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Rheology of Native Dextrans in Relation to Their Primary Structure

J. Sabatié,[a] L. Choplin,[a] J. L. Doublier,[b] J. Arul,[a]
F. Paul[c] & P. Monsan[c]

[a]Department of Chemical Engineering, Université Laval, Quebec, Canada
[b]LPCM, INRA, Rue de la Géraudière, 44072 Nantes, France
[c]BioEurope, 4 Impasse Didier Daurat, Z. I. Montaudran, 31400 Toulouse, France

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ABSTRACT

High molecular weight dextrans were synthesized at five temperatures (3, 10, 20, 25 and 30°C) using an in-vitro enzymatic method. The rheological properties of these dextrans in aqueous solution were assessed through their flow behaviour and their viscoelastic characteristics. The results were interpreted in relation to their primary structure and particularly to their branching.

It was shown that the relatively expanded conformation of the dextrans synthesized at 3, 10 and 20°C gives to these dextrans comparable properties which are not too different from those described in literature for random-coil linear polysaccharides. Dextran synthesized at 30°C exhibited flow properties which are typical of particle suspensions in dilute and semi-dilute solution. In the concentrated domain, this dextran yielded structured systems with properties typical of weak gels. This unexpected behaviour could be related to the highly-ramified structure of this dextran in comparison with the dextrans synthesized between 3 and 20°C. On the other hand, the dextran synthesized at 25°C displayed rheological behaviour which could also be related to an intermediate primary structure between those of dextran synthesized at 20°C and dextran synthesized at 30°C.

INTRODUCTION

Dextran is the generic name for a large family of bacterial polysaccharides synthesized from sucrose by extracellular dextransucrases. The

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synthesis reaction follows the pattern

$$n\text{-sucrose} \longrightarrow \underset{\text{(dextran)}}{[\text{glucose}]_n} + n\text{-fructose}$$

The peculiarity of this reaction is that neither a primer nor a high-energy phosphorylated intermediate is involved in the transfer of one glucose into the dextran chain, as the energy required results from the split of the sucrose molecule.

The mechanism of action of the dextransucrase from *Leuconostoc mesenteroides* NRRL B 512F has been precisely described by Robyt and coworkers (1974; 1976; 1978). The authors emphasized the role of acceptor molecules such as fructose, free dextran, maltose, and their effect on the size and primary structure of the final product.

On the other hand, during the same period of time, the use of new separation techniques involved an important increase of the efficiency of dextransucrase purification processes (Lopez & Monsan, 1980). Recently, highly-purified dextransucrase was obtained by a PEG-dextran, phase-partition process which permits the concentration and stabilisation of the enzyme (Paul *et al.*, 1984).

These two factors, the knowledge of the synthesis mechanism and the availability of dextransucrase, make dextran an interesting microbial polysaccharide for establishing relationships between the conditions of synthesis, the primary structure and the solution properties. Thus one of the long term objectives of this work is the control of solution properties, i.e., rheological properties, of dextran through the choice of specific synthesis conditions.

There are two principal parameters which substantially influence the synthesis reaction: the presence of efficient acceptors and the temperature of synthesis. A first part of this program concerning the direct enzymatic synthesis of controlled molecular weight dextrans by addition of good acceptors (maltose, oligodextrans) in the synthesis medium, has already been described (Paul *et al.*, 1986).

In the present work, the authors chose to synthesize high molecular weight dextrans (without acceptors) and to consider the temperature of synthesis. Indeed, this parameter seems to have substantial effects on dextran structure and properties, because large differences in appearance and texture are observed between the synthesis media obtained at low and high temperatures. A first part of this study, concerning the effect of synthesis temperature on the primary structure of dextran has been reported (Sabatie *et al.*, 1988). It has been concluded that for a synthesis temperature ranging from 3 to 20°C, dextrans have an expanded conformation due to relatively linear structures. At a high synthesis temperature (30°C), the molecule contains more secondary ramifications resulting in a much more compact conformation and a higher density of segments. Between 20 and 30°C branching seems to increase progressively, according to the intermediate results obtained with the dextran synthesized at 25°C. Finally, molecular weights of 7×10^6 for the dextrans synthesized between 3 and 25°C and a molecular weight of 13×10^6 for the dextran synthesized at 30°C were found.

