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Viscoelastic properties of aqueous solutions of an exocellular polysaccharide from cyanobacteria*

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The viscoelastic properties of aqueous solutions of the exocellular polysaccharide of *Cyanospira capsulata* have been studied, over a wide range of polymer concentrations, using small deformation oscillatory, steady and transient shear methods. The viscoelastic spectra generally resemble those of an entangled network, although notable deviations can be observed in the low frequency dependence of G' and G''. At higher polymer concentrations, the viscoelastic spectrum shows solid-like behaviour over a wide range of frequencies. The superposition of $\eta^*(\omega)$ and $\eta(\dot{\gamma})$ curves occurs only at low frequencies, at higher frequencies the slope of $\eta^*(\omega)$ is lower than that of $\eta(\dot{\gamma})$. By studying the time evolution of shear stress after the inception of a steady shear rate (stress overshoot), the recovery of non-linear properties after steady shearing flow is seen to occur after times of *c.* 10^3 s (in the case of 1·1% w/v solutions).

The overall viscoelastic properties appear original in comparison with those of the two structurally limiting types of polysaccharide, the 'ordered' chain xanthan and the 'random coil' guar. A rationale for this 'anomalous' viscoelastic behaviour can be tentatively proposed in terms of flickering intermolecular cross-interactions between semi-flexible segments, which occur in addition to the usual topological constraints.

INTRODUCTION

Gram-negative microbial exopolysaccharides have been extensively studied, both because of their unique physical properties and their commercial potential (Sutherland, 1985). Among these exopolysaccharides, one of the most actively investigated is that from *Xanthomonas campestris*, known as xanthan. The characteristic rheological properties of this polysaccharide have determined its industrial success (Ash, 1985). Xanthan is one of the stiffest molecules currently known and its chain conformation in solution is usually described in terms of the worm-like chain model (Sato *et al.*, 1984; Tinland & Rinaudo, 1989; Shatwell *et al.*, 1990*a*). The rheological behaviour of xanthan reflects the ordered conformation usually adopted in solution and consequent intermolecular interactions (Ross-Murphy *et al.*, 1983; Cuvelier & Launay, 1986; Richardson & Ross-Murphy, 1987*b*; Rochefort & Middleman, 1987).

The physical properties of some cyanobacterial exopolysaccharides have been investigated, and then only in part, quite recently. Exopolysaccharides from *Phormidium* J 1, *Anabaenopsis circularis*, *Calothrix*

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desertica and *Synechocystis* PCC 6803 showed novel flocculating and gelling properties that may be exploited in industrial applications (Bertocchi *et al.*, 1990). Cyanobacteria are photosynthetic prokaryotes present in all types of ecosystems including fresh, salt and alkaline waters as well as soil, rocks, plant roots or leaves (Vincenzini *et al.*, 1992). Like other microorganisms, many cyanobacterial species produce large amounts of exocellular mucilaginous material, mainly of polysaccharidic nature (Bertocchi *et al.*, 1990). It is generally accepted that the synthesis of exocellular polysaccharides can be considered as one of the metabolic strategies for the survival and the growth of cyanobacteria, especially under extreme environmental conditions (Vincenzini *et al.*, 1992).

Cyanospira capsulata is a nitrogen fixing filamentous cyanobacterium recently isolated and comprehensively studied from the microbiological point of view (Sili *et al.*, 1984; Florenzano *et al.*, 1985; De Philippis *et al.*, 1989). This cyanobacterium is able to produce an exopolysaccharide (CC-EPS), in addition to the material which forms a capsule surrounding the micro-organism. In the course of culture the exopolysaccharide is released in large amounts into the medium, independently of the growth phase (De Philippis *et al.*, 1989; Vincenzini *et al.*, 1992), increasing the viscosity of the culture broth. The ability to produce highly viscous solutions at low polymer concentrations has stimulated rheological investigations on both culture broths and CC-EPS aqueous solutions (Lapasin *et al.*, 1992; Navarini *et al.*, 1990). The rheological behaviour of CC-EPS has been found to be similar to that of as good a suspending agent as xanthan. However, in the range of concentration investigated, the time-dependent properties have been found to be more typical of a flexible random coil polysaccharide such as guar, a galactomannan obtained from the seed endosperm of certain legumes (Navarini *et al.*, 1990).

