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Structural aspects and phase behaviour in deacylated and high acyl gellan systems

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Abstract

The physical properties of deacylated and high acyl gellan systems were examined using small deformation rheology, optical rotation and differential scanning calorimetry. It is demonstrated that the drop in strength of deacylated gellan structures at high levels of counterions is caused by the formation of ordered nuclei which require low temperatures ($<$ 50°C) to stabilise a low functionality network whereas the conformational transition leading to nuclei occurs at $\approx$ 70°C. High acyl gellan is capable of gelation in the absence of added cations with structures showing similar cooling and heating profiles. It appears, however, that increasing levels of salt encourage sparse cross-linking between the acyl-free parts of the chain. These intermolecular associations remain stable during heating and support a network of increasingly flexible chains as the acylated helices are gradually melting. Thus the structure shows heating profiles with rubbery characteristics (i.e. increasing storage modulus), also observed in conditions of suppressed aggregation in high-sugar deacylated gellan networks. Chains of the two gellan variants are sterically incompatible and, depending on the level of added salt, yield single-phase systems, or composites with disparate phase behaviour. Finally, blending-law analysis supports the hypothesis that the entrapment of solvent in a polymeric phase is governed by the topology and the morphology of its network.

Keywords: Deacylated gellan; High acyl gellan; Phase behaviour

1. Introduction

Gellan is the carboxylated extracellular polysaccharide secreted by the organism *Sphingomonas elodea* (Kang and Veeder, 1982). Its primary structure is composed of a linear tetrasaccharide repeat unit (O'Neill et al., 1983):

$$\underset{A}{\rightarrow 3)\text{–}\beta\text{–D–Glc}p\text{–}(1\rightarrow 4)}\text{–}\underset{B}{\beta\text{–D–Glc}p\text{A–}(1\rightarrow 4)}\text{–}\underset{C}{\beta\text{–D–Glc}p\text{–}(1\rightarrow 4)}\text{–}\underset{D}{\alpha\text{–}L-\text{Rha}p\text{–}(1\rightarrow)}$$

The polymer is produced with two acyl substituents present on the 3-linked glucose, namely: L-glyceryl, positioned at O(2) and acetyl at O(6). On average there is one glycerate per repeat unit and one acetate per every two repeats (Kuo et al., 1986). Direct recovery of the polysaccharide from the fermentation broth yields the high acyl form whereas deacylation by alkali treatment results in the low acyl counterpart.

Cooling of the disordered deacylated coil in solution induces a conformational transition and the ordered chain is stabilised with three intramolecular hydrogen bonds per repeat sequence, notably the O-3B···O-5A (Chandrasekaran et al., 1988a). Molecules are further stabilised with the formation of three-fold left-handed double helices which involve hydrogen bonding between the glucuronic acid of one chain and the (1 → 4)-linked glucose and rhamnose molecules of its partner (O-6C···O-61B/O-62B and O-3D···O-2B; Chandrasekaran et al., 1988b). X-ray studies also suggest that the available hydroxyl groups of L-rhamnose and the hydroxymethyl groups of D-glucose (A) residues form noteworthy interactions between double helices, but it is the coordination between monovalent cations and the carboxyl group of the D-glucuronate that underpins the lateral packing (Chandrasekaran et al., 1988c). Where the

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distance between two cations is 0.43 nm a water molecule can bridge them with concomitant increase in the stability of the aggregate. These two monovalent ions can be replaced by a calcium ion which is capable of cross-linking directly two adjacent helices (Chandrasekaran and Thailambal, 1990).

Regarding the high acyl gellan, the acetyl group protrudes from the double helix identical to the hydroxymethyl group of residue A thus fitting the standard X-ray structure. The bulky glyceryl group, however, stretches in the opposite direction and forces both the carboxyl group and the adjacent glucuronate residue to turn in order to alleviate the steric clash (Chandrasekaran et al., 1992). The new arrangement reinforces the stability of double helices via additional hydrogen bonding (e.g. the intermolecular O10A···O2B) but makes them incompatible for aggregation. Further, increase in the distance, for example, between potassium ions from 0.43 to 0.62 nm does not permit the formation of water bridges. Mono/divalent ions are also buried by the glycerate and as a result they lose valuable coordination sites required for a cation-driven mechanism of gelation (Chandrasekaran and Radha, 1995).

The striking difference in cation sensitivity should translate into disparate network viscoelasticity. Thus, texture profile analysis of the two variants recorded weak elastic and strong brittle properties for the high acyl and deacylated gellan gels, respectively (Moorhouse, 1987). Ten years later the high acyl gellan is commercially available and, clearly, fundamental understanding of the interactions between the two variants can guide the development of novel product concepts. In addition, salt triggers off the mechanics of deacylated gellan, an event which transforms the 'balance of power' in the binary mixture and provides an ideal medium to advance our long standing interest, namely: the development of a unifying theory that relates the properties of a mixed system to those of its constituents (Kasapis, 1995).

2. Materials and methods

The gellan sample used in this investigation was a gift from Monsanto PLC., Tadworth, UK. It came in the deacylated and the high acyl form, commercially available as Kelcogel-77109A and Kelcogel LT100, respectively. The acyl content of LT100 was measured by HPLC of the acid hydrolysates (Cheetham and Punruckvong, 1985), and was found to be 12.7% glycerate and 3.41% acetate on a pure polysaccharide basis. This corresponds to approximately 0.9 glycerate and 0.4 acetate substituents per repeat unit. The purification and ion-exchanging steps are discussed below.