The aim of the present paper is to study the rheological properties of these native dextrans in aqueous solution. An attempt is also made to interpret the different behaviours on the basis of their primary structure.

METHODS

Five high molecular weight dextrans were synthesized at different temperatures (from 3 to 30°C), using a highly purified dextransucrase (Paul *et al.*, 1984) and under conditions of synthesis as previously reported (Sabatie *et al.*, 1986*a*; 1986*b*). Dextran-3 or D-3 denotes the dextran synthesized at 3°C, dextran-3–20 or D-3–20 denotes the dextrans synthesized between 3 and 20°C inclusively, and so on for the other synthesis temperatures. The molecular weight and radius of gyration of these samples were measured by light-scattering using a semi-logarithmic representation of a Zimm-Plot and the data analysis proposed by Burchard & Pfannemuller (1969). The hydrodynamic volume of these dextrans was estimated through their intrinsic viscosity determined on a Low-Shear estimated through their intrinsic viscosity determined on a Low-Shear (Contraves, Zurich) viscometer. More details on methods and results are found in Sabatie *et al.* (1988).

Aqueous solutions of dextran-3–25 were prepared at 60°C by stirring gently for approximately 3–4 h until as clear a solution as possible was obtained. In fact, high shear would induce a time-dependent increase in viscosity as well as in turbidity. In the case of dextran-30, such a phenomenon was not observed and solution properties were not dependent upon the conditions of dispersion (Sabatie *et al.*, 1986*a*).

Flow curves in dilute solutions were measured using a Low-Shear 30 viscometer (Contraves). In the case of dextran-3, relative viscosity in very dilute solutions was also determined with a capillary (Ubbelohde) viscometer. Flow curves for concentrated solutions and experiments in dynamic mode were carried out on a Rheometrics System-4 rheometer, using the cone and plate fixture (25 mm cone radius and 1 degree cone angle) at 25°C.

RESULTS

Flow properties

Flow curves of dextran-3 in dilute and concentrated solutions are shown in Fig. 1. It is noteworthy that a shear-thinning behaviour is observed for concentrations as low as 0·168% (w/v). At higher concentrations, the overall pattern of the flow curves remains identical. The power law index for the 10% (w/w) solution is $n = 0{\cdot}71$ (n being defined as $\sigma = k\dot{\gamma}^n$, with σ shear stress and $\dot{\gamma}$ shear rate), showing that the shear-thinning behaviour is quite limited. In fact, these properties are usual for polysaccharides in solution and are typical of most macromolecular solutions. However, for the highest concentrations, this behaviour is only observed at relatively low shear rates. As a matter of fact, a threshold-type shear-thickening behaviour would result from shearing at shear rates higher than a critical value indicated by $\dot{\gamma}^*$ (i.e. dextran-3, 10% in water, $\dot{\gamma}^* = 5\ \mathrm{s}^{-1}$). This phenomenon has already been described (Sabatie *et al.*, 1986*a*; 1986*b*) and will not be considered in the present paper since the results reported here do not concern the range of shear rates at which the phenomenon occurs.

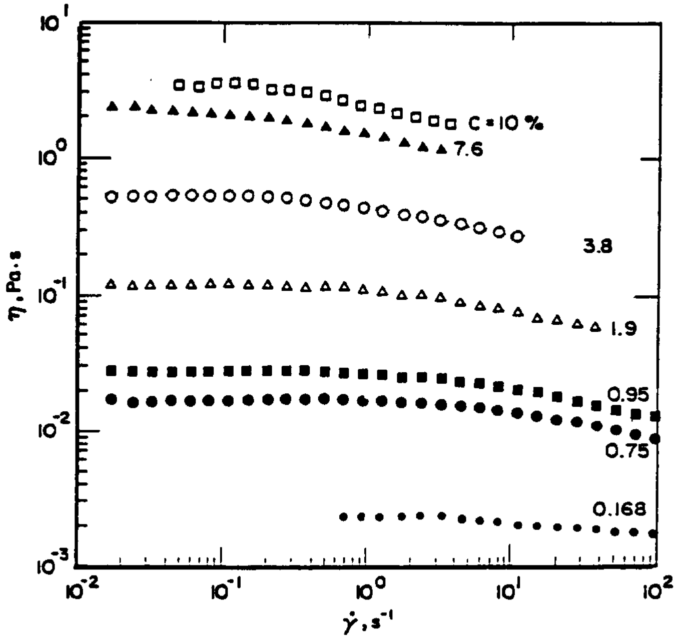

Fig. 1. Flow curves of dextran-3 aqueous solutions at different concentrations.