Although the chemical structure of CC-EPS has not yet been completely identified, the exopolysaccharide consists of arabinose, fucose, glucose, mannose and galacturonic acid in a molar ratio of 1 : 1 : 1 : 1 : 2 with the presence of a small amount of pyruvate residues (*c.* 0·22 nmol/mg) (Cesàro *et al.*, 1990; Vincenzini *et al.*, 1992). The novel rheological behaviour, as well as the presence of uronic acid in the repeat unit of CC-EPS, has motivated a physico-chemical characterization of the solution properties, aimed at investigating both the polyelectrolyte behaviour and the conformation adopted in solution (Cesàro *et al.*, 1990). This experimental data showed that the solution conformation of CC-EPS is that of a random coil of moderate flexibility. The rheological behaviour of CC-EPS was therefore ascribed to these intermediate features and some speculation was made about the level of intermolecular interactions (Cesàro *et al.*, 1990).

It is known that both the conformation adopted in solution and the regularity of the sugar sequence including 'minor substituents' on the basic repeat unit strongly affect the viscoelastic properties of polysaccharides. At sufficiently high concentrations and molecular weights it has been possible to distinguish two *qualitatively* different types of viscoelastic response. The first, typical for flexible random coil polymers including guar, is governed almost exclusively by the entanglement between polymer chains (Ferry, 1980); the second, illustrated by conformationally ordered polymers like xanthan, is known as 'weak gel' behaviour and apparently reflects the contribution of specific intermolecular interactions, over and above the usual 'tube' or topological constraints (Richardson & Ross-Murphy, 1987*b*).

The purpose of the present paper is to investigate the viscoelastic properties of CC-EPS in aqueous electrolyte solution over a wide range of concentrations (up to 1·1% w/v) using a comprehensive variety of rheological experiments including small deformation oscillatory, steady and transient shear techniques. The critical comparison of experimental results with similar ones for the two above-mentioned polysaccharides can be useful in order to elucidate the 'intriguing' rheological behaviour previously observed (Navarini *et al.*, 1990).

EXPERIMENTAL

Materials

The sample of the purified exocellular polysaccharide from *Cyanospira capsulata* (CC-EPS) is the same as that previously used to carry out physico-chemical characterizations in dilute aqueous solution (Sample CC-EPS-2 in Cesàro *et al.*, 1990; $M_w = 1{\cdot}6 \times 10^6$ and $[\eta]$, the intrinsic viscosity = 22·6 dl/g).

Solutions of the polymer at different concentrations (0·25% w/v–1·1% w/v) were prepared by dissolving the polymer in 0·1 M NaCl with overnight stirring at room temperature. Particular attention was paid to the preparation of the solutions at the highest concentration (1·1% w/v): in this case stirring was prolonged until a homogeneous phase was obtained. In all cases heating of the solutions was avoided. The concentration range, well above the concentration c^* (at which the coil overlap occurs) for this system (Navarini *et al.*, 1990), can be expressed by means of the Simha parameter $c[\eta]$, which is an estimate of the volume occupied by the polymer coil (Launay *et al.*, 1986). In the present work, $c[\eta]$ ranges from 5 to 25 and covers semi-dilute up to a less than grossly concentrated regime.

Methods

All rheological measurements were carried out at 25 °C using a Rheometrics Mechanical Spectrometer (RMS

605) with cone and plate geometry (50 mm diameter, 0·04 radian cone angle). Steady shear measurements in the range 10^{-1}–10^{3}/s and dynamic oscillatory measurements in the range 10^{-2}–10^{2} rad/s were made using a sensitive transducer (ST-10). Solvent evaporation was prevented by a layer of low viscosity silicone fluid (Dow Corning 200/10cs) on the exposed surface of the sample.

