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Gelation of chemically cross-linked polygalacturonic acid derivatives

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We have previously developed a process for the cross-linking of glycuronans (tetrabutylammonium, TBA, salt form) with dialkyl or dialkylaryl halides. In the present work we have investigated the effect of such cross-linkers, including 1,6-dibromohexane (DBH), 1,3-diiodopropane and 1,10-diiodododecane on TBA polygalacturonic acid (PGA). In particular, we have employed PGA/DBH at low stoichiometric amounts (R) of DBH so that the cross-linking reaction reaches an equilibrium conversion either just above or below the gel point conversion. At $R = 0.084$ a clear power law region is seen in both shear dynamic moduli G' and G'', and with frequency exponents of 0.657 for G'' and 0.640 for G' (both ±0.004), a condition which has been associated with the gel point. We have also investigated the effect of the other cross-linking agents to assess the significance of different halogenic and chain length effects.

INTRODUCTION

A recent definition by Almdal *et al.* (1993) states that a gel is a soft, solid or solid-like material, which consists of two or more components, one of which is a liquid, present in substantial quantities, and which shows a flat mechanical spectrum in an oscillatory shear experiment, with G' exhibiting a plateau extending to times of the order of seconds, and a G'' which is considerably smaller. This definition, however, does not address the important question of the various processes by which the gel is actually formed.

Regardless of the precise molecular mechanism, gelation occurs if one of the segments of a polymer can 'cross-link' with segments on other chains. Such intermolecular reactions initially form branched chains, with a corresponding increase in M_w. The rise in M_w is, in turn, accompanied by an increase in the number of potential cross-linking sites. In this way M_w increases faster and faster until eventually it becomes 'infinite'. At this point, the so-called *gel point*, the largest individual molecule completely spans the container (or in the case of microgel particles the microvolume) in which it sits. When M_w is infinite, the longest relaxation time is also infinite, so the sample no longer flows but has the properties of an equilibrium solid. This, in turn, implies that the equilibrium modulus G_0, i.e. that which would be measured at zero frequency or infinitely long times now has a finite (rather than zero) value. As we shall see below, this definition is complicated by the effect of finite frequencies and/or lifetime of cross-links. The Almdal 'rule' is, therefore, an operative definition rather than a theoretically based one, but this certainly does not negate its value.

In principle, any process which involves cross-linking chains, can lead to gelation. Chemically there are many such routes, such as cross-linking high molecular weight linear chains or step-addition polymerisation of oligomeric precursors. The gel time is defined as the time taken to achieve the gel point conversion. Measurement of the gel point is not easy whether we use chemical methods to directly evaluate the conversion of functional groups or whether we are using a physical technique, such as a measurement of M_w.

Because of the above details, it was thought to be difficult (if not impossible) to determine the exact gel point by rheological methods, since it would require an infinite time for the gelling system to relax to equilibrium. Further, the incipient network is extremely tenuous, and indeed one may conjecture that the critical strain (to fail) has its minimum value (→0) at this critical point.

Despite this, many authors have tried to use dynamic measurements to assess what we may refer to as the rheological gel point. For example, it may be judged to take place when the sample gelling signal becomes just greater than the background noise, when say G' (the

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shear storage modulus) becomes greater than a preassigned threshold value, or the time when G' becomes greater than G'', the shear loss modulus (i.e. when tan δ becomes just less than 1) sometimes called the crossover point. The latter method has been employed for decades in the adhesives industry.

The method of Winter and Chambon (1986) helps provide a sounder approach. They assumed (and this is a strong assumption, which seems to neglect entanglement effects) that at the gel point the stress relaxation modulus $G(t)$ could be represented by a power law $G(t) \sim t^{-n}$. From the Laplace transform, at the gel point, both $G'(\omega)$ and $G''(\omega) \sim \omega^n$. We can then pose the question: 'What is this exponent?'.

