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0144-8617(95)00026-7

*Carbohydrate Polymers* **27** (1995) 23–36

0144-8617/95/$09.50+.00

# Solubility, solution rheology and salt-induced gelation of welan polysaccharide in organic solvents

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(Received 15 November 1994; revised version received 4 February 1995; accepted 8 February 1995)

Aqueous solutions of welan show gel-like rheology, including a $c^2$-dependence of $G'$ (where $c$ is concentration and $G'$ is elastic modulus). Network formation is attributed to weak interactions between ordered species in the double-helical conformation recently characterised by X-ray fibre diffraction in the solid state. High concentrations of salt cause only a slight increase in modulus. Solutions of welan in dimethylsulphoxide (DMSO), by contrast, have properties broadly similar to those of disordered polysaccharides in water. Loss of 'weak-gel' character in mixed solvents of DMSO and water occurs over a narrow range of composition (between ~86 and ~91% v/v DMSO for 1% w/v welan, increasing somewhat at higher concentrations of polymer).

Addition of salt to disordered welan (in 91% DMSO) causes rapid re-ordering, with slower development of a 'true' gel network by cation-mediated helix–helix aggregation. On exposure to excess water, the gels swell (attributed to reversal of the aggregation process) but remain intact (attributed to direct crosslinking of chains through stable double helices). Gels formed by addition of salt to ordered welan (in 80% DMSO), by contrast, dissolve completely on immersion in water. At moderate salt concentration (100 mM NaCl), both types of network revert to 'weak gels' on heating to ~80°C, but those formed from the disordered state (in 91% DMSO) show a second melting process at higher temperature, attributed to loss of double-helix structure in this more destabilising solvent. Both processes are reversible on cooling, with the thermal hysteresis anticipated for an aggregating system, giving stronger networks than were obtained initially by direct addition of salt.

The solubility of ordered welan in polar organic solvents (ethylene glycol and aqueous DMSO) decreases sharply with increasing concentration of salt. This behaviour, and the salt-induced gelation, are attributed to enhancement of electrostatic attraction between negatively charged helices and positive counterions in solvents with dielectric constants substantially lower than that of water.

## INTRODUCTION

Most of the polysaccharides currently available as 'industrial gums' have a long history of use, often dating back hundreds or, in some cases thousands of years (Whistler & BeMiller, 1993). A notable exception is xanthan, the anionic exopolysaccharide from *Xanthomonas campestris*. Xanthan was discovered during a systematic programme of bacterial screening carried out in the 1950s by Dr Allene Jeanes and her colleagues in the USDA Northern Regional Research Laboratories (Jeanes *et al.*, 1961). It was singled out for detailed study because of its unusual rheological properties, which combined gel-like character at rest with the ability to flow freely under stress. The origin of this 'weak gel' behaviour, which was then unique to xanthan, was subsequently traced to an ordered conformation in solution (Morris, 1973; Holzwarth, 1976; Morris *et al.*, 1977), allowing a tenuous network to be formed by 'low-enthalpy low-entropy' interactions between the rigid strands (Ross-Murphy *et al.*, 1983; Morris, 1991).

Xanthan was developed as a commercial material by Kelco (now a unit of the Monsanto Corporation). Its first major industrial application was in oil recovery, with subsequent clearance for food use. More recently, Kelco conducted an in-house screening programme which identified several other microbial polysaccharides with potential for commercial exploitation (Pettitt,

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1986). It was later found that these new 'biogums', although covering a wide spectrum of physical properties, have the same polymer backbone, a tetrasaccharide repeating sequence (O'Neill *et al.*, 1983; Jansson *et al.*, 1983) of: →3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→. The differences in physical properties arise from the presence of different sidechains or substituent groups.

Welan (which is produced by *Alcaligenes* ATCC 31555 and was formerly known as S-130) has a single-sugar sidechain at O(3) of the 4-linked glucose (O'Neill *et al.*, 1986). The sugar may be either α-L-rhamnose or α-L-mannose, in the approximate ratio 2:1. At least 85% of the repeat units also have an acetyl substituent (Stankowski & Zeller, 1992) at O(2) of the 3-linked glucose. Welan has 'weak gel' solution properties similar to those of xanthan and, like xanthan, was developed initially for use in the oil industry (as indicated by the name). An important characteristic of welan for oilfield applications (e.g. in drilling muds or tertiary recovery) is that its solution rheology remains stable to very high temperature (above ~150°C). Welan is also soluble, and retains its useful rheology, in certain polar organic solvents, opening up a number of other areas of potential major industrial application (e.g. for coating aircraft wings and control surfaces to inhibit build-up of ice).

The present work began with an investigation of why some production batches of welan were insoluble in ethylene glycol while others dissolved readily. The study was widened to other polar organic solvents, and it was found that the viscosity of welan in dimethylsulphoxide (DMSO) was grossly lower than in water (or ethylene glycol), and that the characteristic 'weak gel' properties were abolished. Loss of viscosity in mixed solvents of water and DMSO was found to occur very sharply, between ~85% and 90% v/v DMSO (for 0·5% w/v welan). Solutions prepared at a DMSO concentration just below the critical range showed a typical order–disorder transition on heating, which was fully reversible on cooling. These findings are reported in detail elsewhere (Hember *et al.*, 1994).

We present here a brief account of the solubility of welan in ethylene glycol, followed by more extensive coverage of its behaviour in DMSO and in DMSO–water mixtures. The central conclusion is that ionic interactions have a far greater influence in these organic solvents than in water.

## MATERIALS AND METHODS

Welan samples from three different production batches were kindly supplied by Kelco. Sample 1 (batch number 88074A) was the material used in previous investigations (Robinson *et al.*, 1991; Hember *et al.*, 1994). Sample 2 (batch number 43221A) was selected as representative of welan with good solubility in ethylene glycol. Sample 3 (batch number 424-109) was similarly selected as having very poor solubility in ethylene glycol. DMSO (minimum purity 99·5%) was purchased from Sigma. Salts were Analar grade from BDH. Solutions of welan were prepared by mechanical stirring at ambient temperature, and were made up on a weight-per-volume basis to give equivalent polymer concentrations in solvents of different densities.

Dilute solution viscosity was measured on a Contraves Low Shear 30 viscometer, using cup-and-bob geometry with inner and outer radii of 5·5 and 6·0 mm. Viscosity measurements at higher concentrations were made with cone-and-plate geometry (50 mm diameter; 2° cone-angle) on a Sangamo Viscoelastic Analyser. Rheological response to low-amplitude oscillatory shear was characterised using cone-and-plate geometry (diameter 50 mm; cone angle 0·05 rad) on a sensitive prototype rheometer designed and constructed in this department by Dr R.K. Richardson. To circumvent problems of thermal expansion/contraction during heating and cooling scans, 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 retaining constant strain across the outer portion (which constitutes 80% of the total area). The periphery of the sample was coated with light silicone oil to minimise loss of solvent or absorption of atmospheric moisture. Temperature was controlled by a Haake circulating water bath and measured with a thermocouple attached to the stationary element. A fixed heating or cooling rate of 1 deg/min was used when monitoring the temperature course of rheological change. Compression-testing was carried out on a TA-XT2 Texture Profile Analyser from Stable Microsystems. Gel samples were cast in lubricated cylindrical moulds (10 mm diameter; 12 mm height) sealed by glass endplates. Force–deformation curves (25 data points per second) were recorded under uniaxial compression (0·7 mm per second) at 20°C.

Dielectric constants were determined by measurement of sample capacitance (1 MHz; 20 ± 1°C) between parallel nickel plates (area ≈11 $cm^2$; separation ≈19 mm) on a Hewlett-Packard precision LCR meter (type 4284A). Correction was made for the capacitance of the empty cell (< 3% of the total).

## SOLUBILITY IN ETHYLENE GLYCOL

Sample 2 dissolved readily in ethylene glycol ($CH_2OH$-$CH_2OH$), giving solutions with obvious gel-like character (immobilisation of air bubbles and suspension of small particles). Sample 1, used as standard in our previous research (Robinson *et al.*, 1991; Hember *et al.*, 1994) and in the studies of performance in DMSO/water systems reported below, was also soluble in ethylene glycol, giving mechanical spectra similar to those

obtained in water, but with significant enhancement of moduli (Fig. 1).