Transient experiments on the solutions at the highest polymer concentration were performed using the Rheometrics Data Capture Option, which enables the sampling period to be divided into four zones from an accurately defined start time. Examination of the time-scale for recovery of non-linear properties after steady-shearing flow was performed by shearing the sample for 45 s at 1/s (zone 1), holding stationary for the specified rest time (1–1000 s) (zone 2) and then shearing for a further 45 s at 1/s. All data were plotted in terms of the stress overshoot ratio maximum (i.e. transient maximum stress/steady long-term stress) as a function of the rest time. The principle of this experiment has been previously discussed by Stratton & Butcher (1973).

RESULTS AND DISCUSSION

Dynamic oscillatory experiments

Investigations of the linear shear viscoelastic behaviour of the CC-EPS solutions have been performed using dynamic oscillatory experiments and cone-plate geometry. Such experiments give the frequency-dependent moduli G' and G'', the so-called viscoelastic spectrum. The overall relationship between stress and strain in the dynamic oscillatory experiment is given by the complex viscosity, which is defined as:

$$\eta^* = \frac{\sqrt{(G')^2 + (G'')^2}}{\omega}$$

where ω is the frequency (Ferry, 1980).

In Fig. 1 the complex viscosity has been plotted against strain for 0·4% w/v and 1·1% w/v CC-EPS solutions for each of two different frequencies. The viscoelastic behaviour of CC-EPS solutions is essentially strain-independent in the frequency range investigated.

The viscoelastic spectra of CC-EPS solutions in the range of concentration 0·25–1·1% (w/v) are shown in Figs 2 and 3. The spectra have been recorded over the full frequency range (0·01–100 rad/s) for solutions of 0·7% and 1·1% (w/v). No meaningful response was obtained at low frequency with the most dilute solutions (0·25% and 0·4%), even though a high sensitivity transducer was used.

Solid-like behaviour is apparent at high frequency for all concentrations used and extends down to lower frequencies for the highest concentrations (1·1% w/v). Furthermore a 'cross-over' between the curves of G' and G'' is also evident, showing a tendency to liquid-like behaviour when the frequency decreases. The cross-over transition frequency decreases with increasing concentration, changing from *c.* 20 rad/s to 0·4 rad/s. Another characteristic is that both the limiting slopes of G' and G'' (at low frequency) slightly decrease with increasing concentration, but remain in the range 0·6–0·8 for G' and *c.* 0·5–0·8 for G''; for a Newtonian fluid the corresponding slopes are 2·0 and 1·0, respectively.

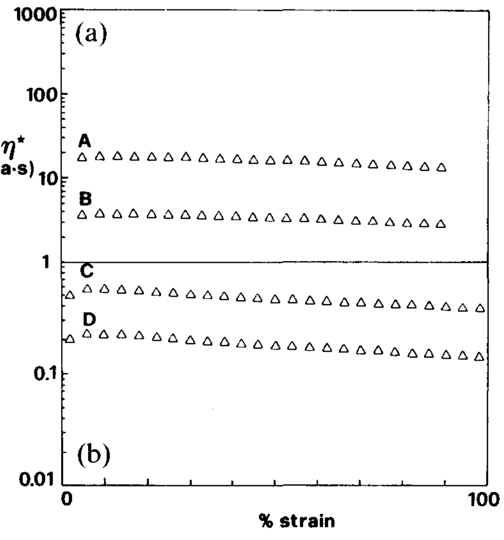

Fig. 1. Complex viscosity, η^*, as a function of % strain (maximum absolute value = 0·04 rad) for CC-EPS solutions in NaCl 0·1 M at two different concentrations and frequency values, ω. (a) 1·1% w/v: (A) ω = 1 rad/s, (B) ω = 10 rad/s; (b) 0·4% w/v: (C) ω = 3 rad/s, (D) ω = 20 rad/s.