Four grams of deacylated gellan were dissolved in 800 ml of distilled water at 90°C. A cellulose membrane tube with a pore diameter of 2.4 nm was filled with the solution, sealed and placed in a water bath at 75°C for five days. For three days the water was changed four times a day and then it was replaced with a 7.5 mN NaCl solution which corresponds to the stoichiometric equivalence of the carboxyl groups for this preparation. Finally, the gellan solution was freeze-dried.

The elevated gelation temperature of the high acyl gellan renders dialysis inappropriate, and instead, a cation-exchanging resin was used (Amberlite IR 120). Two hundred grams of the material was first exchanged into the acid form by eluting with a 0.1 N HCl solution until the eluant was pH 1, washed by distilled water until the pH became almost neutral, and exchanged to the sodium form by elution with 1 N solution of NaCl until the eluant was again neutral (for practical details see Manning, 1992). Five grams of the polymer were dissolved in 1 litre of distilled water at 90°C and then the resin was added and stirred for 30 min. After decanting, the gellan solution was separated from the resin and freeze-dried.

Single and mixed solutions for physical studies were prepared by dissolving the freeze-dried material at 90°C. Following this, the ionic strength was brought to the desired level by mixing with a sodium or calcium chloride solution.

Low-amplitude oscillatory measurements were performed at 10 rad/s on a sensitive in-house rheometer with a cone and plate geometry (cone angle 0.05 rad; 50 mm diameter). As mentioned in the introduction, high acyl gellan forms elastic networks which remain intact at the experimental strain (1%) of temperature and frequency sweeps. Deacylated samples are slippery and require a device of perforated cylinders to ensure proper adhesion to the surface of the measuring geometry (Richardson and Goycoolea, 1994). The presence of high acyl gellan imparts adhesion to the binary mixtures and allows the use of cone and plate geometry for their study. Samples were loaded on to the pre-heated platen/cylinder of the rheometer (90°C), their exposed edges covered with a silicone fluid to minimise water loss and cooled to 20°C at a rate of 1°C/min. This was followed by frequency sweeps from 0.1 to 100 rad/s. The last part of the rheological routine involved strain sweeps or heating scans (1°C/min) to the highest accessible temperature of the machine (95°C).

Differential scanning calorimetry measurements were performed on a BIO-DSC batch and flow microcalorimeter. Sample pans were filled with 0.90 g of the sample and the water-filled reference pan matched this to within 2 mg. At the beginning of each experiment materials were heated to 98°C to eliminate erroneous phenomena due to thermal history during sample preparation and loading, and then cooled and heated at a rate of 0.2°C/min.

Finally, optical rotation (OR) measurements were taken on a Perkin-Elmer 241 polarimeter with sensitivity of 1 mdeg. The machine has filters to set the wavelength at the mercury emission lines of 365 and 436 nm used to record the disorder to order transition of the polysaccharide. Hot solutions (90°C) filled a jacketed cell of pathlength 1 cm and cooled to 5°C at 1°C/min (same temperature regime with the rheology) using a Haake circulating water bath.

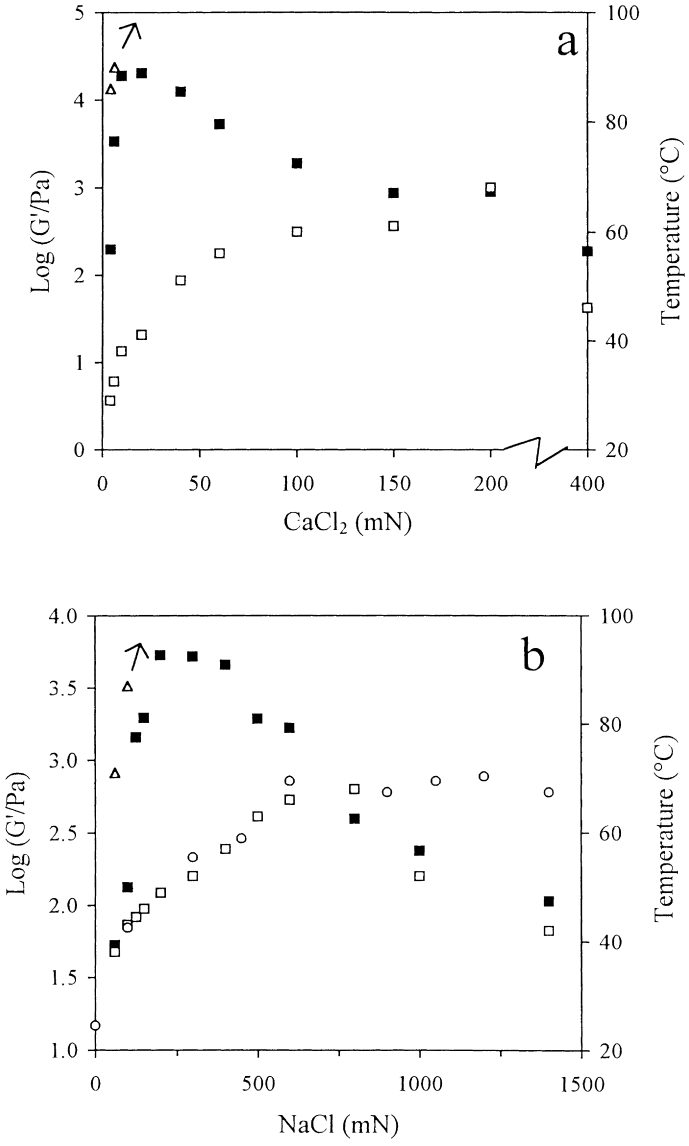

Fig. 1. Development of storage modulus at 20°C (■), rheological onset of network formation (□) and melting temperatures (△), and onset of conformational transitions using polarimetry (○) for 0.5% deacylated gellan with increasing levels of a) calcium and b) sodium ions. The G′ data are on the left y axis.

