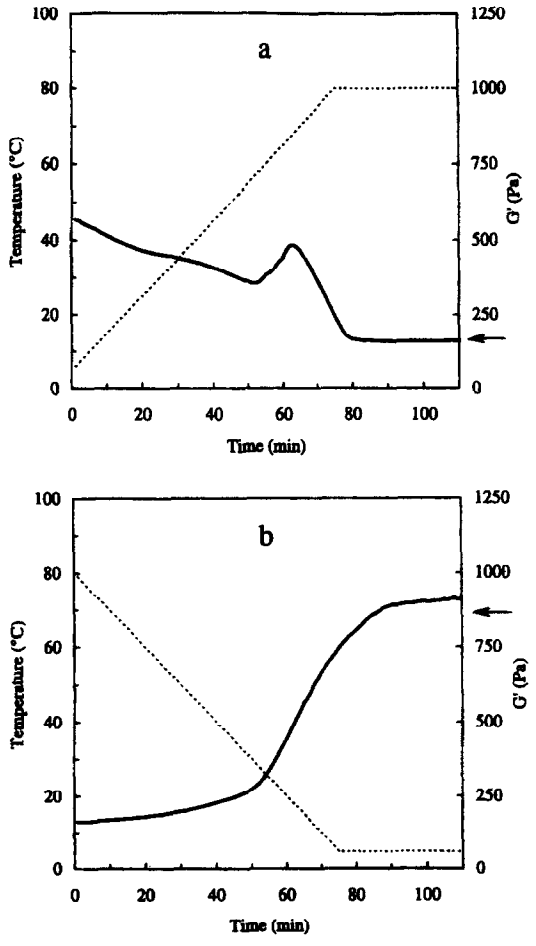

Fig. 9. Gelatinisation of PCS (5 wt%) in an agarose gel matrix (0.25 wt%) formed by controlled cooling (1°C min^{-1}) from the solution state at 45°C. (*a*) Variation of G' (——) with temperature (- - -) during heating from 5 to 80°C (1°C min^{-1}) and holding for 35 min at 80°C. (*b*) Variation of G' with temperature on subsequent cooling and holding at 5°C. Measurements were made at 10 rad s^{-1} and 0.5% strain. The arrows on the right hand axes show G' values calculated by classic theory of network de-swelling

cause only slight reinforcement of the agarose network. Experiments where such anomalously high moduli were observed were terminated after the initial cooling and holding steps. In a few cases, however, the final moduli at 5°C were only slightly higher than the value for agarose alone, indicating a reasonably homogenous dispersion with no significant contribution to overall G' from, for example, associated starch granules bridging between the plates of the rheometer. For these samples, the experiment was continued by heating to 80°C (at 1°C min^{-1}), holding for 35 min, and re-cooling. A typical heating scan is shown in Fig. 9*a* and the subsequent cooling scan is shown in Fig. 9b.

On heating, there is an initial slight reduction in G', as found (Mohammed et al., 1998a) for 0.25 wt% agarose alone, up to ~55°C, which is the onset temperature for gelatinisation of PCS (Abdulmola et al., 1996b). The modulus then rises sharply, consistent with transfer of water from the agarose phase into the starch granules, thus raising the concentration (and hence the modulus) of the continuous matrix. At higher temperatures (above ~65°C) the initial trend to lower moduli with increasing temperature, due to softening of the agarose network, is resumed, with G' then levelling out to a constant value during the holding period at 80°C. Network softening is then reversed during the cooling and holding steps shown in Fig. 9*b*. The final modulus at 5°C, however, is substantially lower than for a composite gel of the same composition (0.25wt% agarose + 5wt% PCS) formed by quench-cooling after gelatinisation of the starch component (~900 Pa, Fig. 9*b*, in comparison with ~2100 Pa, Fig. 6), although the modulus obtained for 0.25% agarose alone after cooling at 1°C min^{-1} is somewhat higher (Mohammed et al., 1998a) than on quenching (~550 Pa, in comparison with ~300 Pa).

A reduction in the final modulus is an expected consequence of reversing the sequence of the gelation and gelatinisation processes. If a gel network with essentially permanent crosslinks is formed at an initial concentration, c_i, and is then taken to a lower or higher final concentration, c_f, by introduction or removal of solvent (swelling or de-swelling) then, from classic swelling theory (Flory, 1953), the associated change in modulus is given by:

$$G_f/G_i = (c_f/c_i)^{2/3} \quad (6)$$

As shown in Fig. 4*b*, the phase volume occupied by 5 wt% gelatinised PCS is ~0.52, thus reducing the agarose phase volume to 0.48; the polymer concentrations in the agarose phase before and after gelatinisation are therefore related by: $c_f/c_i = 1/0.48 \approx 2.083$. Thus, from Eq. (6), the expected increase in modulus when the gelled matrix is 'deswollen' by gelatinisation of 5 wt% PCS is given by:

$$G_f = G_i(1/0.48)^{2/3} \approx 1.63G_i \quad (7)$$

If, by contrast, the increase in concentration is induced prior to gelation, by gelatinisation of the starch granules in agarose solution, the increase in modulus will then be determined by the much higher exponent shown in Eq. (3), giving:

$$G_f = G_i(1/0.48)^{3.204} \approx 10.5G_i \quad (8)$$

The deswelling route would therefore be expected to give a weaker matrix (by a factor of $10.5/1.63 \approx 6.4$), with corresponding reduction in the overall strength of the composite.