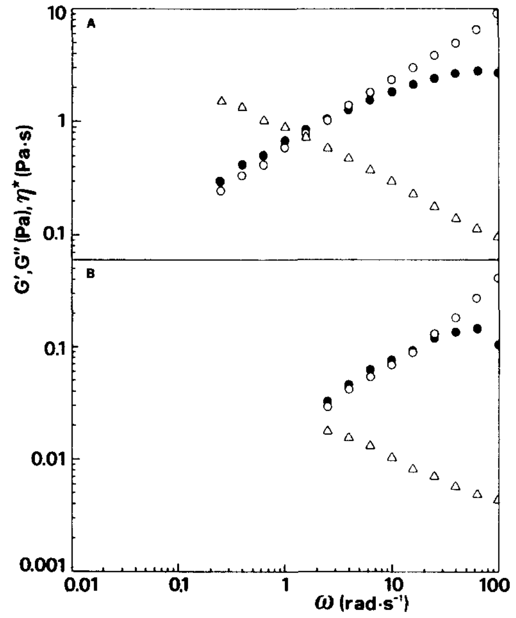

Fig. 2. Frequency sweep for 0·4% w/v (A) and for 0·25% w/v (B) CC-EPS solution in 0·1 M NaCl. Triangles: η^*; open circles: G'; filled circles: G''.

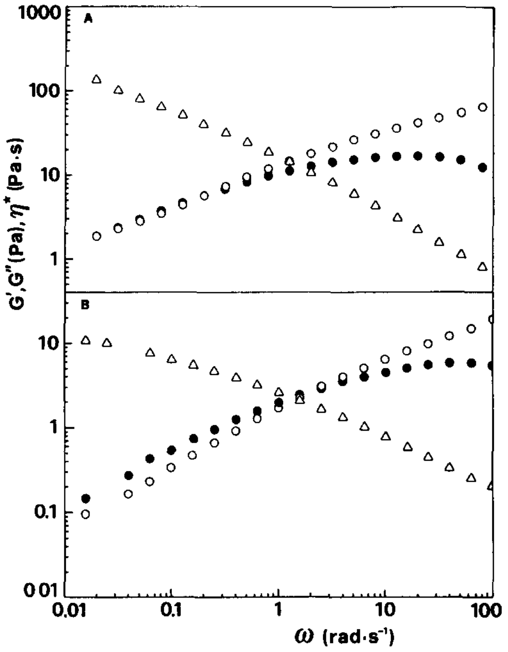

Fig. 3. Frequency sweep for 1·1% w/v (A) and for 0·7% w/v (B) CC-EPS solution in 0·1 M NaCl. Symbols as in Fig. 2.

The complex viscosity, in all cases, assumes approximate power law dependence at high frequencies; in this frequency range, the slopes of the curves increase with concentration. At low frequencies there is no evidence of a Newtonian plateau. More enlightening is the (so-called Cox-Merz) superposition of the $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ curves (Cox & Merz, 1958; Ferry, 1980; Doi & Edwards, 1986) as will be discussed later.

A molecular interpretation of the viscoelastic behaviour of CC-EPS can be made only by comparison with the results obtained with other polymers of known conformational features. Flexible random coil polymers, such as polystyrene, in good solvents at moderate concentration (above the semi-dilute concentration limit, c^*) show typical viscoelastic spectra where at lower frequencies G' approaches $\propto \omega^2$ and $G'' \propto \omega$ (see above) with $G' < G''$. G' increases with frequency faster than does G'', so there is a 'cross-over' at a certain frequency value, and subsequently $G' > G''$. Subsequently the slope of both curves decreases, giving a 'plateau' where $G' > G''$ — this behaviour can be related to existing theories, e.g. reptation. For example, molecular weight and concentration affect the intensity of entanglement coupling and thus the onset of the plateau zone of frequency response (Ferry, 1980; Doi & Edwards; 1986).