Heating runs to 90°C followed, and both temperature routines were pursued as long as the light transmission remained above 80%.

3. Results and discussion

3.1. Order versus network formation in deacylated gellan

The literature on the structural and thermal properties of deacylated gellan is flourishing. Perhaps the best-known aspect of the subject is the continuous reinforcement of the small and large deformation properties of networks with increasing ionic strength at normal levels of food use (Grasdalen and Smidsrød, 1987; Watase and Nishinari, 1993). The purpose of the present exercise is to provide a series of data for the follow up on gellan mixtures, but also to address a point of controversy at higher levels of added counterions.

As shown in Fig. 1a, the strong interactions between calcium ions and the gellan helices in the deacylated form support a rapid network development which achieves maximum network strength at 20 mN $CaCl_2$ (G' is equal to 20.23 kPa at 20°C). At the end of the cooling run, mechanical spectra are typical of hydrogels with frequency independent moduli and tan δ values of about 0.01. On subsequent heating networks remain thermally irreversible in the presence of 10 mN Ca^{2+}; $T_{melt} > 95$°C which is the highest experimentally accessible temperature of our setting. Further, addition of counterions stabilises the coaxial double helices with the onset of network formation (T_{form}) rising steadily to ≈ 68°C at 200 mN of calcium

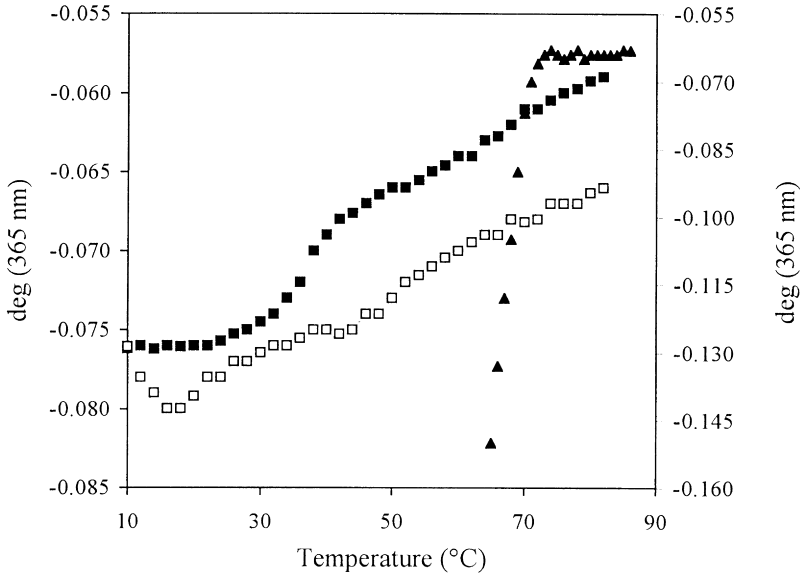

Fig. 2. Optical rotation measurements for 0.5% deacylated gellan with 100 mN NaCl on the left y axis (cooling (■); heating (□)) and in the presence of 1200 mN NaCl on the right y axis (cooling (▲)). Scan rate is 1°C/min, 1 cm cell pathlength.

ions. However, increasing levels of Ca^{2+} have an adverse effect on the gel properties with the network strength and the recorded temperature of network formation eventually falling significantly.

Qualitatively, addition of sodium chloride to the gellan system produces a similar profile. Thus, the values of G' and T_{form} rise and fall with increasing levels of salt and networks become thermally irreversible (Fig. 1b). However, maxima in the structural properties are observed at higher normalities (in the case of G' at 20 and 250 mN $CaCl_2$ and NaCl, respectively), a result which verifies the strong specific interaction between calcium and carboxyl groups whereas sodium ions are seen as a cloud surrounding and thus neutralising the gellan chain. Work by Papageorgiou et al. (1994) on acidic samples (pH 3.8) of deacylated gellan with gelatin used the effect of sodium on gellan networks to rationalise the observed exclusion phenomena in the mixture. They also split the experimental range of NaCl into two distinct patterns of behaviour. Part I extends as far as the point of maximum network strength which is the result of additional ordering in a well balanced structure of aggregated helices and disordered coils. The second stage covers the salt content up to the maximum temperature of network formation, which is accompanied by a drop in the values of storage modulus. It was suggested that ordering at high temperatures initiates the formation of a network (values of T_{form} keep rising) which evolves with cooling and transforms into an assembly of aggregated helices. Macroscopically the system can be seen as an aggregated gel comprising particles of reduced functionality.

An alternative hypothesis, suggested by Tang et al. (1996), considers the excessive positive charges at high levels of counterions to be prohibitive to the formation of intermolecular associations and attributes the diminishing gel strength to reduced order in the gellan chain. Reduction in the degree of cross-linking of a network should also create elastic structures where the flexible chains can stretch to a good measure before they relax. Thus, 60% addition of oligomeric co-solute to gellan destabilises aggregation and results in a spectacular drop in the Young modulus and an increase in the yield strain of the gel (Sworn and Kasapis, 1998). Data by Tang and co-workers, however, show a continuous reduction in the values of deformation at failure with added Na^+ or Ca^{2+} which bottom out at the upper range of the counterion content, hence arguing for strain-sensitive aggregated networks. Further, formation of a three-dimensional network depends on the ability of structural nuclei to cross-link and rheology, being a macromolecular technique, is primarily posed to monitor the process of gelation. Optical rotation (OR), on the other hand, detects the conformational disorder-to-order transition regardless of its macromolecular consequence and in combination with the mechanical characterisation is able to disentangle the two phenomena.

