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Conformation dependent depolymerisation kinetics of polysaccharides studied by viscosity measurements

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Degradation of single- and multiple-stranded polysaccharides by acid hydrolysis or free radical depolymerisation with H_2O_2/Fe^{2+} was monitored by viscosity measurements. Single-stranded polysaccharides (alginate, hydroxyethyl cellulose, carboxymethyl cellulose and κ-carrageenan in its disordered conformation) gave the expected linear relationship between $1/\eta_{sp}^{(1/a)}$ and the degradation time, where a is the Mark–Houwink–Sakurada exponent. Double-stranded xanthan and triple-stranded scleroglucan showed a different pattern. Following an initial period with apparently slow degradation, a second regime was entered where the apparent degradation rate was much higher, and where η_{sp} followed the power law, $\eta_{sp} \sim t^{-\nu a}$. However, the estimated values of the parameter ν differed from those calculated by a Monte Carlo method for double- (xanthan) and triple- (scleroglucan) stranded polymers. κ-Carrageenan in its iodide-induced ordered conformation was very stable in acid as compared to the disordered conformation. In the ordered state the apparent degradation rate was initially constant, but increased in later stages. However, the double-logarithmic plot of η_{sp} versus time showed that there was neither a pronounced stable regime nor a regime following the power law. The degradation of gellan resulted in a rapid and linear increase in $1/\eta_{sp}$ with degradation time, both in the ordered and disordered conformation. The same type of degradation kinetics was obtained for welan.

INTRODUCTION

Polysaccharides are in general susceptible to a variety of degradation mechanisms, including oxidative–reductive, free radical depolymerisation, acid-, alkaline- or enzymically catalysed hydrolysis as well as thermal degradation. The degradation rates depend on the concentrations of reactants and temperature in the usual way. In addition, many polysaccharides may adopt distinct conformational states, e.g. random coils, expanded coils or helices. They may further be either single- or multiple-stranded. Such features are also expected to influence the degradation kinetics. It is well known that polysaccharides such as xanthan and scleroglucan are particularly stable as long as they are in the ordered, multiple-stranded conformations. This is highly valued in industrial processes such as in oil recovery, and forms the basis for the choice of xanthan or scleroglucan as viscosifier for long term use in high temperature reservoirs (Davidson & Mentzer, 1982; Holzwarth, 1985; Seright & Henrici, 1986; Chauveteau & Sorbie, 1991). When such polymers are converted to the disordered state, for instance by lowering the ionic strength and/or elevating the temperature above the characteristic transition temperature (T_m), they are depolymerised more rapidly. This has in particular been demonstrated for xanthan, both for acid hydrolysis (Christensen & Smidsrød, 1991), enzymic hydrolysis (Rinaudo & Milas, 1980) and thermal or free radical degradation (Muller & Lecourtier, 1988).

Cooperative order–disorder transitions in polysaccharides are usually detected by changes in the optical rotation, circular dichroism or heat capacity. Transition parameters such as transition temperature (T_m), cooperativity and transition enthalpy depend on structural factors, including charged groups and chain length, as well as on external factors (solvent, ionic strength, pressure, etc.). For many polysaccharides the

nature of the conformational states has not yet been fully understood, and in some cases diverging or controversial conclusions have been presented. This is particularly so in the question of strandedness of the ordered states of xanthan and κ-carrageenan. A general problem seems to be the extrapolation from the well-defined crystalline or fibrous, solid state examined by X-ray diffraction to aqueous solutions where hydration plays a key role, as well as the ambiguous data analysis of X-ray fibre diffraction patterns. Analysis of the depolymerisation kinetics, in terms of the molecular weight decay, is recently suggested as an independent method to investigate the conformational properties in solution (Christensen *et al.*, 1993*a*). The aim of this work is, therefore, to analyse the depolymerisation kinetics based on a simple viscometric method for monitoring polysaccharide degradation, with special emphasis on the polysaccharide conformation and strandedness on the degradation kinetics.