It must have some value between the absolute limits of 0 and 2 for G', and 0 and 1 for G''. Indeed since the two exponents must be the same, one simple 'guesstimate' would be the arithmetic mean of the two limit exponents $((0.5 \times 2) + (0.5 \times 1)/2 = 0.75$. In practice this, and the corresponding geometric mean (0.72), are quite close to a number of (more soundly based, but not necessarily more useful) theoretical estimates. These lie mostly in the range 0.5–0.75; corresponding experimental results for a number of systems fall mainly in the range 0.4–0.8 (Martin & Adolf, 1991).

For example, recent work by Lopes da Silva and Gonçalves (1994) on the ageing process of high methoxyl pectin/sucrose aqueous gels has investigated the evolution of the power law behaviour, in real time ('on the fly'), at different times and temperatures. They found exponents between ~0.4 and ~0.6 depending upon the precise pregel history. They were also restricted to a relatively narrow range of frequencies (approximately two decades) and measurements seem to be reported at only a single strain amplitude (3%). They do, however, endorse earlier work by Michon *et al.* (1993) on gelatin gelation which suggests the measured 'critical' exponents depend upon concentration and thermal history.

One of the problem with simple time dependent measurements (which must employ so-called 'on-the-fly' frequency measurements) is that, even allowing for those collected using sophisticated 'multi-wave' techniques (see, for example, Ross-Murphy, 1994*a*) the frequency information must become convoluted with the change in moduli from the gelation kinetics. By using a 'lean' mixture of cross-linker it should be possible to prepare systems effectively 'frozen' at different ultimate degrees of cross-linking. The wastage naturally includes an increasing degree of cycles and other processes leading to cross-link dissipation (Ross-Murphy & Stepto, 1986) but since the Winter–Chambon power-law gelation criterion is assumed to be 'universal' (even if the precise exponent is not) this should not affect the appropriateness of this as a determinant of the rheological gel point.

In the present work, rheological results are reported for polygalacturonic acid chains *chemically* cross-linked with 1,6-dibromohexane (DBH). One of the aims was to determine the exponent n that arises from *time-independent* experiments and to compare its value with that previously obtained in time-dependent experiments. G' dependence on cross-linking moieties and the extent of cross-linking are also investigated.

Another aspect which could be investigated by this method is whether or not at (or close to) the rheological gel-point the properties of this chemical gel, based upon polysaccharide chains, becomes noticeably more strain dependent, as might be expected for purely physical polysaccharide gels (Ross-Murphy & Shatwell, 1993). This is easily established by carrying out conventional strain sweep measurements.

The work by Axelos and co-workers (1989; Axelos & Kolb, 1990) is rather closer to the approach we have adopted in that they investigated gelation of a pectin sample (low degree of esterification from apples), and estimated the critical stoichiometry, using a variety of methods in order to make time-independent measurements. This work differs from ours in a number of ways. Firstly it involves a conventional physically cross-linked low methoxy pectin/Ca^{2+} network. Such systems are likely to be more strain dependent (although no detailed strain data are discussed, and results are given only for 4% strain). Secondly, as discussed in more detail below, they measured the slope when G' was equal to G'' over a significant frequency interval, and used this to define the gel point. This definition is not quite the same as the Chambon–Winter criterion we have adopted. Finally, the Ca^{2+} induced complexation is, of course, associated with a conformational change of the pectin macromolecule, which will tend to complicate the overall mechanical response.

MATERIALS AND METHODS

A pectic acid sample, a linear homopolymer of $\alpha(1\rightarrow4)$ linked D-galacturonic acid residues (PGA, Sigma, M_w 70 kDa, polymerisation degree = *c*. 350; galacturonic acid content 98%; 2% rhamnose content as backbone irregularity) was transformed to the tetrabutylammonium (TBA) salt form (equivalent weight = 419) using a Dowex '50 × 8' ion exchange resin.

The sample was then dissolved (polymer concentration $C_p = 6\%$ (w/v); $C_p[\eta] = 4$) in dimethylsulphoxide (DMSO) and added, at 20°C, to a suitable volume of 1,3-diiodopropane, DBH or 1,10-diiodecane (Fluka Chimica, Switzerland) in order to obtain the desired cross-linking system at the appropriate stoichiometric ratio (R = equiv I(or B)/equiv of PGA). The mixture was then transferred to the concentric cylinder cell of a Bohlin CS (controlled stress) Rheometer (Bohlin Couette geometry 'C14', ratio of inner and outer cylinders, $r1/r2 = 0.909$) maintained at 20 ± 0.2°C.