Fig. 2 illustrates the OR profile for a gellan sample with 100 mN added NaCl which has been cooled and then heated at 1°C/min. At the high temperature end there is a linear entropic effect on the optical activity of the disordered gellan chains. When measurements are extended to temperatures below 43°C (T_{conf}) a sigmoidal trace is recorded which corresponds to the enthalpic ordering of the gellan molecule. Subsequent heating follows back the sigmoidal profile albeit with a temperature lag due to the additional energetic requirement of melting the aggregated structures. The thermal hysteresis persists at the top of the temperature range thus rendering part of the gellan network thermally

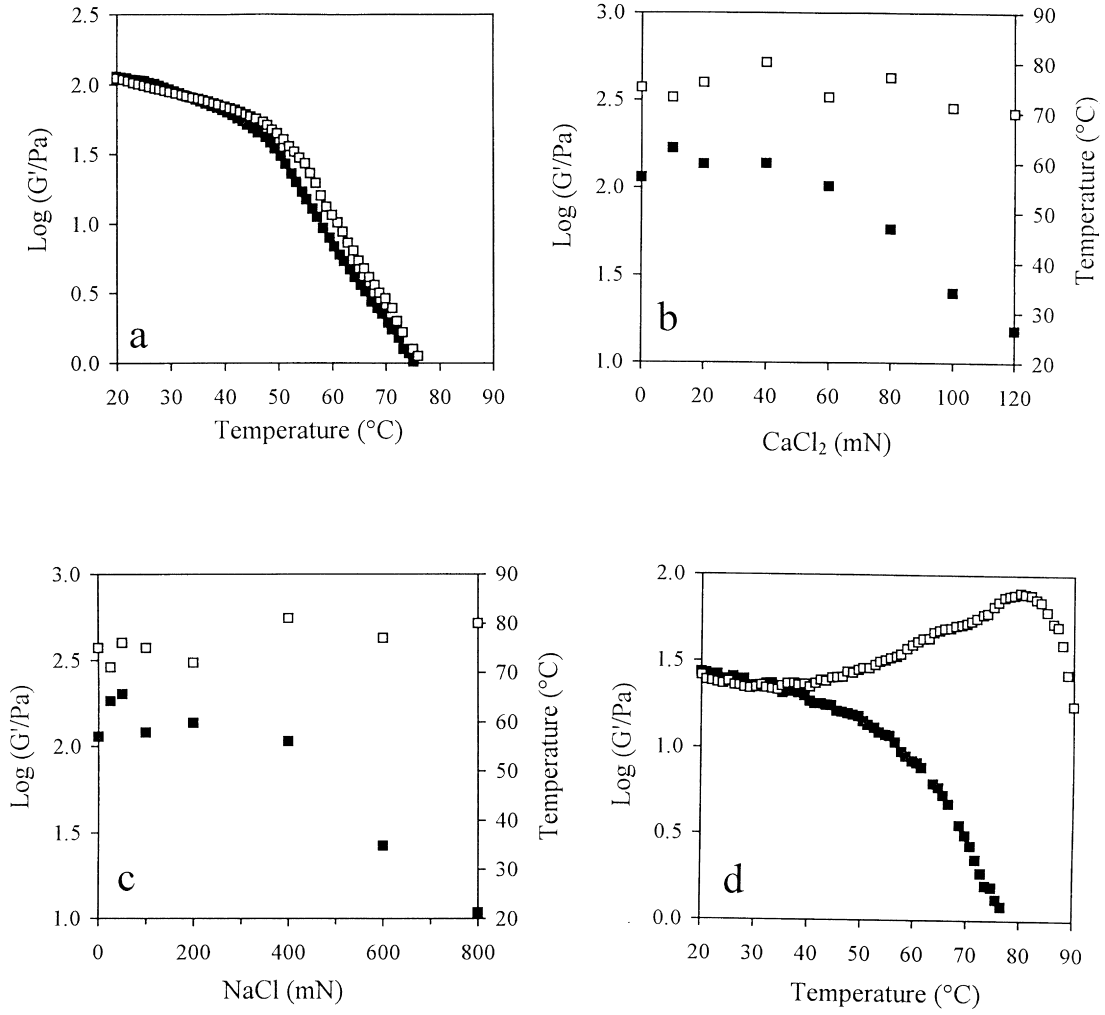

Fig. 3. Data on 0.5% high acyl gellan showing a) cooling (■) and heating (□) profiles at 1°C/min, storage modules at 20°C (■) and temperature at the beginning of network formation (□) as a function of b) $CaCl_2$ and c) NaCl, and d) cooling (■) and heating (□) scans at 600 mN NaCl (1°C/min).

irreversible, in agreement with the rheological T_{melt} in Fig. 1b. Further addition of counterions raises the conformationally sensitive backbone transition, which at 1200 mN NaCl commences at 70°C (Fig. 2). Nevertheless, the end of the transition is lost in a heavily scattering topology (the light transmission falls below 80% at 65°C) which is due to excessive aggregation at the high level of added salt. As shown in Fig. 1b, the temperatures of conformational ordering and gel setting are coincident in the upward part of the salt dependence (0 to 800 mN NaCl) with both processes occurring simultaneously. However, the values of T_{conf} reach and hold a plateau at $\approx$ 70°C thus progressively deviating from the rheological parameters which drop at the upper range of salt concentration. Therefore, the over-abundance of salt encourages a plethora of ordered nuclei which occur early during a cooling run but their alignment in long-range structures only survives in a less vibrant environment at lower temperatures (< 50°C).