The mechanical properties in both solvents are typical of a 'weak gel'. $G'$ (the 'storage modulus', characterising solid-like, elastic response) is substantially higher than $G''$ (the 'loss modulus', which characterises viscous flow) and both moduli show only slight variation with frequency, in contrast to the strong frequency dependence seen for normal polymer solutions. The variation of 'complex dynamic viscosity' ($\eta^*$) with frequency ($\omega$) is also very different from that of normal, conformationally disordered, polysaccharides; log $\eta^*$ vs log $\omega$ is essentially linear, with no indication of levelling out to a horizontal 'Newtonian plateau' at low frequency, and has a slope substantially steeper than the maximum value of −0·76 observed (Morris, 1990) for polysaccharide coils interacting by topological entanglement (−0·82 for sample 1 at 1% w/v and 25°C). The spectra obtained for sample 2 were closely similar in form, but offset to higher values (by about a factor of 2), probably due to a higher molecular weight in this (later) production batch.

Gel-like character is still clearly evident (Fig. 2) in solutions of welan in ethylene glycol at 95°C (the highest temperature at which reliable measurements could be made), but with an approximately 2-fold reduction in absolute values at equivalent frequencies and a slight decrease in the ratio of $G'$ (solid-like character) to $G''$ (liquid-like response).

Sample 3 was readily soluble in water, but showed no detectable solubility in ethylene glycol. Its behaviour when dispersed (at 0·5% w/v) in mixtures of the two solvents was assessed by visual inspection. At a 4:1 (v/v) mixing ratio of ethylene glycol:water, the individual grains of the welan powder swelled slightly, but they remained as discrete particles and there was no significant development of viscosity. When the ethylene glycol content of the mixed solvent was reduced to 3:1, only the cores of the particles remained solid, and sufficient polymer dissolved to give clearly discernable thickening. After storage at ambient temperature for 3 months, the sample had formed a self-supporting gel network. On further reduction of the ethylene glycol content to 2:1, most of the polymer dissolved, giving a viscous solution with 'weak gel' properties (suspension of bubbles) similar to those of welan in water. Gelation was again observed on storage.

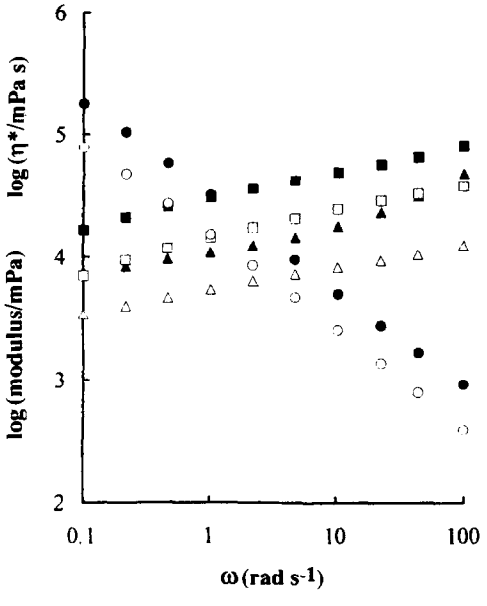


**Fig. 1.** Mechanical spectra (2% strain) for welan (sample 1; 1% w/v; 25°C) in water (open symbols) and in ethylene glycol (filled symbols), showing the variation of $G'$ (squares), $G''$ (triangles) and $\eta^*$ (circles) with frequency ($\omega$).

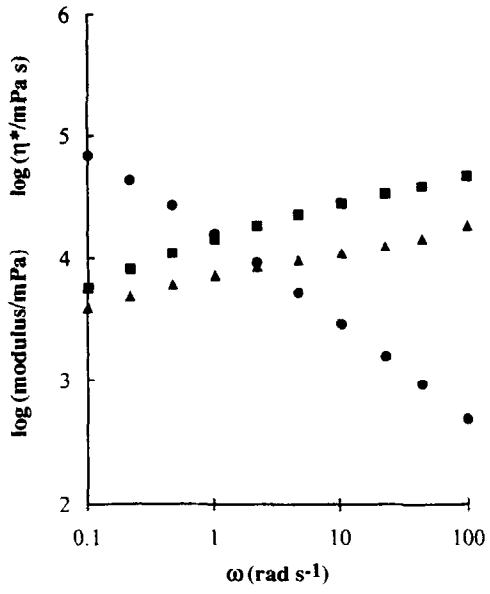


**Fig. 2.** Mechanical spectrum (2% strain) showing the frequency dependence of $G'$ (■), $G''$ (▲) and $\eta^*$ (●) for welan (sample 1; 1% w/v) in ethylene glycol at 95°C.

In a converse series of experiments, the polymer was first dissolved in water and then mixed with ethylene glycol (with volumes arranged to again give a final welan concentration of 0·5% w/v). At 4:1 mixing ratio, some of the polymer precipitated when the ethylene glycol was introduced, but the solution retained appreciable viscosity. At lower levels of addition (3:1 and 2:1 ethylene glycol:water) there was no precipitation, and the 'weak gel' properties were more evident than in the solution prepared by direct addition of sample 3 to 2:1 ethylene glycol:water, as would be expected from the higher concentration of dissolved polymer. All three preparations gelled on storage, but there was a progressive increase in the strength and cohesiveness of the gels with increasing concentration of ethylene glycol.

The most striking observation from these experiments is that welan, which does not gel in water (although giving 'weak gel' properties in aqueous solution), can form 'true' gel networks in the presence of ethylene glycol. It is also clear that its solubility in mixtures of

ethylene glycol and water is controlled by kinetic factors as well as by equilibrium thermodynamics, since the amount of pre-dissolved polymer remaining in solution after addition of ethylene glycol was consistently higher than the amount that would dissolve on prolonged exposure to the same mixed solvent.

Gelation from these 'supersaturated' solutions can be explained as slow reversion towards a stable equilibrium between associated and solvated chain segments. Gelation of polymer dissolved directly in mixtures of ethylene glycol and water cannot, however, be explained in the same way. A likely interpretation is that the gel state represents the best compromise (in terms of overall free energy) between the enthalpic advantage of chain-packing (maximised in the solid state) and the entropic advantage of polymer mobility (maximised in solution).

The initial aim of the investigation, however, was to trace the origin of the striking differences in solubility of different welan samples in ethylene glycol. One possibility we considered was that differences might arise from subtle variations in primary structure (such as the extent or pattern of acetylation, or the sequence of rhamnose and mannose sidechains). Another, simpler source of variation between samples, however, is the content and type of counterions and salt carried over into the final product from the fermentation and extraction processes.

To explore the effect of ion content on solubility, an aqueous solution of sample 3 (which, in its original form, is insoluble in ethylene glycol) was dialysed extensively against deionised water, and freeze dried. The final product was found to be readily soluble in ethylene glycol, giving a viscous solution (at 0·5% w/v) with typical weak gel properties. In a converse experiment, sample 2 (which has good solubility in ethylene glycol) was dissolved at 0·5% w/v in deionised water, calcium chloride (~3 mM) was added, and the solution was freeze dried. The resulting material was almost totally insoluble in ethylene glycol.

The obvious conclusion that salts, and in particular divalent cations, decrease the solubility of welan in ethylene glycol agrees with the findings of a concurrent investigation by Kelco, which showed that solubility can be promoted by EDTA (Baird & Talashek, 1992).

## SOLUTION PROPERTIES IN DMSO

As reported previously (Hember *et al.*, 1994), dissolving welan in DMSO, rather than in water, causes a gross reduction in viscosity, in contrast to the slight enhancement observed (Fig. 1) in ethylene glycol. The characteristic 'weak gel' response in mechanical spectroscopy is also replaced (Fig. 3) by behaviour similar to that of disordered polysaccharides in water, with $G'' > G'$, strong frequency dependence in both moduli, and a Newtonian plateau in $\eta^*$ at low frequency.

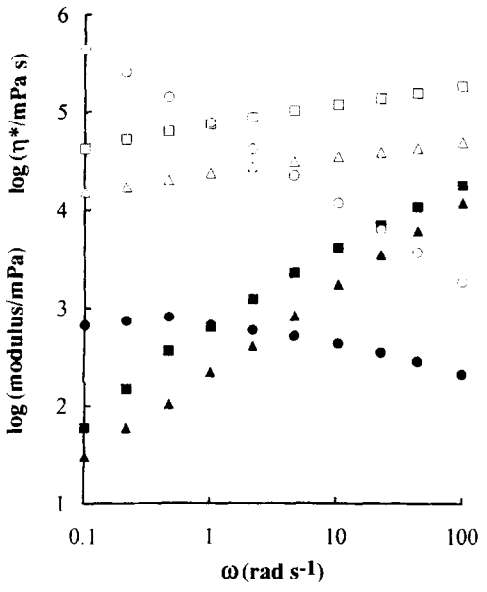


**Fig. 3.** Mechanical spectra (2% strain) for welan (sample 1; 1% w/v; 25°C) in water (open symbols) and in DMSO (filled symbols), showing the variation of $G'$ (squares), $G''$ (triangles) and $\eta^*$ (circles) with frequency ($\omega$).