Frequency sweep measurements (Ross-Murphy, 1994*b*) over the range 1×10^{-3}–8 Hz, ($\sim 6 \times 10^{-3}$–50 rad s^{-1}) and strain sweep measurements (strictly stress sweeps under a feedback control loop) over the range 0–10% were performed for gels formed at different values of stoichiometric ratio of DBH, 1,3-diiodopropane and 1,10-diiodecane to PGA (range 0.1–12). To avoid moisture contamination, the entire measuring system was protected with a home made dry-box and fluxed with pure, dry nitrogen.

RESULTS AND DISCUSSION

In our laboratory we have previously developed a process for the partial esterification of glycuronans (TBA) with alkyl or alkylaryl halides. This procedure can be performed in homogeneous solutions (e.g. in dimethyl sulphoxide) at room temperature and brings about only moderate, if any, chain degradation (Della Valle & Romeo, 1989; Crescenzi & Della Valle, 1991; Crescenzi *et al.*, 1992*a*,*b*, 1993). Cross-linking can then be achieved simply by using e.g., DBH, the degree of cross-linking being controlled by the stoichiometric ratio of reactants.

In a recent paper (Matricardi *et al.*, 1993) we have described measurements on such a partially esterified PGA system (TBA salt form) prepared by dissolving PGA in DMSO and cross-linking with DBH (Fig. 1). Kinetic gelation measurements were made directly in the rheometer. Frequency sweep experiments were made during the cross-linking reaction using a stoichiometric ratio $R = 1$ (per mole of polymer) of DBH to PGA. Gelation occurs under these conditions after approximately 1100 s, and the slopes of G' and G'' at this time were measured as approximately 0.70.

Although these results were very acceptable, as mentioned above, the kinetic gelation experiment is complicated by the convolution of time and frequency (and of concomitant rheological history) because cross-linking is still proceeding during the time required for the frequency sweep. A sounder technique is to chemically stop gelation after different degrees of cross-linking and then make frequency measurements. This is precisely how Winter's own measurements on end-linked poly(dimethylsiloxane)s were carried out. An alternative technique is to adjust the amount of cross-linker so that the reaction reaches an equilibrium conversion either just above or below the gel conversion. This is how the present measurements were carried out.

An initial series of measurements using a lower than stoichiometric ratio of DBH were used to estimate the critical ratio R_c. Gel times t_c (in the range 500–5000 s) were obtained by varying R from approximately 10 to 0.2 (Fig. 2), and assessing the time at which log (tan δ) became independent of frequency (a procedure used by Winter). From this, R_c could be estimated by plotting, as reported in the inset of Figure 2, $1/t_c$ vs R and extrapolating to $1/t_c \rightarrow 0$. From this plot, R_c was estimated to lie close to 0.1. (Although strictly, the dependence of R_c on $1/t_c$ would not be expected to be linear, we are using the extrapolation merely to determine an estimate of R_c. A more precise value can be determined as shown below.)