3.2. Salt-induced network transmutations in high acyl gellan

The second requirement for the investigation of binary mixtures was to rationalise the structural behaviour of high acyl gellan samples which produced an unexpected result. Following the approach of Fig. 1, appropriate amounts of sodium and calcium chloride were added to hot gellan solutions and the rheological routine was implemented. As shown in Fig. 3a, network formation is possible in the absence of added counterions ($T_{form} \approx 75$°C) emanating from a rather broad transition. This pattern of behaviour is repeated on heating and, effectively, there is no thermal hysteresis between the two runs, a result which suggests absence of aggregation. The lack of cation-mediated aggregation was also clear from the coincident cooling/heating OR profiles of the polysaccharide in deionised water and 100 mM NaCl (Morris et al., 1996). There is, therefore, a remarkable contrast between the temperature profiles of the two gellan variants, since the deacylated form requires salt for the formation of an aggregated network with substantial

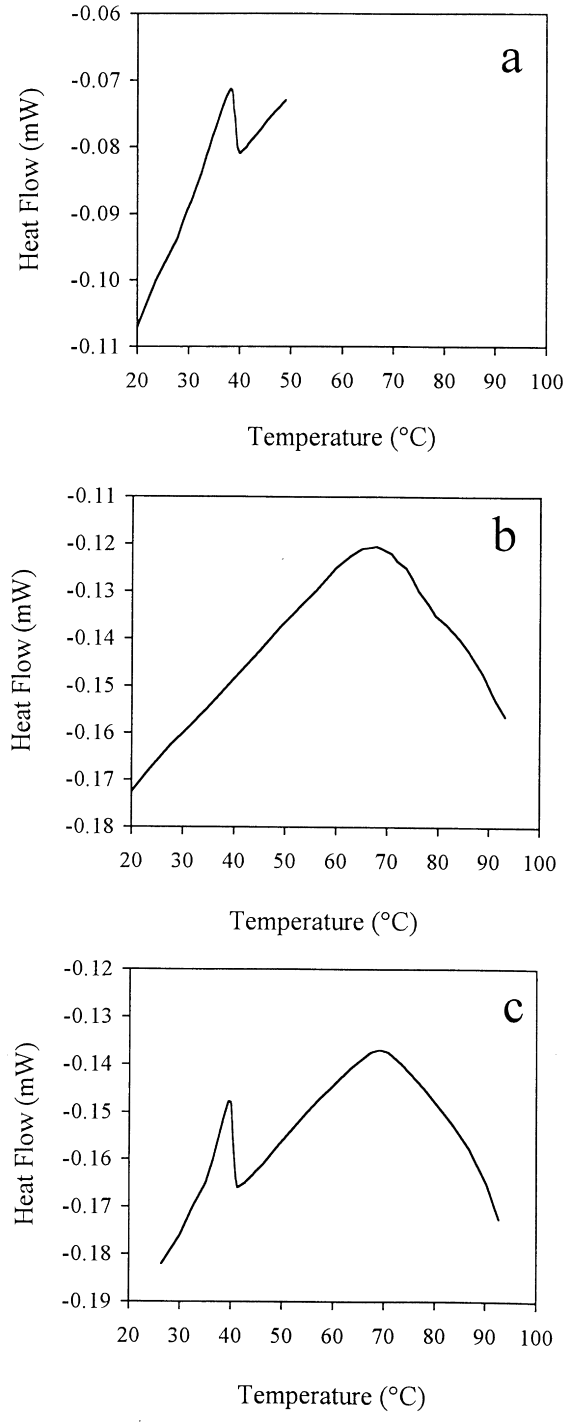

Fig. 4. DSC exothermic peaks for a) 0.5% deacylated gellan b) 0.5% high acyl gellan and c) 0.5% deacylated plus 0.5% high acyl gellan samples at 80 mN NaCl (cooling rate: 0.2°C/min).

thermal hysteresis (Fig. 1). Figs 3b and 3c demonstrate that counterions do not influence the onset of network formation in high acyl preparations which is confined within the temperature range of 70° to 80°C. Further, in the absence of aggregation, gels are much weaker than their deacylated counterparts in Fig. 1.

Increasing addition of counterions appears to affect adversely the high acyl network leading to a tenfold reduction in the values of the elastic component. It is unlikely that over-aggregation, as seen for the deacylated sample, is the cause behind this event since the acyl groups will prevent it from happening and gels remain as elastic as ever. Surprisingly, heating profiles of the gels at high levels of salt (e.g. 600 mN NaCl in Fig. 3d) show a spectacular rise in G' values followed by a drop at the high temperature end, a result which is reproducible at these levels of added NaCl. A similar response was obtained for high sugar ($>$ 60%) deacylated gellan gels where it was argued that the presence of co-solute prevents aggregation and transforms the network into an assembly of sparsely cross-linked (via cations) flexible chains (Papageorgiou and Kasapis, 1995). It appears, therefore, that the acyl groups have the same effect as sugar and prevent polymer aggregation, with the high levels of salt managing to set up limited junction zones in the acyl-free parts of the chain. As a result, heating demolishes gradually the acyl part of the network but the cation-mediated interactions support a rubbery morphology until their energetic threshold is reached ($>$ 80°C) and the whole edifice collapses. The exploration of the structural properties in single gellan preparations will now be used for characterisation of the interactions in mixtures of the two materials.

3.3. Phase behaviour of deacylated/high acyl gellan mixtures

Depending on the conformational characteristics and the ionic groups of a macromolecule, mixing of two species can result in heterotypic systems where positive interactions dominate or in a composite with well defined phases for each component. Changes in topology are clearly demonstrated in the deacylated gellan/gelatin blend where addition of salt neutralises the carboxyl groups and negates the electrostatic interactions between the two constituents thus turning the coacervate into a phase separated gel (Chilvers and Morris, 1987; Papageorgiou et al., 1994). Differential scanning calorimetry (DSC) is used to detect the transmutations in a mixed system with the heterotypic interactions usually manifesting themselves by distorting the peaks of the individual gels and generating a new thermal event.