Newtonian response at low rates of deformation is also clearly evident (Fig. 4) in the shear rate ($\dot{\gamma}$) dependence of steady shear viscosity ($\eta$). The reduction in viscosity at higher rates, however, is somewhat different from that observed for disordered polysaccharides in water. Experimental 'flow curves' for entangled polysaccharide coils can be matched with good precision (Morris, 1990) by the equation:

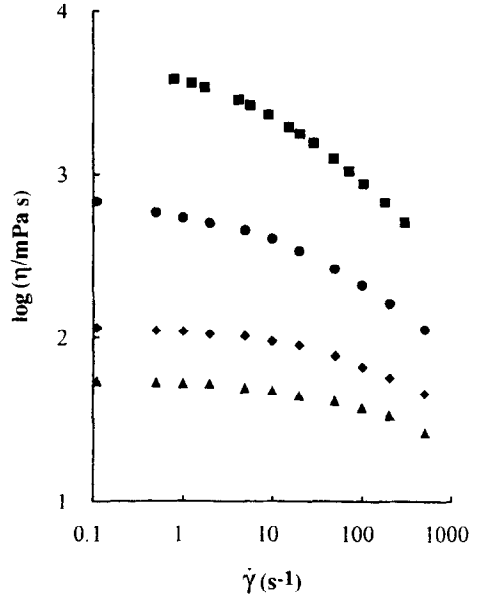


**Fig. 4.** Shear thinning of welan (sample 1; 25°C) in DMSO, showing the decrease in steady shear viscosity ($\eta$) with increasing shear rate ($\dot{\gamma}$) for polymer concentrations (% w/v) of 4 (■), 2 (●), 1 (◆) and 0·5 (▲).

$$\eta = \eta_0/[1 + (\dot{\gamma}/\dot{\gamma}_{1/2})^p], \quad (1)$$

where $\eta_0$ is the maximum 'Newtonian' viscosity at low shear rate and $\dot{\gamma}_{1/2}$ is the shear rate required to reduce the viscosity to $\eta_0/2$. The equation can be recast in the form of a linear relationship between $\eta$ and $\eta\dot{\gamma}^p$:

$$\eta = \eta_0 - [(1/\dot{\gamma}_{1/2})^p]\eta\dot{\gamma}^p. \quad (2)$$

It is found empirically that $p$ has a constant value of 0·76 for samples of wide polydispersity interacting solely by topological entanglement. Thus solutions of most commercial polysaccharide 'thickeners' give linear plots of $\eta$ vs $\eta\dot{\gamma}^{0.76}$, with slope $-(1/\dot{\gamma}_{1/2})^{0.76}$ and intercept $\eta_0$.

As illustrated in Fig. 5, corresponding plots for welan in DMSO showed obvious curvature. The deviation from 'normal' polysaccharide behaviour was quantified using a simple computer program to vary the parameters $\eta_0$, $\dot{\gamma}_{1/2}$ and $p$ in eqn (1) until the root-mean-square difference between observed and fitted values of $\eta$ was minimised. The best fit to the data for the highest concentration of welan shown in Fig. 4 (4% w/v) was obtained with $p = 0{\cdot}58$. Substituting this value in eqn (2) then gives a good linear plot for $\eta$ vs $\eta\dot{\gamma}^p$ (Fig. 5). Similar values were obtained for lower concentrations of polymer, but with a less robust fit because of the smaller variation in $\eta$. Thus although the flow properties of welan in DMSO are broadly similar to those of normal disordered polysaccharides in aqueous solution, the precise form of shear thinning is significantly different.

The concentration dependence of viscosity is also broadly similar to that of disordered polysaccharides in water, but again differs in detail. Figure 6 shows the maximum 'zero shear' viscosity ($\eta_0$) for welan in DMSO, expressed as specific viscosity ($\eta_{sp} = (\eta - \eta_S)/\eta_S$, where $\eta_S$ is the viscosity of the solvent) and plotted

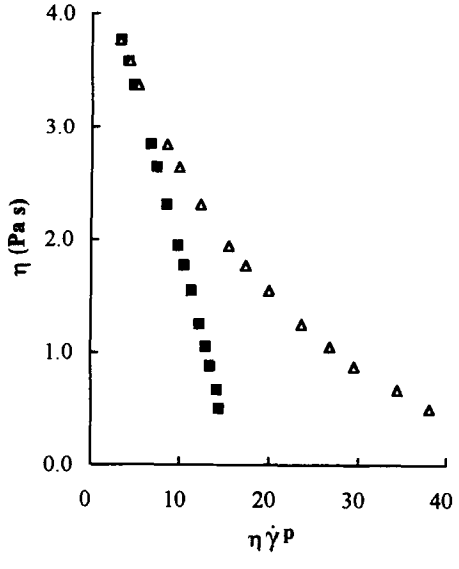


**Fig. 5.** Shear rate ($\dot{\gamma}$) dependence of viscosity ($\eta$) for welan (4% w/v) in DMSO (25°C) plotted according to eqn (2), with $p = 0{\cdot}76$ (△) and $p = 0{\cdot}58$ (■).

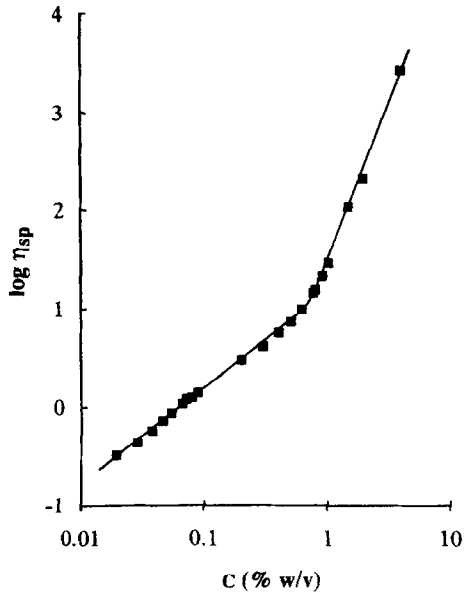


**Fig. 6.** Concentration dependence of 'zero shear' specific viscosity (25°C) for welan in DMSO.

double-logarithmically against concentration ($c$). Analogous plots for disordered polysaccharide coils fall into two linear regions, with an abrupt change of slope (from ~1·4 to ~3·3) at $\eta_{sp} \approx 10$, as the individual coils overlap to form an entangled network (Morris *et al.*, 1981). The 'breakpoint' in Fig. 6 also occurs at $\eta_{sp} \approx 10$, and the slope at higher concentrations is again $\approx 3{\cdot}3$. The slope of log $\eta_{sp}$ vs log $c$ for the dilute solution regime, however, is lower than normal, having a value of ~1·0, so that the 'reduced viscosity' ($\eta_{sp}/c$) remains essentially constant up to the onset of entanglement.

The origin of these departures from typical 'random coil' behaviour will be discussed later, in the light of our findings on the effect of solvent composition and salt.

## RHEOLOGY IN AQUEOUS SOLUTION

As shown in Fig. 7, measured values of log $G'$ for 'weak gels' of welan (sample 1) in water at ambient temperature increase linearly with the logarithm of polymer concentration. The slope of the double-logarithmic plots is close to 2 (ranging from ~1·9 for $G'$ values at 100 rad $s^{-1}$ to ~2·3 at 0·1 rad $s^{-1}$). Thus the elastic response of welan in aqueous solution is approximately proportional to the square of its concentration. A $c^2$-dependence of modulus is often observed for 'true' gelling systems, and is anticipated theoretically (Clark & Ross-Murphy, 1987) for biopolymers with a large number of potential binding sites along each chain (i.e. high 'functionality') at concentrations substantially higher than the minimum required for formation of a continuous network (i.e. at $c \gg c_0$, the minimum critical gelling concentration).