Among the polysaccharides studied, a linear random-coil glycan, like guar, exhibits the viscoelastic behaviour described above (Richardson & Ross-Murphy, 1987*a*). In the same concentration regime (i.e. at comparable values of the product $c[\eta]$) the anionic exopolysaccharide xanthan exhibits a quite different viscoelastic spectrum. The behaviour is very similar to that obtained for a gel system (although the *strain* dependence is much more marked) showing a minimal dependence of the storage and loss moduli (G', G'') on the imposed frequency and G' is much greater than G'' in the whole range of frequencies investigated (Clark & Ross-Murphy, 1987; Richardson & Ross-Murphy, 1987*b*). The spectra of the xanthan solutions do not change significantly on dilution until quite low concentrations are reached (Clark & Ross-Murphy, 1987; Milas *et al.*, 1990); then the behaviour becomes identical to that of an entangled polymer solution. This concentration dependence, and, in addition, the ability to flow i.e. to sustain steady shear rate experiments without fracture — albeit without Cox-Merz superposition of $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ — at higher concentrations, are the most important differences between a typical gel system and xanthan solutions (Clark & Ross-Murphy, 1987). Such behaviour is consistent with the picture of a tenuous network or 'weak gel' and it has been suggested that this could be due to more specific interchain couplings between xanthan molecules in addition to the topological restraints (Ross-Murphy *et al.*, 1983; Richardson & Ross-Murphy, 1987*b*). In fact, it is known that, at low temperatures and/or in the presence of salt, xanthan is a rather stiff and conformationally ordered polysaccharide (Morris *et al.*, 1977; Dentini *et al.*, 1984). The level of intermolecular interactions is the most important difference between the two polysaccharide systems discussed above. The flexibility and the lack of regularity of the repeating unit of the guar chain, on the one hand, and the stiffness and the comparative regularity of xanthan on the other, are the molecular parameters which possibly prevent (guar) or stabilize (xanthan) rheologically relevant intermolecular interactions.

The viscoelastic behaviour of CC-EPS aqueous solutions, in the range of concentration investigated, shows a number of peculiarities. In fact the frequency dependence of G' and G'' is quite different from those described above. The experimental data clearly indicate that the viscoelastic properties are reminiscent of those of random coil polymers in good solvent, i.e. guar, and the presence of a cross-over frequency supports this assertion. Nevertheless, at low frequency, the viscoelastic behaviour can be described as intermediate between that of an entanglement network and that of a 'weak gel' system (xanthan) when both are in the same concentration regime.

Although the long time (terminal) relaxations of a polymer solution depend upon molecular weight and distribution (Ferry, 1980), the deviation from the random coil viscoelastic behaviour cannot be ascribed exclusively to the polydispersity of the unfractionated sample. Indeed, the 'intermediate' nature of CC-EPS is apparent by comparing the viscoelastic behaviour of 1·1% w/v CC-EPS solutions and that of xanthan/guar blends (Clark, 1988; Shatwell *et al.*, 1991). Normal xanthan/guar blend viscoelastic spectra show gel-like behaviour at all frequencies, but at low frequency G' values are remarkably close to the G'' values ($G' = G''$ at frequencies below 0·2 rad/s), and the frequency dependence of the moduli lies between that of pure xanthan and that of pure guar solutions (Clark, 1988; Shatwell *et al.*, 1991). This behaviour may be related either to a weak interaction between xanthan and guar molecules (Clark, 1988), and/or to the segmental interactions occurring between the xanthan chain molecules, i.e. guar may modulate the level of xanthan intermolecular interactions (Tako & Nakamura, 1985; Shatwell *et al.*, 1990*b*). Such an effect is apparently sensitive to the proportion of acetyl and pyruvyl substituents on the xanthan (Shatwell *et al.*, 1991).

CC-EPS, polymeric structure is characterized by a regular sugar sequence (Cesàro *et al.*, 1990), but it appears to be a conformationally disordered polysaccharide with moderate chain flexibility more similar to that of alginate (if not to that of xanthan) than that of amylose or guar. The intermediate viscoelastic behaviour of CC-EPS is consistent with the proposed conformation in solution as well as its chain flexibility, and supports the hypothesis that, in addition to entanglement coupling, the existence of 'flickering interchain cross-interactions between semi-flexible segments' (Cesàro *et al.*, 1990) affects the terminal zone of frequency response.