Following this approach, we loaded the gellan samples on to the compartment of our calorimeter, run them to 98°C to melt the gel and then scanned them down at 0.2°C/min. Fig. 4 reproduces typical exothermic peaks for gellan samples at 80 mN NaCl which remain thermally reversible at temperatures below the boiling point (see T_{melt} data in Fig. 1b). A relatively sharp peak with a maximum heat flow temperature (T_{max}) of 39°C denotes the co-operative conformational transition of the deacylated gellan molecule (Fig. 4a). In contrast, the gradual development of the high acyl network is reflected in a broad exothermic event culminating at higher temperatures (T_{max} 70°C in Fig. 4b). Upon mixing,

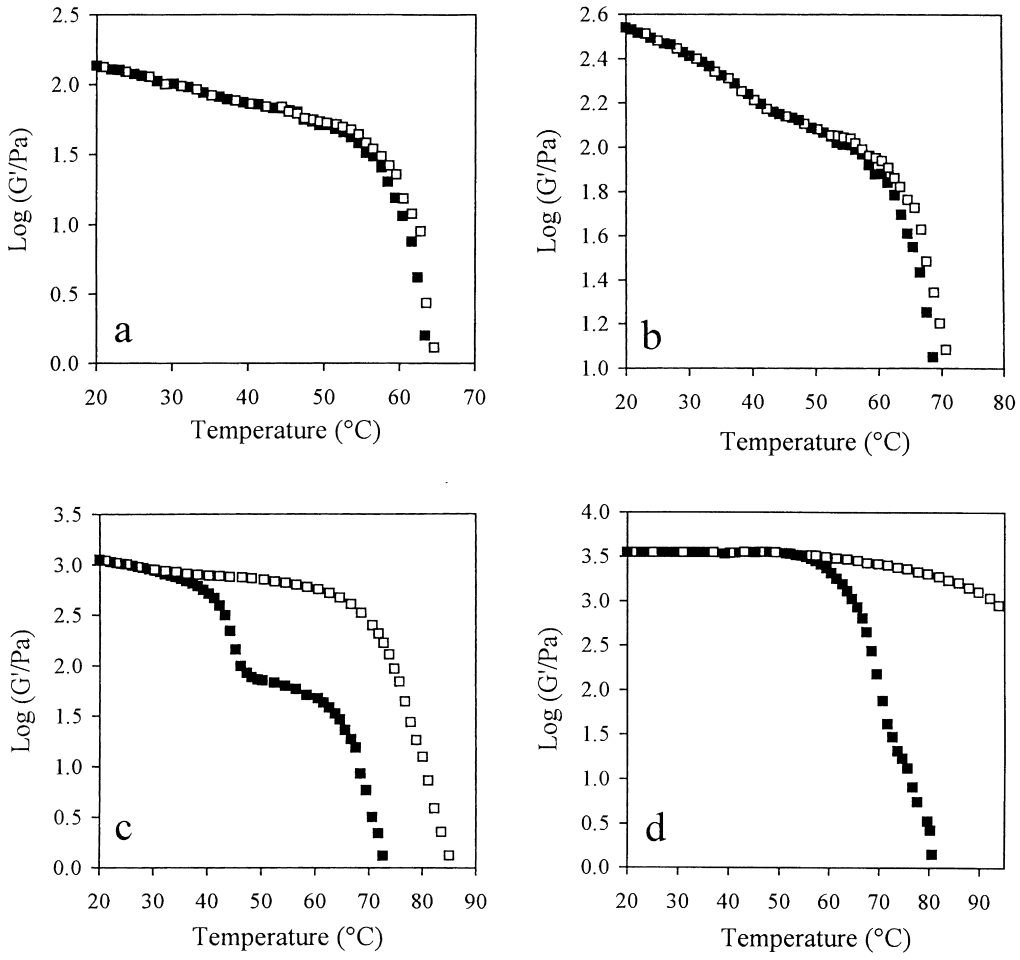

Fig. 5. Cooling (■) and heating (□) profiles of 0.5% deacylated gellan plus 0.5% high acyl gellan with (a) no added counterions, (b) 50 mN NaCl, (c) 100 mN NaCl and (d) 400 mN NaCl (scan rate of 1°C/min; frequency 10 rad/s).

the temperature band and the overall peak form for each transition was maintained (Fig. 4c), a result which argues that the two components gel independently as in isolation.

Having established the non-interactive nature of the two gellan variants, we set about exploring the phase behaviour of the mixture as a function of increasing content of NaCl. Fig. 5 reproduces the cooling and heating profiles of 0.5% deacylated plus 0.5% high acyl gellan blends scanned within the range of 20 to 95°C at a rate of 1°C/min. As shown in Fig. 5a, in the absence of added counterions the single-transition response is determined by the gel strength and the onset/melting of the high acyl counterpart, since the deacylated polymer undergoes conformational transition (OR work) but does not form a continuous network (Fig. 1b). It appears, therefore, that the system is a single-phase of a supporting high acyl matrix with the ordered deacylated chains being distributed uniformly throughout the medium. A similar step of gel formation is recorded at 50 mN NaCl but further cooling sees the advent of a second wave of structure which is the result of gelation of deacylated gellan (Fig. 5b). As shown in Fig. 1b, the polymer does not form a continuous network at this level of salt (vestigial structure is first recorded at 60 mN NaCl), but steric exclusion concentrates up its phase and allows gelation to occur. The composite comprises a high acyl matrix but, this time, the topology of the deacylated phase (i.e. discontinuous inclusions or a second continuous network) cannot be ascertained by pictorial rheology. However, modelling of the phase behaviour using polymer blending laws clarifies this issue, as discussed in Fig. 7b.