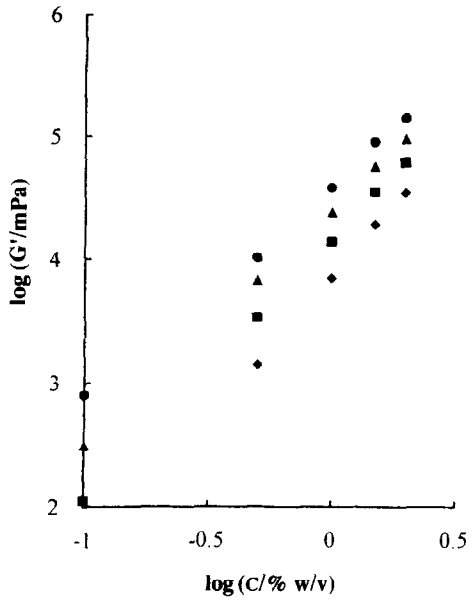


**Fig. 7.** Concentration dependence of $G'$ (25°C; 2% strain) for welan in water, measured at frequencies (rad $s^{-1}$) of 0·1 (◆), 1·0 (■), 10 (▲) and 100 (●).

At constant polymer concentration, the modulus is raised (Fig. 8) by addition of salt, indicating enhanced association of welan helices by suppression of electrostatic repulsion and/or cation-mediated aggregation. The magnitude of the enhancement, however, is slight (~25% increase between $G'$ values for 2% welan in water and in 1 M NaCl).

At lower concentrations, in the range used for determination of intrinsic viscosity ($0{\cdot}2 < \eta_{sp} < 1{\cdot}0$), the viscosity of welan decreases with increasing ionic strength. Such behaviour is general for dilute solutions of polyelectrolytes, and is caused by suppression of intramolecular electrostatic repulsions, allowing the molecules to adopt a more compact conformation. Indeed, the magnitude of the reduction in intrinsic viscosity with increasing ionic strength can be used as a convenient index of chain stiffness (Smidsrød & Haug, 1971).

Previous studies are in agreement in finding a very small salt dependence of intrinsic viscosity for welan, consistent with our recent direct evidence that it exists in aqueous solution in a rigid, ordered conformation (Hember *et al.*, 1994). The absolute values of intrinsic viscosity measured at low shear rate (Robinson *et al.*, 1991), however, are substantially higher (by about a factor of 2) than those obtained using capillary viscometers (Crescenzi *et al.*, 1987; Urbani & Brant, 1989).

The origin of the discrepancy is demonstrated in Fig. 9. Dilute aqueous solutions of welan show obvious shear thinning even at shear rates very much lower than those generated in capillary viscometers (typically $\sim 1000\,s^{-1}$), in contrast to the essentially Newtonian behaviour of disordered polymers over the same range of viscosity. The reduction in viscosity of welan with increasing shear rate is probably due to progressive alignment of the stiff, ordered species in the direction of flow.

A more pronounced example of the same effect has been reported previously (Robinson *et al.*, 1991) for another polysaccharide in the gellan series, rhamsan. The intrinsic viscosity of rhamsan, measured in the Newtonian plateau region at low shear rate, is huge ($[\eta] \approx 150\,dl\,g^{-1}$), but the values obtained from capil-

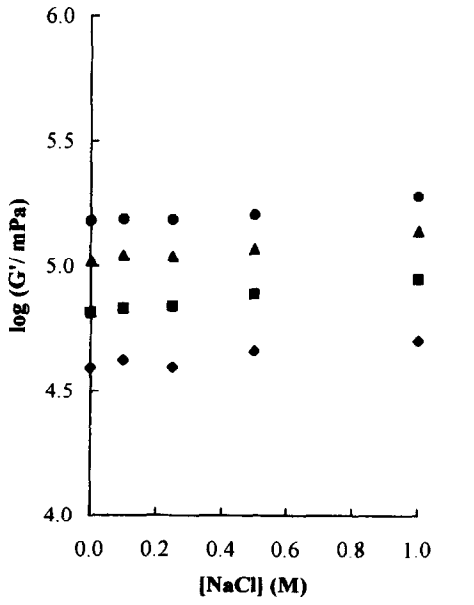


**Fig. 8.** Effect of salt concentration on the 'weak gel' properties of welan (2% w/v; 25°C) in water, as monitored by measurements of $G'$ (2% strain) at frequencies (rad $s^{-1}$) of 0·1 (◆), 1·0 (■), 10 (▲) and 100 (●).

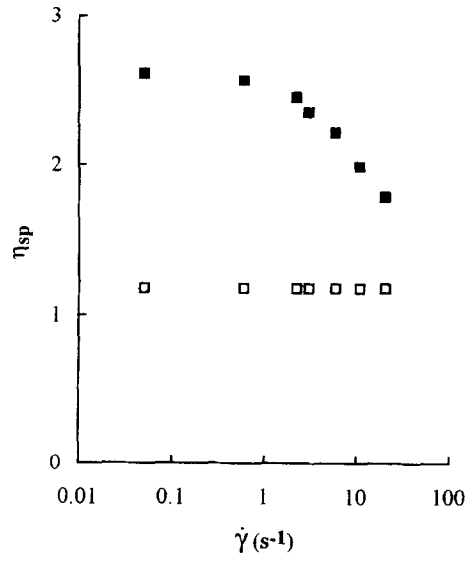


**Fig. 9.** Shear rate ($\dot{\gamma}$) dependence of specific viscosity ($\eta_{sp}$) for ordered and disordered welan in dilute solution (25°C), illustrated for polymer concentrations of 0·0125% w/v in water (■) and 0·015% w/v in DMSO (□).

lary measurements (Crescenzi *et al.*, 1987) are about 20 times smaller. When the ordered structure of welan is disrupted by dissolving the polymer in DMSO rather than in water, however, the dilute-solution viscosity is essentially Newtonian (Fig. 9), as found for 'random coil' polysaccharides in aqueous solution.

## RHEOLOGY IN DMSO–WATER MIXTURES

Figure 10 shows the response of welan (2% w/v) to changes in solvent composition in mixtures of water and DMSO. At DMSO concentrations up to ~91% v/v, $G'$ remains constant at the value observed for the 'weak gel' state in pure water. On slight further increase of DMSO content, however, the modulus drops sharply, reaching the value observed in pure DMSO by ~94% v/v (i.e. loss of 'weak gel' structure at this concentration of welan is centred around 12:1 DMSO:water). The reduction in $G'$ is accompanied by a smaller reduction in $G''$, and by a sharp increase in tan $\delta$ ($G''/G'$).

As shown in Fig. 11, there is also a substantial decrease in steady shear viscosity over the same range of solvent composition, and this was used to follow loss of structure at lower concentrations of welan, where the solutions in DMSO have moduli too low to be measured with acceptable precision under low-amplitude oscillation.

On decreasing the polymer concentration from 2 to 1% w/v, viscosity reduction occurred at a lower content of DMSO, centred around 8:1 DMSO:water (~89% v/v DMSO) rather than 12:1 (~92% v/v). At lower concentration of welan (0·5% w/v), however, there was

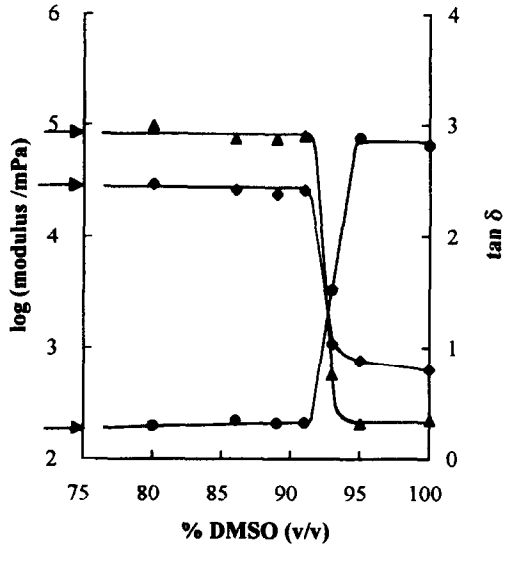


**Fig. 10.** Variation of $G'$ (▲), $G''$ (◆) and tan $\delta$ (●) with solvent composition for 2% w/v welan (25°C) in mixtures of DMSO and water. Measurements were made at 1 rad $s^{-1}$ and 2% strain. The arrows at the left-hand axis show the values obtained in pure water.