Steady shear experiments

The viscosity of polymeric solutions in steady shear flow is independent of the rate of shear, at vanishingly small values of the latter (Newtonian behaviour). By increasing the shear rate, the viscosity approaches a power law dependence with $\eta \propto [\dot{\gamma}]^n$, where n is the slope of the flow curve ($\log \eta$ vs $\log \dot{\gamma}$ plot) at high values of the shear rate. Such non-Newtonian behaviour, known as shear thinning or pseudoplastic, is shown in Fig. 4 for CC-EPS solutions, in the range of concentration investigated. With a solution of 0·25% w/v a Newtonian plateau is reached at low values of the shear rate; accordingly, it is possible to estimate the zero-shear viscosity (η_0 = 0·2 Pa s) and to observe the onset of the shear thinning behaviour at a shear rate of about 2/s. By increasing the concentration the onset of non-Newtonian behaviour shifts to lower shear rate values, i.e. below the accessible range of the instrument, obscuring the Newtonian plateau and the shear rate value at which the shear thinning behaviour occurs. In the range of shear thinning behaviour, the slope of the flow curves increases with the concentration. In particular, at the highest concentration (1·1% w/v), characterized by a coil overlap parameter ≈ 24, the slope of the flow curve reaches a value of 0·70, in agreement with the values found for guar (Robinson *et al.*, 1982), and xanthan (Milas *et al.*, 1990), at the same degree of coil overlap.

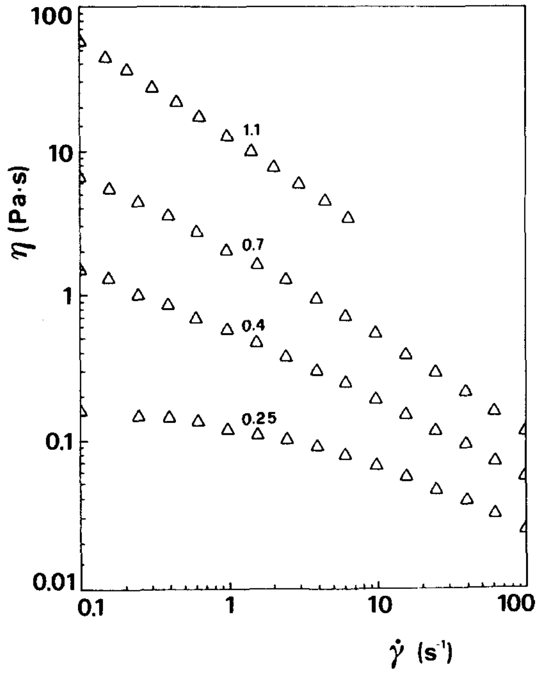

Fig. 4. Steady shear viscosity η for CC-EPS solutions in 0·1 M NaCl plotted against shear rate $\dot{\gamma}$; polymer concentration (% w/v) is indicated on the figure.

From the experimental results obtained for CC-EPS solutions, the superposition of $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ traces shows some peculiarities, consistent with those observed for the viscoelastic spectra. At low shear rate and frequency, the curves $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ can be superposed; on increasing the shear rate and/or frequency, the slopes of $\eta^*(\omega)$ become lower than that of $\eta(\dot{\gamma})$ (Fig. 5). This deviation from the Cox–Merz rule is observed only at high frequency and high shear rate, and not over the whole range of ω and $\dot{\gamma}$ investigated, as observed for xanthan for a comparable degree of coil overlaps (Richardson & Ross-Murphy, 1987*b*). At low shear rate

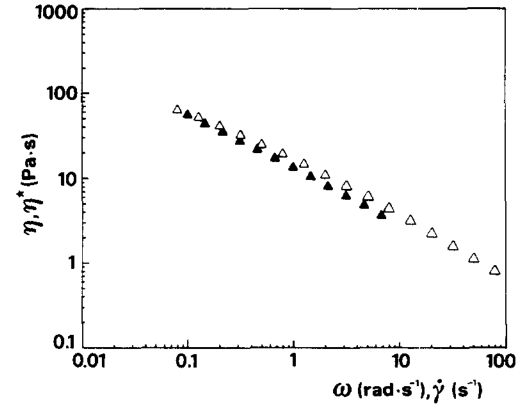

Fig. 5. Cox–Merz plot for 1·1% w/v CC-EPS solution in 0·1 M NaCl. Open triangles: η^*; filled triangles: η.

and low frequency, such correspondence is expected since, although different flow patterns are involved, the effects on the molecular rearrangements cannot be considered significantly perturbative. However the Cox–Merz rule should also be obeyed at high shear rate and high frequency, as has been found experimentally for synthetic random coil polymers (Cox & Merz, 1958; Graessley, 1974). The overall behaviour is qualitatively consistent with behaviour intermediate between that of an entanglement network and that of a 'weak gel'.