Dextran-10–20 display very similar rheological properties to dextran-3 except that $\dot{\gamma}^*$ takes higher values.

By plotting the zero shear rate specific viscosity versus concentration for dextran-3, two straight lines are obtained which correspond to the dilute and more concentrated domains (Fig. 2). The two straight lines join at the critical concentration $c^* = 0{\cdot}48\%$. Data and straight lines from a commercial dextran (T2000) as reported by Morris *et al.* (1981) are also shown. It is seen that at a given concentration, the zero shear rate viscosity, η_0 is much lower for T2000 than for D-3. On the other hand, T2000 seems to display three straight lines with slopes of 1·4, 2·0 and 4 respectively, as was mentioned by Launay *et al.* (1986), and the critical concentrations for T2000 were 3·7% and 18%, respectively. It is worthwhile to note that originally Morris *et al.* (1981) reported only two straight lines with slopes of 1·4 and 3·3, the crosspoint being at a concentration of 7·7%.

Dextran synthesized at 30°C exhibits very specific flow properties as shown in Fig. 3. Newtonian behaviour is observed even for relatively high concentrations (1%). Between 3% and 4% dextran concentration,

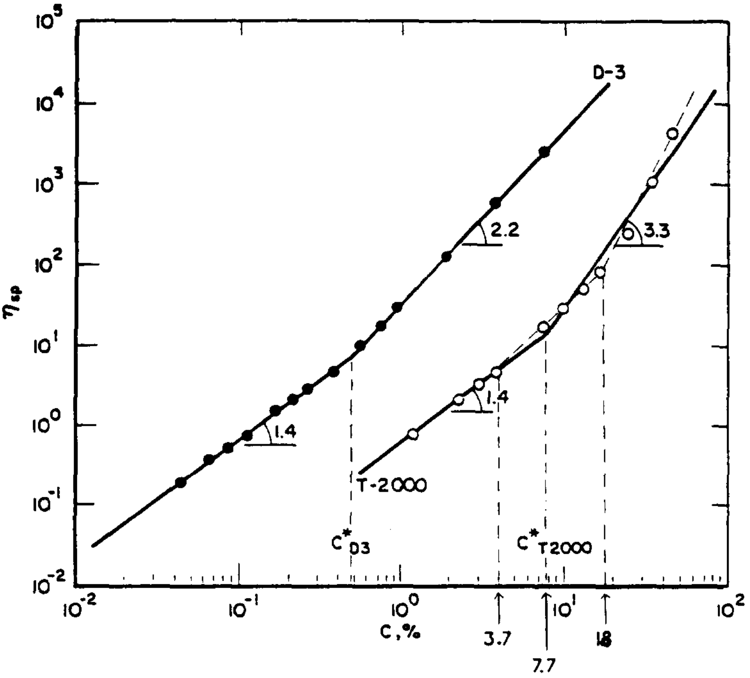

Fig. 2. Zero shear rate specific viscosity versus dextran-3 concentration and data for commercial dextran T2000 from Morris *et al.* (1981).