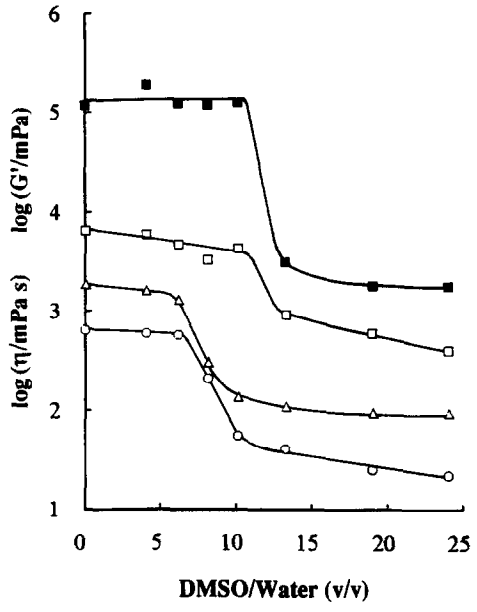


**Fig. 11.** Variation of solution viscosity (10 $s^{-1}$; 25°C) with solvent composition in mixtures of DMSO and water for welan at concentrations (% w/v) of 0·5 (○), 1·0 (△) and 2·0 (□). The variation of $G'$ (1 rad $s^{-1}$; 2% strain; Fig. 10) for the 2% solutions is included for direct comparison (■).

no significant further change in the concentration of DMSO required to destabilise the ordered structure.

## DIELECTRIC CONSTANT OF DMSO–WATER MIXTURES

The effectiveness of water as a solvent for polysaccharides may be explained in part by its ability to form bonds with the hydroxyl groups of the sugar rings, in competition with bonding between the polymer chains. Another important consideration, however, is that water has an extremely high relative permittivity or 'dielectric constant' ($\varepsilon_r = 80{\cdot}4$, in comparison with 1·0 *in vacuo*), thus drastically reducing the force ($F$) between charged or partially charged (i.e. polar) groups:

$$F = q_1 q_2 / r^2 \varepsilon_r \varepsilon_0, \qquad (3)$$

where $r$ is the separation between charges $q_1$ and $q_2$, and $\varepsilon_0$ is the vacuum permittivity.

The dielectric constant of DMSO ($\varepsilon_r = 48$), although higher than those of most other organic solvents, is substantially lower than the value for water. Electrostatic interactions would, therefore, be expected to have a greater effect in DMSO, and in DMSO/water mixtures, than in pure water.

The variation in dielectric constant with solvent composition was quantified experimentally from capacitance measurements on a range of DMSO–water mixtures, using the literature values for the pure materials to obtain the constant of proportionality between

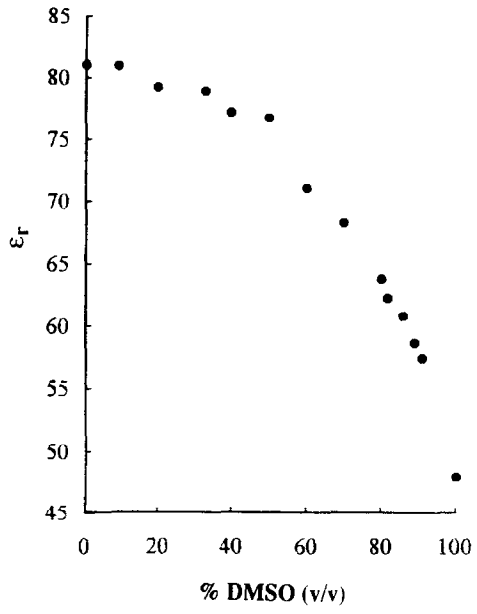


**Fig. 12.** Composition dependence of dielectric constant ($\varepsilon_r$) for mixtures of DMSO and water (20 ± 1 °C).

measured capacitance and $\varepsilon_r$. As shown in Fig. 12, the dielectric constant remains close to the value for pure water up to ~50% v/v DMSO, but then shows an increasingly rapid fall towards the lower value for pure DMSO, with the critical range of solvent composition for 'denaturation' of welan (Fig. 11) falling in the region of steepest descent (around 90% v/v).

## SOLUBILITY OF WELAN IN AQUEOUS DMSO

It is evident from Fig. 8 that salt has little effect on welan in aqueous solution. As described above, however, it has a dominant influence on solubility in ethylene glycol which, like DMSO, has a much lower dielectric constant than water ($\varepsilon_r$ = 37·7). We therefore carried out a brief investigation of the effect of salt on the solubility of welan in mixtures of DMSO and water.

The solvent used was 4:1 DMSO:water. This has sufficient water to preserve the ordered structure of the polymer (Fig. 11) and to dissolve high concentrations of salt, while still having a much lower dielectric constant than water alone (Fig. 12). Welan (sample 1; 1% w/v) was dispersed in preparations of this solvent, containing 5, 10, 20, 30, 50 and 100 mM NaCl, and the extent of dissolution was observed by visual inspection.

At salt concentrations up to 20 mM, the polymer dissolved completely. Partial dissolution was also observed at 30 mM, but at higher concentrations (50 and 100 mM) solvation was limited to swelling of the welan powder. The progressive loss of solubility with increasing concentration of salt is demonstrated directly in Fig. 13, which shows photographs of the samples after prolonged (12 months) storage.

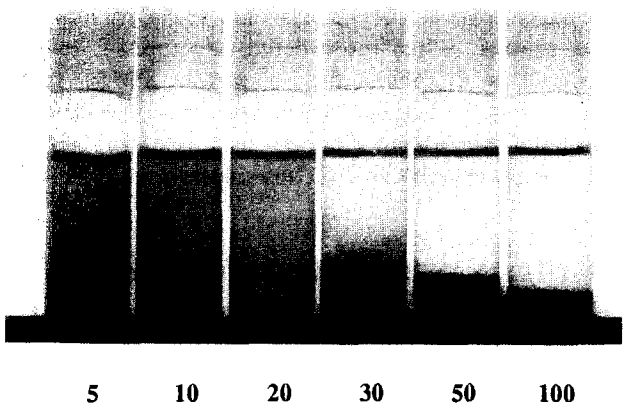


**Fig. 13.** Effect of salt on the solubility of welan in 80% v/v DMSO. The samples were photographed about 1 year after dispersal of welan powder (sample 1) in the mixed solvent. The concentration (mM) of salt (NaCl) present is shown under each vial.

## SALT-INDUCED GELATION OF ORDERED WELAN

Having established that salt causes a drastic reduction in the solubility of welan in 80% DMSO, we next explored the effect of direct addition of salt to welan pre-dissolved in the same solvent. The procedure used was as follows. A plastic vial was charged electrostatically by mechanical friction, and the inner surface coated with the required amount of salt (NaCl) ground to a fine powder in a mortar and pestle. Welan solution (sample 1; 1% w/v in 4:1 DSMO:water) was then injected into the vial by syringe, and stirred vigorously to disperse the salt. The resulting solution was loaded immediately onto the rheometer, and the first readings obtained within ~1 min of initial mixing.

As shown in Fig. 14, there was a rapid increase in $G'$ over the first 10–15 min, with the modulus then levelling out towards a final, stable value within ~1 h. Increasing the concentration of salt gave an almost proportional increase in the final value of $G'$ (Fig. 15), with a corresponding systematic reduction in tan $\delta$.

10 mM NaCl was sufficient to give a self-supporting 'true' gel network (as judged by visual inspection). By 100 mM, the gels were strong enough to be removed from cylindrical moulds and characterised by compression testing. Figure 16 shows an illustrative force-deformation profile, which is typical of the failure properties of a normal polysaccharide gel. At the highest salt concentration studied (500 mM) gelation occurred before the moulds could be completely filled.