The failure of the Cox–Merz rule has been reported in the case of those systems having long-range ordering and structure, such as highly filled polymer melts, polymer gels (Santore & Prud'homme, 1990), and 'weak gels' such as xanthan (Richardson & Ross-Murphy, 1987*b*; Rochefort & Middleman, 1987; Milas *et al.*, 1990). In particular, deviations from the Cox–Merz rule are important for concentrated xanthan solutions and have been interpreted in terms of specific interactions between chain segments, occurring in addition to normal topological entanglements (Richardson & Ross-Murphy, 1987*b*).

Transient experiments

By perturbing a viscoelastic liquid from rest at a constant shear rate, the stress rises, finally approaching the steady-state value as steady shear flow conditions pertain. If the viscoelastic behaviour is linear, stress growth is monotonic, independent of the shear rate imposed. However, concentrated solutions of synthetic polymers (Ferry, 1980), as well as those of polysaccharides (Richardson & Ross-Murphy, 1987*a*,*b*), exhibit monotonic stress growth upon inception of steady shear flow only at low shear rates. By increasing the shear rate, the stress curve shows a maximum before approaching this steady-state value and the phenomenon of stress overshoot can be observed. Such non-linear behaviour is predicted by current molecular theories (Doi & Edwards, 1986).

The stress overshoot phenomena, in terms of recovery of non-linear properties after steady shearing flow, have already been observed in the case of CC-EPS in 0·1 M NaCl at a concentration of 0·5% w/v (Navarini *et al.*, 1990). These results were compared with those observed for concentrated solutions of guar and of xanthan—as far as we are aware the only such polysaccharide data available in the literature (Richardson & Ross-Murphy, 1987*a*,*b*). Nevertheless, due to the different concentration regime and experimental set-up, only a qualitative comparison was attempted.

In order to compare appropriately the time-dependent properties of CC-EPS solutions with those of guar and xanthan, transient experiments have been carried out on CC-EPS solutions at the highest concentration (1·1% w/v), by using the same instrumental and experimental procedure (and the same precautions to ensure good data reproducibility) as in the earlier reported data for guar and xanthan. CC-EPS solutions in NaCl (0·1 M) and at this concentration show stress overshoot at shear rates higher than 0·1/s. As expected, the overshoot is an increasing function of the shear rate, at least in the range of sensitivity of the transducer presently used, and the maximum stress value shifts to smaller values of time upon increasing the shear rate. At a constant shear rate value, the stress overshoot sensibly increases with the rest time preceding the shear rate application.

By plotting the ratio between the maximum stress and the steady stress (overshoot ratio maximum) as a function of the rest time preceding the shear rate application ($\dot{\gamma}$ = 1/s), as shown in Fig. 6, it is possible to observe that the overshoot ratio maximum approaches an asymptotic value, ≈1·28 after *c.* 1000 s. Accordingly, such a delay time is necessary to remove the shear memory. In agreement with the other results, the time-dependent properties of CC-EPS indicate a deviation from typical random coil polysaccharide behaviour in the same concentration regime. As a matter of fact, the behaviour of concentrated solutions of guar (3% w/w) seem reminiscent of, but not identical to, that exhibited by CC-EPS (Richardson & Ross-Murphy, 1987*a*). In both cases, the overshoot ratio maximum approaches an asymptotic value, but in the case of guar a rest time in excess of 100 s is sufficient to delete the previous rheological history, a value one order of magnitude smaller than that obtained for CC-EPS solutions. For xanthan concentrated solutions (1% w/w), where topological constraints are less important than chain–chain specific interactions, 'the recovery extends out to (and probably beyond) times of 10^4 s' (Richardson & Ross-Murphy, 1987*b*).