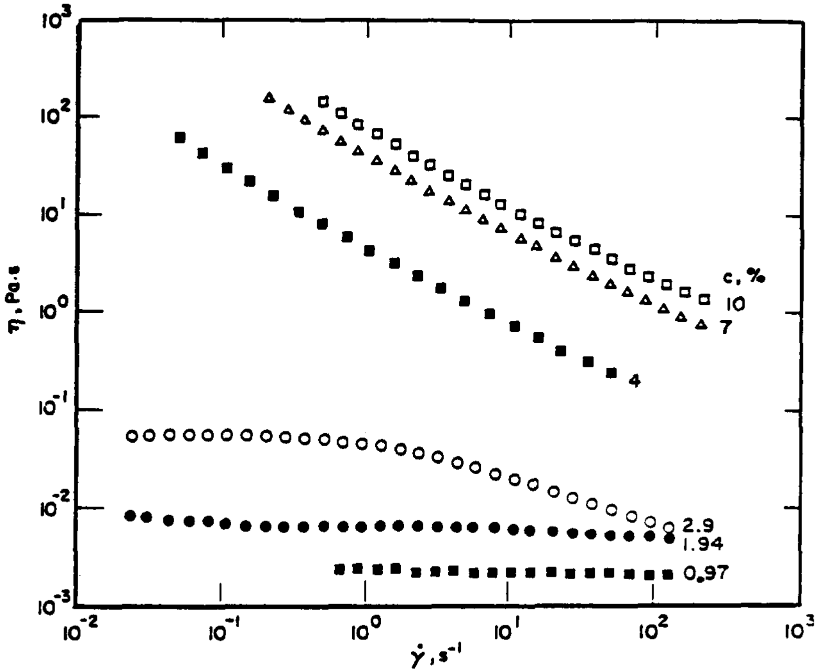

Fig. 3. Flow curves of dextran-30 aqueous solutions at different concentrations.

the flow curve changes dramatically and a behaviour with a pseudo yield-stress and a very steep shear-thinning region ($n = 0{\cdot}15$) is displayed.

The zero shear rate specific viscosity versus dextran-30 concentration gives the graph in Fig. 4. At low concentrations, the slope of 1 is low as compared with the corresponding slope of 1·4 observed with the dextran-3 and also reported for other random-coil polysaccharides. At higher concentrations, the specific viscosity increases rapidly up to 4% dextran concentration. The experimental point determined for this 4% concentration was deduced from the viscosity at $\dot{\gamma} = 0{\cdot}05\ \mathrm{s}^{-1}$ and was added only in order to give a qualitative illustration of the whole behaviour.

A generalization of random-coil polysaccharide behaviours has been proposed by plotting the zero shear rate specific viscosity versus reduced concentration, $c[\eta]$ (Morris *et al.*, 1981). Applied to the dextran-3–30, this representation gives the graph displayed in Fig. 5.

In the dilute domain, all the data, except those for dextran-30, superimpose on the same straight line with a slope of 1·4. This is not surprising, since the flow properties in dilute solutions are directly related to the hydrodynamic volume of the molecules, which is taken into account by the term $c[\eta]$.

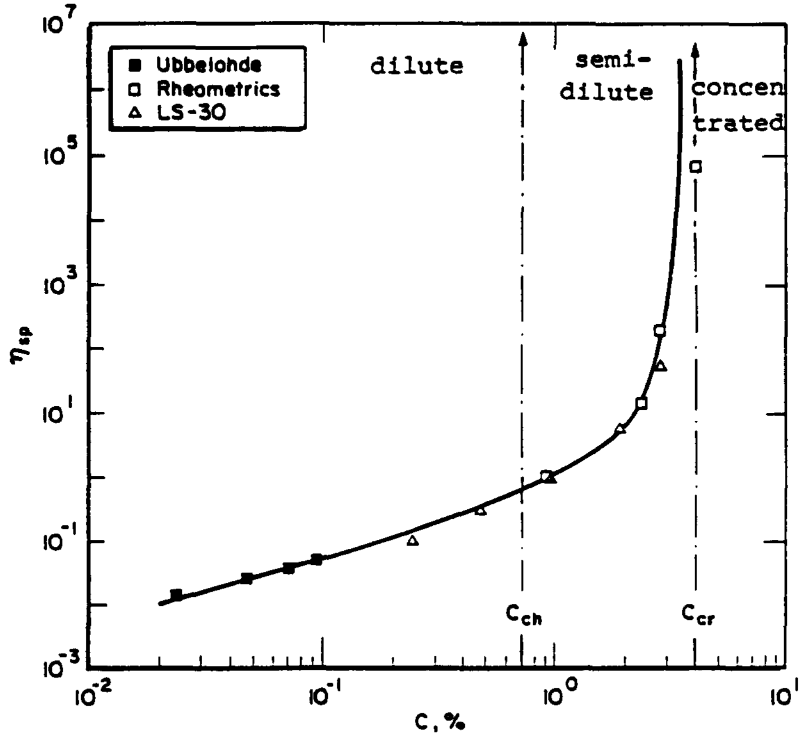

Fig. 4. Zero shear rate specific viscosity versus dextran-30 concentration. Line: Model of Menjivar & Rha (1980) applied to dextran-30; $1/\ln \eta_r = 1/0{\cdot}52\,C - 1/0{\cdot}52\,C_{cr}$ (C in % w/v)

In the more concentrated region, it is worth noting that the slope increases with the synthesis temperature. The dextran-25 gives a straight line with a slope of 3·6, which is intermediate between the slope of 2·2 observed with the dextran-3–20 and the steep increase of viscosity of dextran-30. Moreover, the breakpoint is at $c[\eta] = 0{\cdot}8$ for D-25 and $c[\eta] = 2{\cdot}2$ for D-3, D-10 and D-20.