As shown in Fig. 17, raising the temperature of the gels formed with 100 mM NaCl caused a progressive reduction of $G'$ towards the value found for the salt-free 'weak gel' state, with an accompanying smaller reduction in $G''$. At higher salt (500 mM NaCl) network collapse does not occur until higher temperature

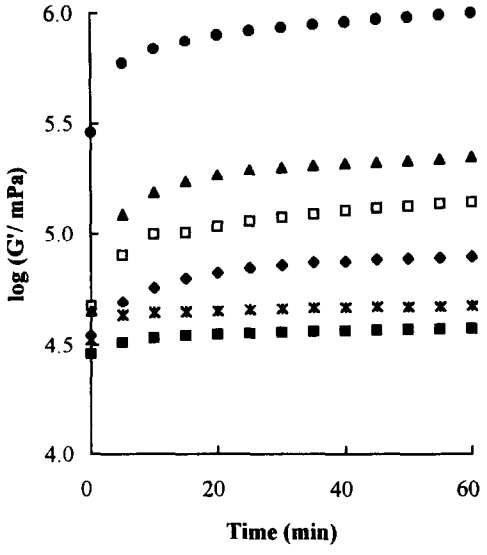


**Fig. 14.** Time course of gelation (as monitored by measurements of $G'$ at 1 rad $s^{-1}$ and 2% strain) for ordered welan (1% w/v in 80% v/v DMSO; 25°C) after rapid mixing with finely powdered salt (NaCl) at concentrations (mM) of 500 (●), 100 (▲), 50 (□), 25 (◆), 10 (*) and 0 (■).

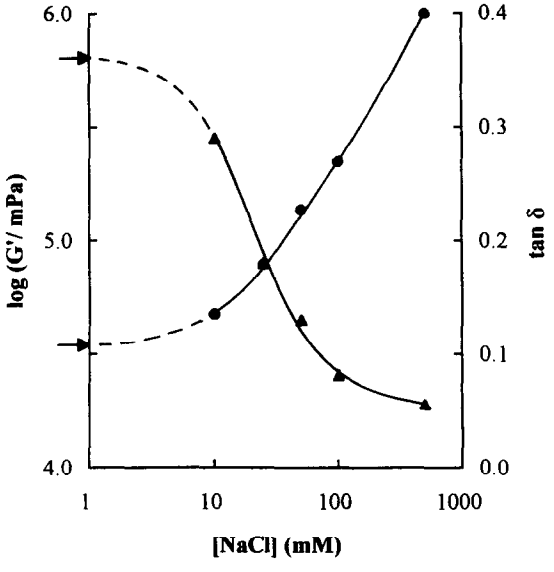


**Fig. 15.** Salt dependence of $G'$ (●) and tan $\delta$ (▲) for welan (1% w/v; 25°C) in 80% v/v DMSO. Measurements were made at 1 rad $s^{-1}$ and 2% strain, 60 min after rapid mixing of finely powdered NaCl with the polymer solution. The arrows at the left-hand axis show values obtained in the absence of added salt.

(Fig. 17), and is obviously incomplete at the highest temperature at which measurements could be made (~95°C).

On cooling the partially melted gel (Fig. 18) the network re-forms, although at somewhat lower temperature (by ~10°), and becomes stronger (higher values of $G'$ and $G''$) than the gel obtained initially by direct addition of salt (presumably due to formation of a more homogeneous network). The extent of hysteresis

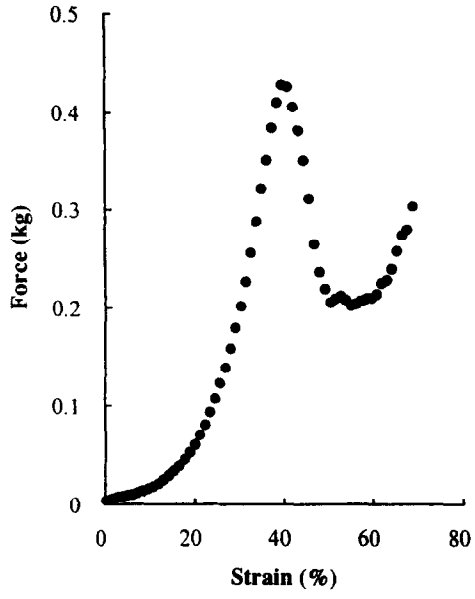


**Fig. 16.** Illustrative force–deformation curve for 1% for 1% w/v welan in 80% w/v DMSO 3 h after rapid mixing with finely powdered salt (100 mM NaCl). Measurements (20°C) were made by compression (0·7 mm per second) of a cylindrical sample (10 mm diameter; 12 mm height) between parallel plates.

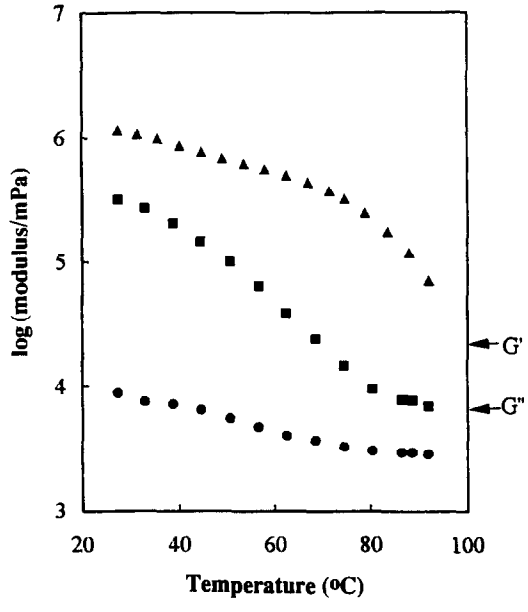


**Fig. 17.** Reduction in $G'$ (■) and $G''$ (●) on heating (1°/min) for 1% w/v welan in 80% w/v DMSO 60 min after rapid mixing with finely powdered salt (100 mM NaCl). The arrows at the right-hand axis show the corresponding moduli for the 'weak gel' state (1% w/v welan in water at 25°C). The temperature dependence of $G'$ at higher salt (500 mM NaCl) is also shown (▲). Measurements were made at 1 rad $s^{-1}$ and 2% strain.

between heating and cooling scans and the enhancement of moduli by thermal 'annealing' both decreased with decreasing concentration of salt.

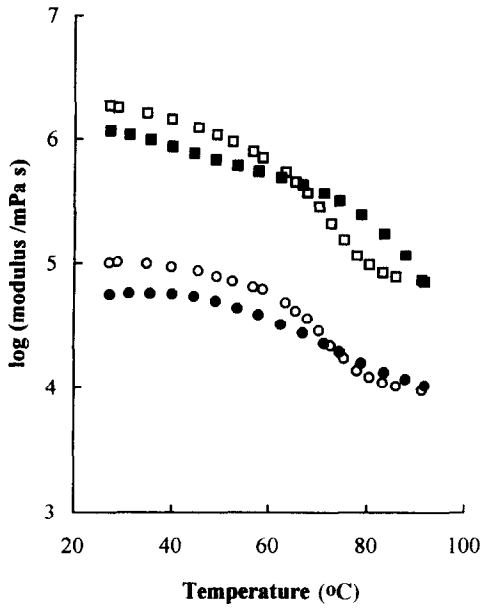


**Fig. 18.** Temperature dependence of $G'$ (squares) and $G''$ (circles) on heating (filled symbols) and cooling (open symbols) for 1% w/v welan in 80% v/v DMSO after salt-induced gelation with 500 mM NaCl. Measurements were made at 1 rad $s^{-1}$ and 2% strain, and commenced 1 h after mixing.

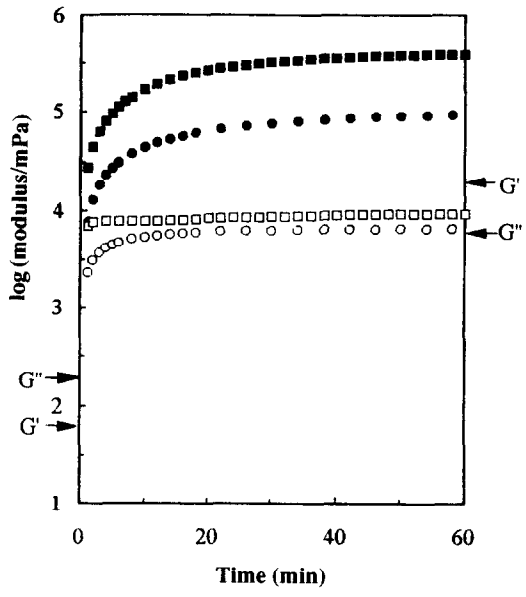


**Fig. 19.** Time course of gelation (25°C) for disordered welan (1% w/v in 91% DMSO) after rapid mixing with finely powdered NaCl at concentrations of 50 mM (circles) or 100 mM (squares), as monitored by $G'$ (filled symbols) and $G''$ (open symbols) at 1 rad $s^{-1}$ and 2% strain. The arrows at the left-hand axis show the initial moduli before addition of salt; those at the right-hand axis show the corresponding moduli for the 'weak gel' state (1% w/v welan in water).