The equilibrium moduli obtained by Mohammed et al. (1998a) for 0.25 wt% agarose alone (using the same sample and experimental conditions as in the present work) were $G' = 545$ Pa at 5°C and $G' = 55.4$ Pa at 80°C. The corresponding values (Eq. (7)) after deswelling to $\phi_X = 0.48$ are $G'_X = 888$ Pa at 5°C and $G'_X = 90$ Pa at 80°C, which are, respectively, slightly higher and much lower than the value of $G'_Y = 850$ Pa obtained for the swollen PCS granules (Fig. 5). The expected modulus of the composite (G'_C) can therefore be calculated (Eq. (9) and Eq. (10)) using the isostrain model (weak filler in strong matrix) for 5°C and the isostress model for 80°C.

$$5°C: \quad G'_c = (0.48 \times 888) + (0.52 \times 850) \quad (9)$$

$$80°C: \quad 1/G'_C = (0.48/90) + (0.52/850) \tag{10}$$

yielding $G'_C = 868$ Pa at 5°C and $G'_C = 168$ Pa at 80°C. As shown in Fig. 9, these values, indicated by arrows on the right-hand axes of the graphs, agree remarkably well with the observed moduli at the end of the holding periods at high and low temperature.

4. Discussion and conclusions

An implicit assumption in the above analysis of network deswelling is that intermolecular associations in the agarose matrix are unaffected by the loss of solvent. In the extreme case of an 'infinitely labile' network (i.e. with crosslinks forming and dissociating very rapidly in comparison with the experimental timescale), the modulus–concentration relationship for swollen/deswollen gels would be the same as for gels formed directly from the solution state at the same final concentrations. Eq. (6) applies to the opposite extreme of permanent crosslinking. The time–temperature curve in Fig. 9*a* suggests that it is reasonably valid to use this relationship for agarose, since in the holding period at 80°C, immediately after deswelling, the measured values of G' remain essentially constant, with no indication of any significant re-arrangement to the stronger network that would be formed on direct gelation at the final, higher, concentration.

It was also assumed implicitly that PCS granules gelatinised in an agarose gel swell to the same extent as they would in solution, and that they have the same modulus at 80°C as at 5°C. The second of these assumptions is unlikely to have introduced any significant error since, as discussed below, the starch phase makes little direct contribution to the overall modulus. The first assumption, however, is far more critical, since the extent of deswelling dictates the concentration (and hence the modulus) of the dominant continuous phase, and it can be justified only by the insensitivity of swelling volume to polymer concentration in solution (Abdulmola et al., 1996a,b).

In view of the untested assumptions involved in the analysis, and of the experimental difficulties discussed previously, the standard of agreement between the observed and calculated moduli in Fig. 9 must obviously be treated with caution, although it does seem reasonable to conclude that application of classic swelling/deswelling theory, in conjuction with the Takayanagi blending laws, gives values of G'_C that are at least broadly consistent with those observed experimentally.

The conclusions from the investigation of composites formed by initial gelatinisation of the PCS 'filler' and subsequent gelation of the agarose matrix, however, are much firmer, and may be of general applicability in understanding the rheology of biphasic co-gels. As shown in Fig. 7, progressive incorporation of PCS in composites with 0.1 wt% agarose caused a large (~20-fold) increase in G' across the range of starch concentrations studied (0–5 wt%), but it is evident that most of the change comes from the increase in concentration of the agarose matrix (giving about a 10-fold increase in G'), with the presence of the (much stronger) starch granules causing only slight further reinforcement (by about a factor of 2 at 5 wt% PCS). At higher agarose concentration (0.25 wt%; Fig. 6), where the modulus of the agarose phase rises above that of the starch phase, the presence of the swollen granules has the opposite effect of making the composite weaker than the continuous matrix, but the difference is again limited to about a factor of 2 at 5 wt% PCS.

The relative insensitivity of composite modulus to the strength of the filler phase is entirely consistent with the behaviour anticipated from the Takayanagi blending laws (Eq. (1) and Eq. (2)). For composites where the filler particles are much softer or much harder than the surrounding matrix (by more than about a factor of 10), the isostrain and isostress equations reduce to:

$$G_C \approx G_X \phi_X \quad \text{if } G_Y \ll G_X \tag{11}$$

$$1/G_C \approx \phi_X / G_X \quad \text{if } G_Y \gg G_X \tag{12}$$

As shown in Fig. 4*b*, gelatinised PCS at the highest concentration used (5 wt%) occupies about half the total volume of the composite ($\phi_Y = 0.52$; $\phi_X = 0.48$), so the maximum expected variation in composite modulus (G'_C) relative to the modulus of the continuous matrix (G'_X) would be confined to the approximate range $G'_C \approx 0.5G'_X$ (Eq. (11)) to $G'_C \approx 2G'_X$ (Eq. (12)), as observed. The same conclusion can be extended to phase-separated biopolymer co-gels, since the component with the larger phase volume is likely to form the continuous matrix, giving $\phi_X > \sim 0.5$.

To our knowledge, this is the first study of a biphasic biopolymer system in which the Takayanagi blending laws have been applied with no adjustable parameters. The phase volumes (ϕ_X and ϕ_Y) come directly from experimental determination of the swelling volume of PCS (Fig. 4*b*), the modulus of the continuous matrix (G'_X) is then obtained from the concentration-dependence of G' for agarose alone (Eq. (3)), and the modulus of the dispersed granules (G'_Y) is derived by the 'model independent' procedure shown in Fig. 5. The central conclusions are as follows:

(1) Overall rheology is determined predominantly by distribution of solvent, with the dispersed phase acting mainly by increasing the concentration, and hence the modulus, of the continuous matrix.

(2) The direct contribution of the dispersed particles is likely to be limited to increasing or decreasing the overall modulus of the composite relative to that of the continuous phase by, at most, a factor of ~2.

(3) The errors introduced by literal application of the relevant blending law (isostrain when the filler is weaker than the matrix; isostress when the matrix is weaker than the filler) are no greater than the experimental scatter in measured values of composite moduli (Fig. 8).

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