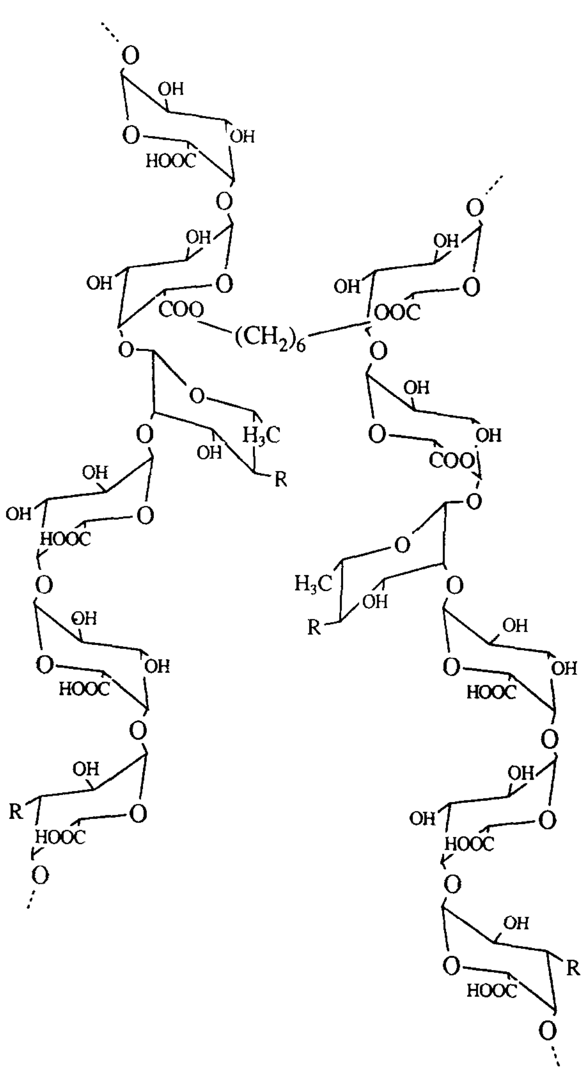

Fig. 1. Dibromohexane cross-linked polygalacturonic acid chains.

Consequently measurements were performed for different values of the ratio R from 0.080 to 0.100 in approximate increments of 0.004. It is worth mentioning that a linear viscoelastic response for all the gels under study was retained up to deformations lying in the range 5–10%.

The corresponding frequency sweeps performed from 8×10^{-2} to 8 Hz, and at a strain of 0.01 (= 1%), well within this pre-established linear region show a sharp transition from pregel ($R = 0.080$) to postgel ($R = 0.090$) behaviour as shown in Fig. 3a–d. At $R = 0.084$, a clear power law region is seen in both G' and G'', and with $G'' \sim 2 \times G'$ extending over the

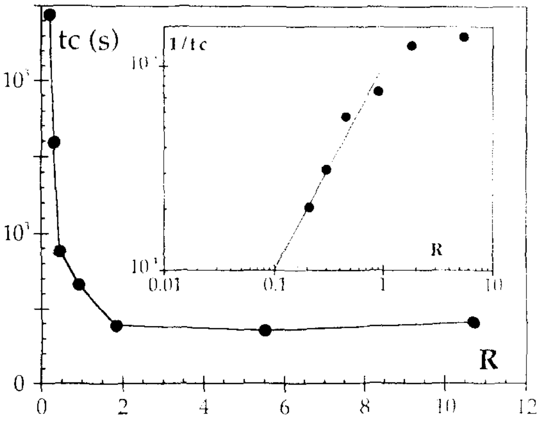

Fig. 2. Gel time (t_c) dependence on R for PGA–dibromohexane cross-linking. R is the equivalent ratio between polymer and cross-linker. Polymer concentration $C_p = 6\%$ (w/v) in DMSO at 20°C.

complete frequency range (three decades of frequency). The frequency exponents were 0.657 for G'' and 0.640 for G' (both ±0.004). The mean value of these two exponents gives a gel point slope of 0.648 ± 0.006, in good agreement with previous results both from our own work (Matricardi *et al.*, 1993) and other systems (Winter, 1987*a*, 1987*b*; Winter *et al.*, 1988; Scanlan & Winter, 1991; Hodgson & Amis, 1990; Adolf *et al.*, 1990), although the absolute precision, coupled with the range of frequency over which the data are obtained, is arguably better than almost all previously published results. There is some indication of systematic oscillations in $G'(\omega)$ in these traces, but we regard these as due to minor instrumental artefacts, rather than a real effect. Certainly the overall slope is well fitted to a straight line form.

From R_c, the number of cross-links per PGA chain at the gel point was found to be 10. The theoretical value is two per chain, and the difference reflects the number of potential cross-links 'wasted' by formation of intramolecular cross-links (cycles) and in other chemical side reactions. Similar ratios between the theoretical and practical degree of cross-linking are expected from other work (Ross-Murphy & Stepto, 1986).