The mechanism of recovery of non-linear properties after steady shearing flow of the CC-EPS seems to be slower than that of guar, which is apparently governed simply by the time scale of re-entanglement (∝ the tube reestablishment time in the reptation model; Doi & Edwards, 1986). The finding that CC-EPS' viscoelastic

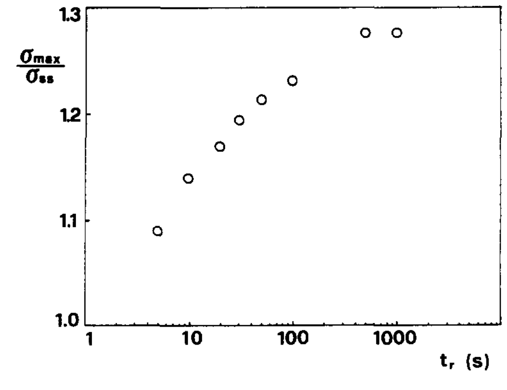

Fig. 6. Plot of overshoot ratio maximum, σ_{max}/σ_{ss}, versus rest time t_r for the 1·1% CC-EPS solution in 0·1 M NaCl.

behaviour can be described as qualitatively intermediate between that of guar and xanthan, is therefore confirmed by the results of transient experiments. We suggest that, in addition to the more traditional dynamic and steady shear experiments, transient experiments, which have not yet become routine, can be very useful in the characterization of polymeric liquids. The caveat is, unfortunately, that such experiments are instrumentally rather testing.

The overall behaviour described above, in which interactions over and above the purely topological contribute to the rheological properties, was originally designated by Newlin *et al.* as due to 'hyperentanglements' (Newlin *et al.*, 1962). Theoretical work by Joanny (1980) and Gonzalez (1983) has tried to modify the reptation model by including complexing groups of finite lifetime, so as to create a 'sticky' tube. According to their work the terminal relaxation time of a chain carrying a small number of functional groups is related to that of the free chain by:

$$\tau_d = (1 - x_2)^{-Nx_2} \tau_d^0$$

where N is the number of groups per chain, and x_2 is the mole fraction of complexing groups.

Experimental studies by Stadler & de Lucca Freitas (1990) on model polybutadienes modified to include hydrogen bond sites, have suggested this approach goes only part way to explaining the behaviour they observed. In the present case, N is presumably constant, and x_2 will be a function of polysaccharide concentration. What is clear is that this equation predicts a finite, though presumably large, zero shear viscosity (since this will depend upon τ_d; Doi & Edwards, 1986), rather than the apparent 'yield stress' (indeterminate viscosity) behaviour we have observed. To investigate this aspect further, future time movement experiments would need to be performed for very long periods.

CONCLUSIONS

In a previous paper (Navarini *et al.*, 1990), somewhat unusual behaviour has been observed in the rheology of aqueous solutions of CC-EPS at concentrations up to 0·5% w/v. In particular, the shear-dependent (concentration dependence of the steady shear viscosity) properties were similar to those exhibited by the microbial polysaccharide xanthan, but the time-dependent properties were more similar to those of the plant galactomannan guar. In order to explain this peculiar rheological behaviour, the existence of flickering interchain cross-interactions between semi-flexible segments was proposed (Navarini *et al.*, 1990). Such a hypothesis is also supported by results from the physico-chemical characterization, which suggests that the solution conformation of CC-EPS is that of a random coil with moderate chain flexibility (Cesàro *et al.*, 1990).

The experimental data reported in this paper can be summarized as follows:

- the viscoelastic behaviour of CC-EPS aqueous solutions at high frequencies resembles that of an entanglement system with G' significantly greater than G'', and the two approximately frequency-independent. Nevertheless, at lower frequencies, this dependence increases in a way not comparable with that of an entanglement system, and G' and G'' approach one another, with a cross-over at a frequency which is strongly concentration-dependent;
- the superposition of $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ curves shows noticeable deviations from the Cox–Merz rule at high frequencies;
- the recovery of the non-linear properties after steady shearing flow is reminiscent of that of flexible random coil polymers. However, in the case of CC-EPS, the process is exceptionally slow;
- the viscoelastic spectra are qualitatively similar to those obtained for xanthan/guar blends.

All these findings are consistent with the picture of a random coil polysaccharide able to form, on increasing concentration, an entanglement network locally stabilized through specific non-covalent intermolecular interactions between semi-flexible segments of the polymeric chains, and is thus in full agreement with the hypothesis previously formulated (Cesàro *et al.*, 1990; Navarini *et al.*, 1990). Further studies are necessary to confirm this view, amongst which clearly one of the most important is the complete elucidation of the primary structure.

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