Further increase in the salt content (from 50 to 100 mN NaCl) unveils another development. Thus, in Fig. 5c the bimodal cooling profile is at each most spectacular and in combination with the prolonged melting trace result in significant thermal hysteresis which characterises the heating of deacylated gellan gels at 100 mN NaCl (Fig. 1b). This finding argues that the gelation and melting mechanisms of deacylated gellan are substantially reinforced at 100 mN of Na^+ and the polymer is now capable of forming a continuous network, the composite as a whole being a bicontinuous phase separated system. Finally, we quadrupled the level of salt and observed a comprehensive domination of the deacylated polymer in the system with the transition of the high acyl gellan only appearing as an early

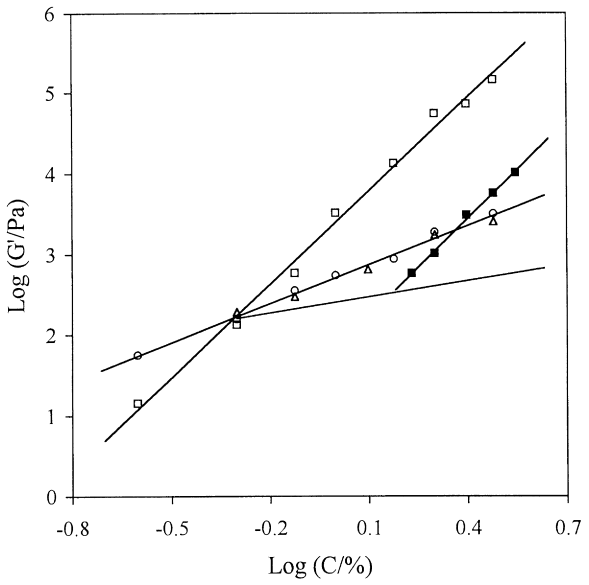

Fig. 6. Concentration-modulus relationships for deacylated gellan gels (20°C) in the presence of 50 (■) and 100 (□) mN NaCl, for high acyl gellan gels at 50 (△) and 100 (○) mN NaCl, and the prediction for a deswelled high acyl gellan gel (——).

feature at 80°C (Fig. 5d). This is also reflected in the heating profile of the composite which becomes thermally irreversible at temperatures below 95°C.

3.4. Quantitative analysis of the phase behaviour

This part of our study focuses on the prediction of solvent partition between the two phases using the isostrain and isostress blending laws of Takayanagi et al. (1963). The approach allows estimation of the effective concentration of a polymer within its phase and hence of the viscoelasticity of two networks in a mixture. It requires complete phase separation in the gel but there is evidence that this is a reasonable assumption, as polymer-entrapping gelation is minimised and complete phase separation is allowed to develop by slowing down the rate of cooling of a binary solution from about 30 to 1°C/min (Alevisopoulos et al., 1996 where details can be found about the Takayanagi equations and the computerised algorithm used to generate Fig. 7). The model was used extensively to assess the effect of relative polymer concentration and conformational characteristics on the 'ergonomics' of phase separation in a mixture (Kasapis, 1995). Preparations containing a neutral polymer as one component (e.g. maltodextrin with gelatin or whey protein) or denatured globular proteins which gel upon reconstitution (e.g. milk and soya proteins) were studied using the solvent partition as the variable parameter.

In the present mixture, however, the strength of each gellan network is also a function, albeit disparate, of the salt distribution between the two phases. Based on the approximate equimolar density of carboxyl groups in the two gellan variants, our modelling assumes a uniform distribution of salt in the 'water lattice'. This keeps the normality of salt in each phase constant and allows construction of a baseline behaviour which follows the network strength as a function of polymer content. Initially, the quantitative treatment was implemented on mixtures of 100 mN NaCl and Fig. 6 illustrates the good linear relationships obtained for the single deacylated and high acyl gellan preparations at this level of salt. Use of the calibration curve of modulus versus concentration for the high acyl sample, however, implies that, upon cooling, gelation occurs simultaneously for both constituents which form networks at their effective concentrations as a result of greatly increased steric exclusion. Instead, the high acyl gellan network is formed first and is then deswelled as the gelation of the deacylated phase below 50°C (Fig. 5c) creates aggregated segments with elongated configurations. For the case of ionic networks with a relatively low degree of intermolecular associations the classic approach by Flory (1953) predicts that the modulus of the contracted network is a function of concentration in the power of 2/3 (deswelling factor). The high acyl gellan network appears to be an appropriate example for this methodology[1] which has been used in the present discussion in conjunction with the experimentally derived fit for the deacylated network (Fig. 6).

The modulus-concentration relationships for each polymer were used as part of a computation that spans every possible distribution of solvent between the two phases (yielding each time the corresponding effective polymer concentrations) until the estimated modulus matches the experimental network strength of the composite. Fig. 7a reproduces the computerised output for the upper and lower bounds of the mixture at 100 mN NaCl plotted against the solvent fraction in the deacylated gellan phase. In Fig. 5c it was argued that this macromolecular arrangement corresponds to a bicontinuous phase separated system which by definition behaves as an isostrain mechanical analogue (Takayanagi et al., 1963). Gratifyingly, the experimental value of the composite crosses the upper bound thus being congruent with the isostrain prediction. A straightforward calculation recasts this finding into the relative amount of water held at each polymer phase using the so-called p factor (Clark et al., 1983):

$$P = (S_{DG}/x_{DG})/(S_{AG}/x_{AG})$$

where S_{DG} and S_{AG} refer respectively to the amount of solvent in the phases of deacylated and high acyl gellan ($S_{DG} + S_{AG} = 1$), with x_{DG} and x_{AG} being their original (nominal) concentrations (0.5%). The p estimate is 1.2 which argues that the deacylated component captures 20% extra solvent in its network, and yields values of $\approx 0.9\%$

[1] In addition, the networks of high acyl gellan are permanent within our experimental timescale, i.e. they cannot retract by abolishing and reforming their junction zones under the swelling process of the deacylated phase. Thus high acyl gels fail by far to recover there network strength once they have been sheared.