Dynamic mode

Figure 6 presents a typical frequency dependence of the dynamic moduli, G' and G'', and the complex viscosity magnitude obtained with dextran-10. The same pattern was observed with dextran-3–25. The strain amplitudes mentioned in this figure were chosen from strain-sweep experiments at the upper limit of the region where the dynamic moduli G' and G'' are amplitude independent, that is, in the domain of linearity. It is observed that in dilute and concentrated solutions, the

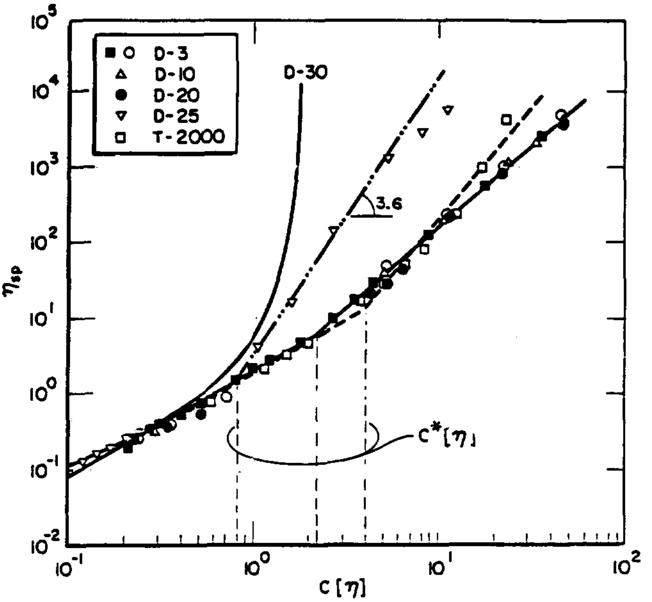

Fig. 5. Zero shear rate specific viscosity versus dextran-3-30 reduced concentration and data for commercial dextran T2000 from Morris *et al.* (1981).

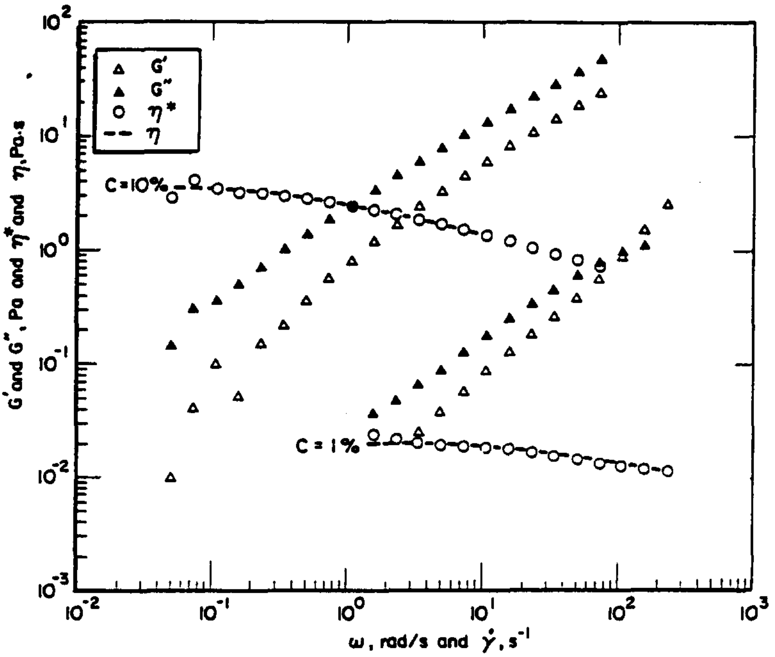

Fig. 6. Dynamic moduli G' and G'' and magnitude of the complex viscosity versus frequency for dextran-10 in water. Strain amplitude = 30% for 10% solution, 100% for 1% solution.