The samples measured for R values just $> R_c$ also show interest. For example, Fig. 3c ($R = 0.088$, $R/R_c \approx 1.048$) strongly suggests that a plateau at low frequencies has just developed (which when extrapolated to $\omega \to 0$ would suggest G_0 was > 0). At $R/R_c \approx 1.071$ (Fig. 3d) the cross-over frequency has moved up the scale, and the absolute value of G' at the

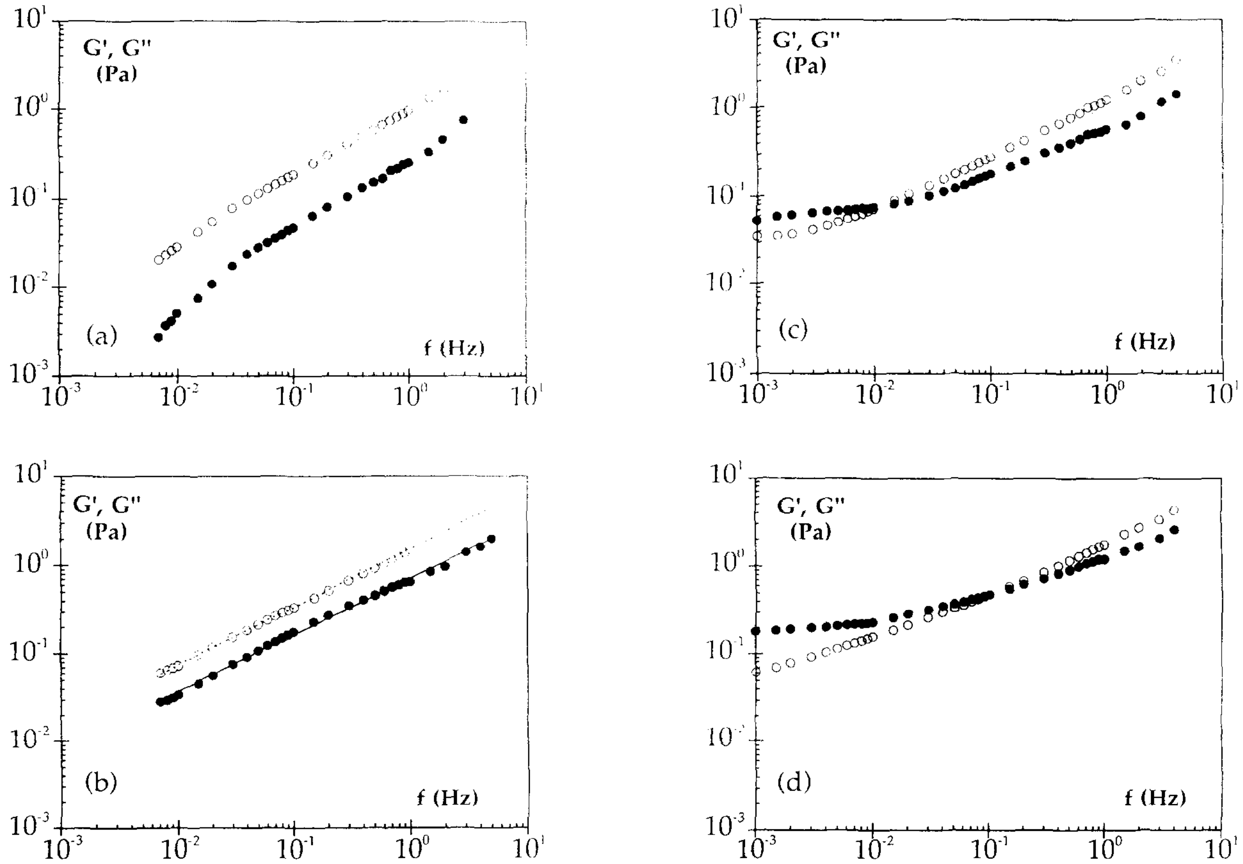

Fig. 3. Mechanical spectra of PGA-1,6-dibromohexane cross-linked systems at different equivalent ratios between polymer and cross-linker (R). (a) $R = 0.080$; (b) $R = 0.084$; (c) $R = 0.088$; (d) $R = 0.090$. Polymer concentration $C_p = 6\%$ (w/v) in DMSO at 20°C. G', filled circles; G'', open circles.

lowest frequency has also increased, precisely as expected.