On immersion in excess water, these gels (formed by salt-induced association of ordered welan) gradually lost cohesion, and eventually dissolved completely, in contrast to the behaviour reported below for gels formed by addition of salt to welan dissolved in a solvent containing sufficient DMSO to disrupt the ordered structure.

## SALT-INDUCED GELATION OF DISORDERED WELAN

The solvent chosen for these investigations was 10:1 DMSO:water (~91% v/v DMSO), just above the critical range for loss of 'weak gel' character (Fig. 11) at the polymer concentration used (1% w/v), but the experimental procedures were otherwise identical to those described in the previous section.

As shown in Fig. 19, the values of $G'$ and $G''$ obtained for 1% w/v welan in this solvent within ~1 min of rapid mixing with finely powdered salt (50 and 100 mM NaCl) were already about two orders of magnitude higher than the corresponding values for the salt-free solution, and comparable to the moduli for the ordered 'weak gel' state. The subsequent time course of network development was closely similar in form to those shown in Fig. 14 for salt-induced gelation of ordered welan (in 80% DMSO), and the final moduli were again higher at higher salt concentration (Fig. 19).

The final gels were self-supporting and demouldable (i.e. 'true' gels) and, as illustrated in Fig. 20, had more

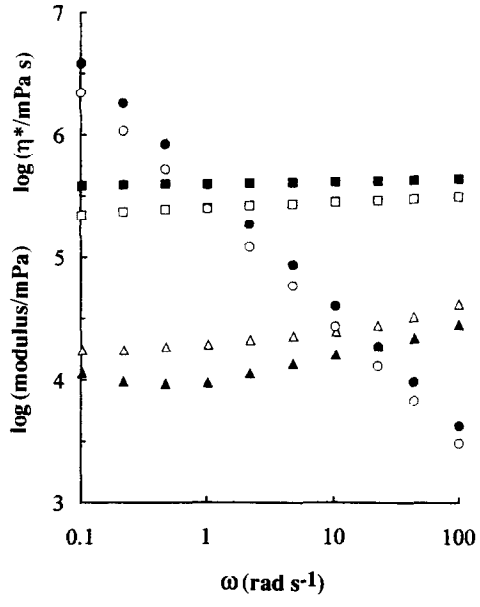


**Fig. 20.** Mechanical spectra (25°C; 2% strain) for salt-induced gels of welan 1 h after rapid mixing with finely powdered salt (100 mM NaCl). Measured values of $G'$ (squares), $G''$ (triangles) and $\eta^*$ (circles) are shown for gels formed from the ordered polymer in 80% DMSO (open symbols) and from the disordered polymer in 91% DMSO (filled symbols).

pronounced gel-like character than those prepared from the ordered state at the same concentration of salt (higher $G'$; lower $G''$ and, therefore, lower tan $\delta$). A more striking difference, however, is that on prolonged

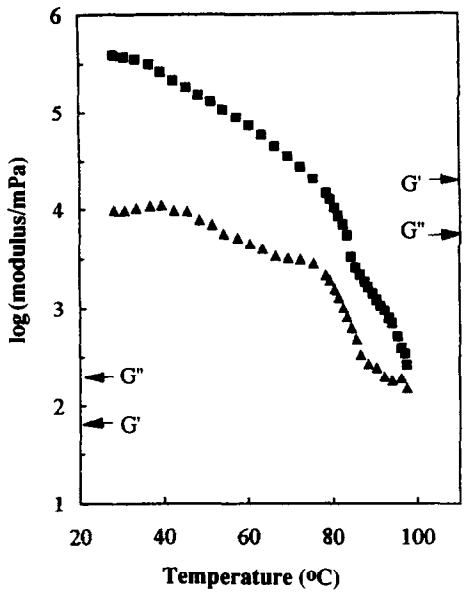


**Fig. 21.** Reduction in $G'$ (■) and $G''$ (▲) on heating (1°/min) for 1% w/v welan in 91% DMSO 60 min after rapid mixing with finely powdered salt (100 mM NaCl). Measurements were made at 1 rad $s^{-1}$ and 2% strain. The arrows at the left-hand and right-hand axes show the corresponding moduli (at 25°C) for, respectively, the starting solution before addition of salt, and the 'weak gel' state in water.

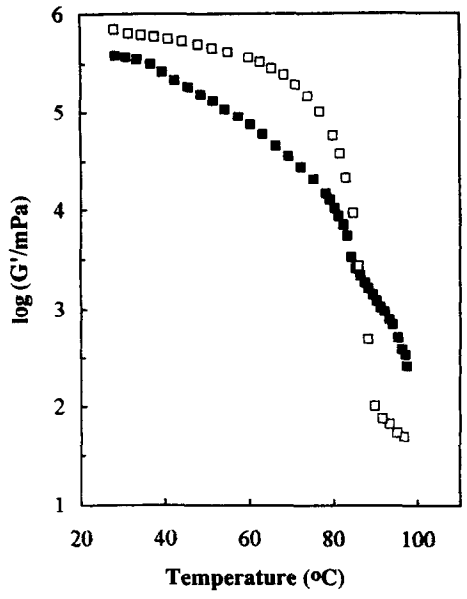


**Fig. 22.** Temperature dependence of $G'$ (1 rad $s^{-1}$; 2% strain) on heating (■) and cooling (□) at 1°/min for 1% w/v welan in 91% v/v DMSO 1 h after salt-induced gelation with 100 mM NaCl.

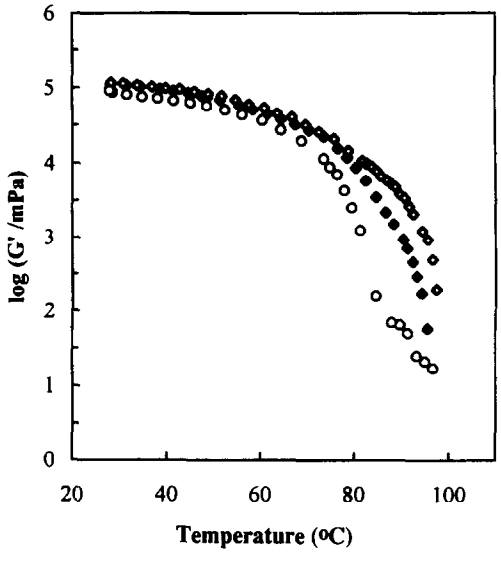


**Fig. 23.** Temperature dependence of $G'$ (1 rad $s^{-1}$; 2% strain) for 1% w/v welan in 91% v/v DMSO after gelation with 50 mM NaCl. Measurements commenced 1 h after initial mixing (at 25°C), and were recorded during successive heating (◆), cooling (○) and reheating (◇) scans (at 1°/min).

immersion in excess water, the gels formed from denatured welan (i.e. in 91% DMSO) swelled, but remained intact.

Figure 21 shows the rheological changes observed on heating the gel obtained by addition of 100 mM NaCl to 1% w/v welan in 91% v/v DMSO. Loss of network structure occurs in two distinct 'waves'. The first is closely similar to the melting process shown in Fig. 17 for the corresponding sample in 80% DMSO, reaching $G'$ values comparable to those in the salt-free 'weak gel' state by ~80°C. At higher temperature, however, there is a second, sharper, decrease, with the moduli dropping towards the values observed for disordered welan in DMSO.

As shown in Fig. 22, the final value of $G'$ attained on re-cooling to room temperature is again substantially higher than the corresponding value before heating, and the first sharp increase in $G'$ in the cooling direction occurs at lower temperature (by about 10°) than the final loss of structure on heating.

Similar thermal hysteresis between heating and cooling scans was also observed (Fig. 23) at lower salt concentration (50 mM NaCl), but with no significant enhancement of modulus at lower temperature after heating and cooling. On subsequent reheating of the resulting gel, however, final melting occurred at higher temperature (by ~5°) than the corresponding melting process for the gel formed initially by direct addition of salt.

## DISCUSSION

Several anionic polysaccharides, including carrageenan (Morris *et al.*, 1980; Norton *et al.*, 1984), agarose sulphate (Norton *et al.*, 1986) and gellan (Robinson *et al.*, 1991) have been shown to form gels by cation-mediated aggregation of co-axial double helices. The novel salt-induced gelation of welan in organic solvents,

reported here, can be explained by a closely analogous mechanism of intermolecular association.