dextran-3–25 present dynamic moduli whose dependence upon frequency is typical of macromolecular solution without specific interaction. This is also illustrated by verifying that the so-called Cox–Merz rule illustrated by equation (1) is obeyed (see Fig. 6). It must be noted that the regions where $G' \propto \omega^2$ and $G'' \propto \omega$ are hardly observed because the values correspond to the limit of the transducer sensitivity.

$$|\eta^*|_\omega = \eta(\dot{\gamma} = \omega) \tag{1}$$

Dextran-30 exhibits in dilute solutions similar properties to the other dextrans with a storage modulus G' much smaller than the loss modulus G'' (Fig. 7). However, above 4%, the properties are drastically changed and G' becomes higher than G'', both moduli being almost frequency independent. Moreover, the Cox–Merz rule is no longer obeyed. This behaviour is typical of structured media and the concentrated solutions of dextran-30 can be considered as weak gels.

DISCUSSION

It is clearly demonstrated in Fig. 5 that three different behaviours are obtained depending upon the synthesis temperature. Dextrans synthe-

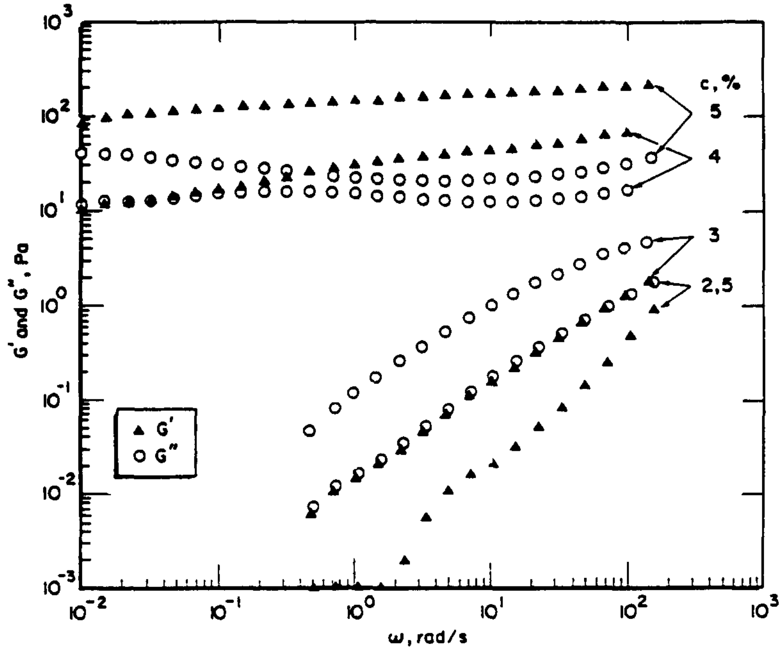

Fig. 7. Dynamic moduli G' and G'' versus frequency at different concentrations of dextran-30 in water. Strain amplitude = 5% for 4% and 5% solutions, 50% for 2·5% and 3% solutions.

sized at 3, 10 and 20°C display the same pattern and the data can be superimposed onto the results for dextran T2000 reported by Morris *et al.* (1981) with the exception of the highest reduced concentrations ($c[\eta]$ above 10). The slope of 1·4 in the dilute domain is equal to that reported for different random-coil linear polysaccharides in aqueous solution (Morris *et al.*, 1981; Castelain *et al.*, 1987). The slope of 2·2 obtained in the more concentrated region for D-3, D-10 and D-20 is to be compared with the slope of 2·0 within the intermediate semi-dilute region, between c^* and c^{**}, evidenced in the case of some linear polysaccharides (Cuvelier & Launay, 1986; Castelain *et al.*, 1987). The breakpoint at $c[\eta] = 2{\cdot}2$ thus determines the upper limit of the dilute regime characterized by the critical concentration $c^* = 2{\cdot}2/[\eta]$. This value is slightly higher than those reported for other polysaccharides (0·6 for Hydroxyethyl cellulose HEC, 1·4 for Carboxymethyl cellulose CMC and xanthan gum as examples) (Castelain *et al.*, 1987). It remains however of the same order and is to be compared with theoretical estimates proposed by several authors: $c^*[\eta] = 1{\cdot}1$ for hexagonal packing of spherical coils (Simha & Zakin, 1960) or $c^*[\eta] = 1{\cdot}5$ for total occupancy of the available volume by coils (Morris *et al.*, 1980). It has been proposed (Graessley, 1980) that above c^* a progressive compression of the coils occurs with increasing concentration. Contraction of the coils progressively brings the chains to their unperturbed dimensions above a second critical c^{**}. This is not observed for D-3, D-10 and D-20, in contrast to dextran T2000 and linear polysaccharides of much lower molecular weight (Launay *et al.*, 1986). It is more likely that, for these three dextrans, c^* equals c^{**}. This is conceivable provided that dextran adopts unperturbed dimensions whatever the concentration. In fact, results obtained through light scattering measurements have shown that water is a Θ solvent for these high molecular weight dextrans (Sabatie, 1987).