Comparison with the previously mentioned measurements of Axelos and co-workers (1989) is particularly interesting. As alluded to above, they used a subtly different definition of the gel point, i.e. the conversion when $G''/G' = 1$. They measured this (common) slope to be 0.71, a value which corresponds very closely to that suggested by others (Derrida *et al.*, 1983; Clerc *et al.*, 1984). This exponent is rather greater than we have found, but their gel point frequency sweep bears a close resemblance to Fig. 3d, where the slope cited corresponds to the upper part of this figure. Since, by the common slope definition we have employed here, their sample is already past the gel point, we would obviously expect their reported gel point slope to be slightly greater than ours. Despite this, their overall critical degree of cross-linking ($R_c = 2[Ca^{2+}]/[COO^-]$), 0.086, is remarkably close to ours, 0.084. In view of the very different gelation mechanisms involved in the two methods, and more particularly the different number of redundant cross-links this implies, the agreement may be fortuitous.

In Fig. 4, the inverse of the gel time vs stoichiometric ratio (cross-linker equivalents vs polymer equivalents) is reported for three PGA systems prepared with differing extents of cross-linking and different cross-linking moieties like 1,3 diiodopropane, DBH and 1,10 diiododecane. It appears that the absolute efficiency of the cross-linking agent depends inversely on the chain-length (λ) of the final cross-link (or the length of the ester alkyl chain), although saturation appears to occur when $\lambda > 6$. Whether this reflects simply a chain-length dependence, the halogenic form of the cross-linkers, or the relative susceptibility to side-reaction and wastage remains to be established.

In Fig. 5a and b the mechanical spectra of PGA-1,3-diiodopropane and PGA-1,10-diiodododecane derivatives, respectively, are reported. These show typical behaviour of samples in the gel state, with G' and G'' largely independent of frequency. In this case, both R and the cross-linker chain length are varied. Shear moduli of gels where the cross-linking had proceeded to almost complete reaction, were determined for various stoichiometric ratios R. As reported in Fig. 6, all the systems studied showed a quite sharp maximum of G' when R was in the range 0.5–0.9. Furthermore, G' decreases within this range as the cross-linking chain length increases. This effect is quite commonly observed for other cross-linked gel systems. For very high degrees of cross-linking, the crosslinks cannot be entirely randomly distributed. This leads to a higher proportion of network defects, elastically ineffective cycles etc. This sometimes results in a decrease in modulus, although this effect is exaggerated by employing a linear scale in Fig. 6. Significantly there is little or no evidence of inhomogeneity at high R values, compared with the corresponding Ca^{2+} cross-linked gels. All the original frequency sweeps are quite flat, so that $G'(1)$, measured at 1 Hz, and the extrapolated G_0 ($= G'(\omega \to 0)$) hardly differ.

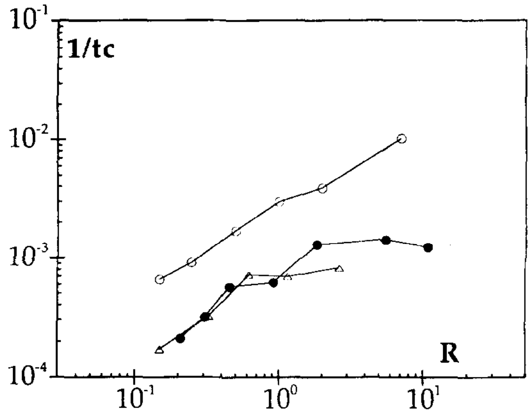

Fig. 4. Gel time dependence on R for the PGA cross-linked with 1,3-diiodopropane (open circles), 1,6-dibromohexane (filled circles) and 1,10-diiodododecane (triangles) in DMSO at 20°C. Polymer concentration $C_p = 6\%$ (w/v).