It has been established conclusively from recent X-ray fibre diffraction studies that welan, in the solid state, also exists as a co-axial double helix (Chandrasekaran *et al.*, 1994). Our own observations of sharp changes in solution rheology in response to changes in solvent environment (Figs 10 and 11) and of a typical, thermally reversible, order–disorder transition on heating and cooling in aqueous DMSO (86% v/v; Hember *et al.*, 1994) strongly indicate that, under appropriate solvent conditions, the double-helix structure persists in solution, and gives rise to characteristic 'weak gel' properties.

Addition of salt can facilitate association of charged helical structures in two different ways: by non-specific screening of electrostatic repulsion between the helices, and by incorporation of counterions as an integral part of the aggregate structure, contributing to its stability by electrostatic attraction. Our present results indicate that the latter is the dominant mechanism in salt-induced gelation of welan.

In water, where charge–charge interactions are decreased by the very high dielectric constant, the effect of addition of high concentrations of salt is limited to a modest enhancement of 'weak gel' character (Fig. 8). In solvents of substantially lower dielectric constant (ethylene glycol and 80% DMSO), where electrostatic repulsion between the helices will be greater, much lower concentrations of salt can induce formation of 'true' gels. It therefore seems reasonable to conclude that reducing the dielectric constant of the solvent promotes gelation of welan by enhancing the electrostatic attraction between negatively charged helices and positively charged counterions. Indeed, the increased importance of ionic interactions in organic solvents is well established for synthetic ionomers (Utracki & Weiss, 1989) and widely exploited in industry. The increase in gel-like character when welan is dissolved in ethylene glycol rather than in water (Fig. 1) and loss of solubility in organic solvents as the salt content is increased can, of course, be explained in the same way.

Enhancement of electrostatic interactions is also likely to be a significant factor in destabilisation of the welan double helix by DMSO, alone and in mixed solvents with water (Figs 10 and 11), with reduction in dielectric constant (Fig. 12) causing a progressive increase in repulsion between carboxyl groups on the participating strands. Other factors however, must also be involved, since the 'weak gel' character indicative of conformational order is retained (Figs 1 and 2) in solutions of welan in ethylene glycol, which has a lower dielectric constant than pure DMSO. A likely explanation is that DMSO, because of its extreme polarity, will be a particularly strong acceptor for hydrogen bonds but, being aprotic, is incapable of bonding to other DMSO molecules. It may, therefore, be more effective in disrupting the hydrogen-bonding scheme involved (Chandrasekaran *et al.*, 1994) in stabilisation of the welan double helix than either ethylene glycol or water, both of which can form hydrogen bonds to other molecules of the same solvent.

As demonstrated in Fig. 17, the cation-mediated junctions between welan helices are thermally labile, with gels formed by moderate concentrations of salt (100 mM NaCl) in 80% DMSO losing their 'true gel' character and reverting to 'weak gels' over an experimentally accessible temperature range (i.e. below 100°C). The extent (Fig. 15) and thermal stability (Fig. 17) of helix–helix aggregation increase with salt concentration, as would be expected from simple mass-action considerations. Loss of network cohesion on exposure to excess water can then be explained directly by dilution of the salt concentration to a level where it is no longer sufficient to maintain the stability of the aggregate structure.

The more complex gelation and melting behaviour seen at higher concentration of DMSO (91% v/v) can be interpreted as follows. Dissolving welan in this solvent disrupts the ordered structure. The first effect of addition of salt is to promote rapid re-ordering, by reducing electrostatic repulsion between the polymer chains, giving rise to the massive increase (Fig. 19) between the moduli observed under salt-free conditions and the first values recorded after mixing with NaCl. Analogous salt-induced disorder–order transitions are commonly observed for charged polysaccharides in aqueous solution. Subsequent development of network structure (Fig. 19), and the initial reduction in moduli on heating (Fig. 21), can then be attributed to, respectively, formation and thermal disruption of cation-mediated associations between the welan helices, as before.

The obvious interpretation of the second, major, loss of network structure at higher temperature (Fig. 21) is that it corresponds to dissociation of double helices into disordered coils. The thermal hysteresis observed (Fig. 22) between heating and cooling scans over this temperature range is again directly analogous to the behaviour of other polysaccharides that form gel networks by helix–helix association, and is attributed (Morris & Norton, 1983) to aggregation stabilising the individual helices to temperatures higher than those at which they will form in isolation on cooling from the disordered state.

Since direct addition of salt to a polymer solution is likely to give rise to a rather heterogeneous network, enhancement of gel strength after thermal dissociation and slow re-gelation (Figs 18 and 22) can be readily explained by formation of a more homogeneous structure. As might be anticipated, enhancement is greatest (Figs 22 and 23) at high concentrations of salt, where the rate of network formation is also greatest (Figs 14 and 19). The enhancement of thermal stability observed (Fig. 23) after melting and re-setting gels produced

initially by addition of salt to disordered welan (in 91% DMSO) can similarly be explained by formation of longer helixes when ordering is induced by slow cooling rather than by a rapid increase in ionic strength.

One of the most striking results from this investigation, however, is the difference in properties between the salt-induced gels formed from welan in the ordered state (in ethylene glycol or 80% DMSO) and from the disordered state (in 91% DMSO). Gels of the latter type are stronger (higher $G'$; lower tan $\delta$; Fig. 20) and, more significantly, resist dissociation in excess water.

In a previous study (Hember *et al.*, 1994) we found that when solutions of disordered welan in DMSO were poured into water, they formed strings of gel which, like the gels obtained by addition of salt to welan in 91% DMSO, remained intact on prolonged immersion in water. Our interpretation was that welan, as biosynthesised, comprises 'perfect' double helices, with each chain associated with a single partner along its full length; dissolving in DMSO separates the strands; sudden exposure to excess water induces rapid re-ordering, with each chain forming several shorter stretches of double helix structure with different chain partners, to give a crosslinked network. Since, as demonstrated by the persistence of 'weak gel' properties to very high temperature, the welan double helix is extremely stable under aqueous conditions, gels formed by this mechanism of crosslinking cannot be dissociated by exposure to water.

The behaviour observed in the present work can be explained in the same way. Gelation of ordered welan, by addition of salt to solutions in ethylene glycol or in 80% DMSO, occurs solely by cation-mediated association of the original 'perfect' helices, giving networks that can, therefore, be dissolved in water. Denaturation in 91% DMSO and subsequent re-ordering by addition of salt allows formation of a network crosslinked both by direct participation of chains in double helical junctions with different partners and by salt-induced association of the helices. The helix aggregates can again be dissociated by immersion in water, allowing the network to swell, but the helical junctions remain intact, preventing complete dissolution.

Finally, the solution properties of welan in DMSO, although qualitatively similar to those of disordered polysaccharides in water, show quantitative differences in concentration dependence and shear thinning. The anomalies in concentration dependence are largely confined to the dilute solution regime, and can be readily explained. When a polyelectrolyte is dissolved in water, increasing the polymer concentration will also increase the concentration of counterions, and therefore reduce coil dimensions by screening electrostatic repulsions between different segments of the polymer chain. The complication can be removed by preparing solutions at constant ionic strength (normally by dialysing a stock solution against an appropriate concentration of salt, and using the dialysate for all subsequent dilutions). Because of the obvious problems of solubility of inorganic salts in organic solvents, however, the viscosities shown in Fig. 6 refer to solutions prepared directly in DMSO. The unusually low slope of $\eta_{sp}$ vs log $c$ before the onset of entanglement can therefore be attributed to progressive reduction in coil dimensions with increasing concentration of polymer, because of the accompanying increase in the concentration of counterions (and of any extraneous salts present in the original sample).

The origin of the anomalous shear thinning behaviour (Fig. 5), however, is less obvious. One possibility is that the water content of 'dry' welan (which, like other polysaccharides, incorporates water molecules as an integral part of the solid-state structure) is sufficient to preserve some limited double helical association in DMSO, so that the solutions are not truly 'random coil'. An alternative possibility is that, because of the lower dielectric constant of DMSO, dipolar interactions between polysaccharide chains are stronger than in water, so that topological entanglement is augmented by weak, but significant, enthalpic attraction.

## ACKNOWLEDGEMENTS

We thank Kelco Inc., San Diego, USA, for many stimulating discussions and for financial support of this investigation. We also thank Dr R. Chandrasekaran for communication of research results prior to publication, and our colleagues Dr R.K. Richardson and Mr G. Stone for generous access to their equipment for measurement of, respectively, rheological response to low-amplitude oscillatory shear and solvent capacitance.

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