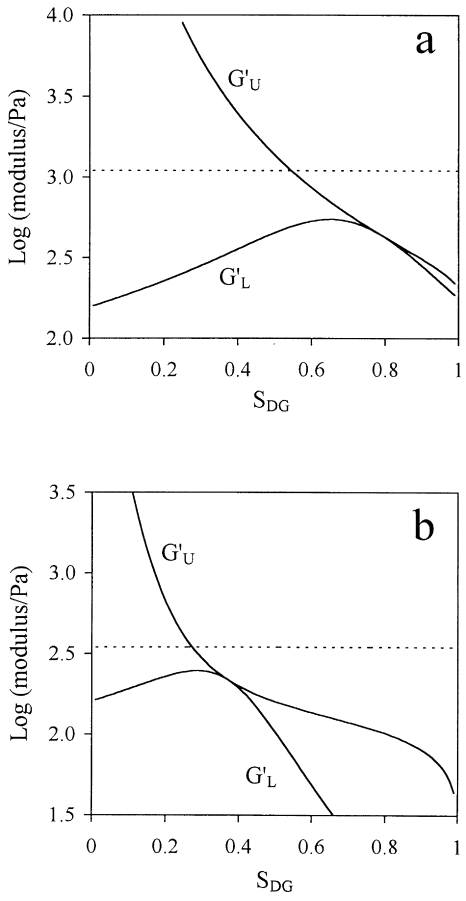

Fig. 7. Upper and lower bounds for the storage modulus of 0.5% deacylated gellan plus 0.5% high acyl gellan mixtures as a function of the solvent fraction of the deacylated phase at a) 100 and b) 50 mN NaCl. The experimental values of the composite gels are shown as dashed lines parallel to the abscissa (modelling and experimental values refer to 20°C).

and 1.1% for the final (effective) concentrations of the deacylated and high acyl gellan phases.

In previous explorations of the phase behaviour of composite gels we proposed that the topology of a network (filler or continuous phase) and its actual morphology (e.g. balanced cross-linking or precipitated gels) are the main determinants of the solvent partition in a binary mixture. Gradual increase in the concentration of the filler raises its phase volume and allows it to phase invert the mixture thus forming the continuous matrix which entraps relatively higher levels of solvent (Kasapis, 1995). Further, manipulation of the cooling rates triggers gelation and consolidation of one network at the expense of the other with the former claiming a favourable redistribution of solvent (Alevisopoulos et al., 1996). As mentioned in the introduction, the present system offers a third avenue of checking this hypothesis since dramatic transformations of the macrostructure of the deacylated gellan can be induced by varying the level of counterions. The above solvent partition ($p = 1.2$), it soon transpired, is not a general rule, as we shall demonstrate below for the case of 50 mN NaCl. Thus, 0.9% deacylated gellan with 50 mN added salt hardly gels (verified but not shown), a response which is not compatible with the second wave of structure recorded in the cooling profile of Fig. 5b.

To identify the pattern of solvent partition between the two phases at 50 mN NaCl, we derived calibration curves of modulus versus concentration for the individual polymers at this level of salt. Fig. 6 demonstrates the low sensitivity of high acyl structures to the ionic environment, with the network strength being primarily determined by the macromolecular content (coincidence of G' values at 50 and 100 mN NaCl). In the case of deacylated gellan, however, the presence of 100 as compared to 50 mN NaCl facilitates the formation of a functional (lower gelling concentrations) and cohesive (stronger networks) polymeric cobweb.

Fig. 7b illustrates the results of the blending-law analysis at 20°C carried out using the deswelling argument for the high acyl gellan and the experimental fit of G' versus concentration for the deacylated counterpart at 50 mN NaCl. The experimental modulus of the mixture in the presence of 50 mN salt remains well above the values of the lower bound and intersects the trace calculated for the isostrain arrangement at the S_{DG} point of 0.27. Thus the effective concentrations of deacylated and high acyl phases are 1.85% and 0.69%, respectively, with their mechanical strengths being equal to 0.77 and 0.2 kPa. Therefore, the upper bound prediction in combination with the phase characteristics of the mixture suggest that the second step of structure formation in Fig. 5b generates a continuous phase for the deacylated gellan.

In terms of the solvent partition, a p value of about 0.4 was obtained, corresponding to the deacylated component capturing only 40% of the solvent held in the high acyl phase. This, of course, is totally different from the previous estimate at 100 mN NaCl ($p = 1.2$). In agreement with previous investigations on binary biopolymer mixtures, there is a stark contrast in the distribution of solvent which can be rationalised on the basis of the direct contribution of added counterions to the cohesion of deacylated gels. Increasing the salt content from 50 to 100 mN encourages the formation of an extended network of deacylated threads which can exclude effectively the high acyl chains thus claiming most of the solvent in the system. This outcome further reinforces the hypothesis that the phase arrangement and the viscoelasticity of a network will control the phase volume and hence its share of solvent in a binary mixture.

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