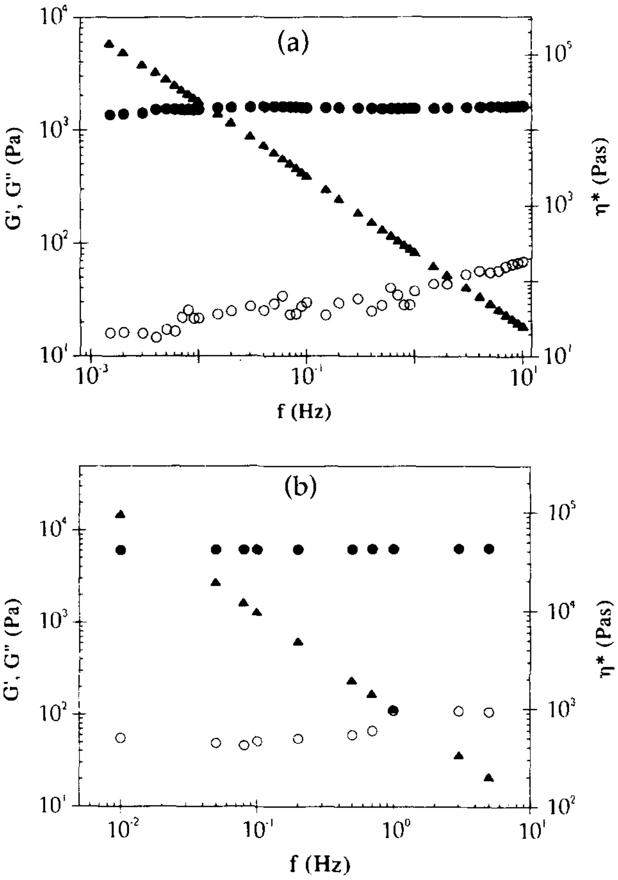

Fig. 5. Mechanical spectra of PGA-1,3-diiodopropane at (a) $R = 0.250$; and (b) PGA-1,10-diiodododecane at $R = 0.622$ systems. Polymer concentration $C_p = 6\%$ (w/v) in DMSO at 20°C. G', filled circles; G'', open circles; η^*, triangles.

The origin of the overall behaviour of the PGA gels

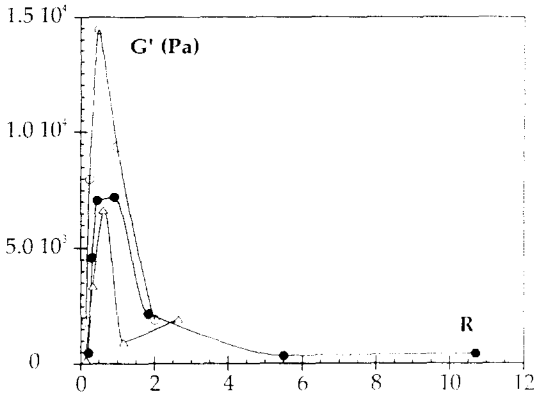

Fig. 6. G' (at 1 Hz) dependence on R for the PGA cross-linked with 1,3-diiodopropane (open circles), 1,6-dibromohexane (filled circles) and 1,10-diiodododecane (triangles) in DMSO at 20°C. Polymer concentration $C_p = 6\%$ (w/v).

(excluding side-effects like loop formation or inhomogeneities of the resulting gels) probably lies in the complex interactions between moieties having such different structural elements as alkyl bridges and polysaccharidic chains. The final result depends upon the stoichiometry of the system, but in a reliable, and even predictable manner.

CONCLUSION

The use of lean cross-linker stoichiometry to prepare stable pre- and postgel TBA–PGA/DBH systems very close to the critical R_c proved very successful, and enabled both frequency and strain sweeps to be carried out without complications due to time (or temperature) effects. The frequency dependence was within the (albeit broad) range observed by other workers, but the comparative strain insensitivity was encouraging. Whether or not the rheological gel point corresponds to the gel point determined, for example, by the divergence of M_w remains to be established. The effect of the different cross-linkers DBH, 1,3-diiodopropane and 1,10-diiodododecane on gel modulus was in the order of increasing chain length, but separating the effect of chain length from that of different halogens derivatives clearly requires further experiments. These should include classical kinetic studies, molecular (nuclear magnetic resonance) work and further rheological experiments.

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