In the more concentrated domain, the difference, in terms of slope, is not surprising. As a matter of fact, dextran is unusual as it contains long branches which are only partly taken into account by the term $c[\eta]$ (Graessley *et al.*, 1976).

The specificity of dextran synthesized at 30°C is clearly illustrated in Fig. 5. The pattern of the curve in Fig. 4 is very similar to that of particle suspensions or of globular protein solutions as described by Menjivar & Rha (1980). These authors have proposed a model that describes the flow properties of globular proteins in solution over a wide range of concentrations. This model assumes each macromolecule to be composed of a molecular core and an interactive volume. On this basis, a characteristic concentration C_{ch} is defined up to which the Einstein equation is obeyed ($\eta_r = 1 + 2{\cdot}5\Phi$). Similarly, a critical concentration C_{Cr}

is assumed from which an apparent yield stress is observed. These two concentrations, C_{ch} and C_{cr}, which correspond to the maximum and the minimum interactive volume of the macromolecule, respectively, determine the three concentration domains shown in Fig. 4. Based on the Mooney equation for particle suspensions, Menjivar & Rha (1980) have also derived the following mathematical expression:

$$1/\ln(\eta_r) = 1/[\eta]C - 1/[\eta]C_{cr} \qquad (2)$$

The intrinsic viscosity of dextran-30 was found to be 52 ml/g (Sabatie, *et al.*, 1988). By using this value in the above equation, a good fit of experimental data is obtained in the dilute and semi-dilute domains (Fig. 4). This model also allows a more accurate estimate of the critical concentration ($C_{cr} = 4\%$ (w/w)) to be obtained. This predicts η_0 to be infinite above 4%, which is verified experimentally, as illustrated for the last point in Fig. 4. Above this value, dextran-30 thus exhibits flow behaviour (Fig. 3) and viscoelastic properties (Fig. 7) typical of structured media.

These unexpected properties of dextran-30 must be ascribed to its very compact conformation due to a high density of segments (Sabatie *et al.*, 1988). It was shown that dextran-30 is highly branched, particularly through secondary ramifications. This highly ramified structure yields macromolecules which are much more compact than dextran-3–20. This compactness can prevent the overlap of the molecules which, for other dextrans, takes place in a relatively low concentration, in the vicinity of c^*. This effect of branching on the state of entanglements was proposed by Fujimoto *et al.* (1970) to explain the failure of the Bueche and Graessley theories to predict the flow properties of branched macromolecules.

However, the overlap of molecules is forced to occur above the critical concentration ($C_{cr} = 4\%$) which leads to a large increase of the characteristic time of the medium (Fig. 7). At still higher concentrations, it is believed that the molecules are superficially entangled to yield dynamic properties which are typical of structured media. This is consistent with the prediction of Graessley (1977) on the behaviour of entangled branched polymers, based on the concept of reptation from De Gennes (1971).

As illustrated in Fig. 5, dextran-25 is found to have an intermediate behaviour. The structure of dextran-25 in terms of branching and compactness was shown to be intermediate between dextran-3–20 and dextran-30 (Sabatie *et al.*, 1988). Here also, there is an evident connection between structural characteristics and rheological properties. The result is the dependence of viscosity upon reduced concentration (Fig. 5) which is steeper than for dextran-3–20 but less dramatic than for dextran-30. This suggests that between the synthesis temperatures of 20 and 30°C, the properties may change progressively. Thus, data plotted in Fig. 5 give an interesting illustration of the effect of branching on the flow properties of dextrans.

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