A nonspecific (random) depolymerisation of a single-stranded polymer obeys the following equations (Tanford, 1961):

$$\frac{1}{\bar{x}_{n,t}} = \frac{1}{\bar{x}_{n\,0}} + kt \qquad (1a)$$

and

$$\frac{1}{\bar{x}_{w,t}} = \frac{1}{\bar{x}_{w,0}} + \frac{kt}{2} \qquad (1b)$$

where $\bar{x}_{n,t}$ and $\bar{x}_{n,0}$ and $\bar{x}_{w,t}$ and $\bar{x}_{w,0}$ are number- and weight-average degrees of polymerisation, respectively, at times t and 0, and k is the rate constant for bond cleavage. As $\bar{x}$ is proportional to the molecular weight, eqns (1a) and (1b) indicate that the inverse of the molecular weight should increase linearly with the depolymerisation time. This has previously been demonstrated for alginate (Haug *et al.*, 1963; Smidsrød *et al.*, 1963) and hyaluronate (Rickards *et al.*, 1967). Accordingly, a single-stranded polysaccharide can be applied as a model substance for a given degradation method (e.g. H_2O_2/Fe^{2+}), to examine whether the degradation is random with respect to chain cleavage.

In the case of multiple-stranded polymers the molecular weight decay will deviate from linearity. This was shown by Thomas (1956), who performed random enzymic degradation of DNA with deoxyribonuclease (DNase). This enzyme binds nonspecifically to the DNA molecules and cleaves only one strand at a time. The strand cleavage leads to the release of a proton from the phosphate–ester bond, and the number of broken bonds is determined by titration (Thomas, 1956). The observation that about 50 breaks per molecule were needed before a reduction of M_w could be observed was supposed to be caused by the double-helical conformation, in accordance with the assumption that cleavage in both strands within a critical minimum distance (DP_{min}) was necessary for the whole molecule to break.

Based on this and other reported differences in stability of single- and multiple-stranded polymers, a theoretical study using a Monte Carlo method was undertaken (Stokke *et al.*, 1992). This analysis predicted a distinct degradation behaviour upon random depolymerisation, which was strongly dependent upon the strandedness. As for DNA an induction period was found where the cooperative intermolecular forces within the multiple-stranded structure to a large extent prevented strand separation and, consequently, a decrease in the observable M_w. This was followed by a regime at $M_{w,t}/M_{w,0} < 0{\cdot}1$ where the decrease in molecular weight followed a power law dependence on the depolymerisation time (t):

$$\frac{M_{w,t}}{M_{w,0}} \sim t^{-v} \qquad (2)$$

The exponent v was found to be 1·0 (as also expected from eqn (1)) for single-stranded polymers, 1·66 for double-stranded polymers and 2·3 in the triple-stranded case. Hence, information about strandedness is in principle obtained by measuring the molecular weight as a function of depolymerisation time. Experimental verification of such calculations has, for xanthan (acid hydrolysis), been attempted by converting intrinsic viscosity data to molecular weights (Stokke *et al.*, 1992) and recently, from molecular weight measurements by low angle laser light scattering (Christensen *et al.*, 1993*a*).

In order to investigate the degradation behaviour of a series of different polysaccharides we have adopted a simple method based on continuous measurements of the viscosity using a capillary viscometer, where data can be collected with great accuracy over a large range of relative viscosities down to $\eta_{rel} \approx 1{\cdot}01$. At such a low η_{rel} the most critical issue is temperature control. The problem with shear-thinning effect in solutions containing stiff molecules at high molecular weights can be avoided by using sonicated low-M_w samples.

The relationship between the molecular weight and the intrinsic viscosity $[\eta]$ is usually given by the Mark–Houwink–Sakurada (MHS) equation:

$$[\eta] = K{\cdot}M^a \qquad (3)$$

In the single-stranded cases, combination of eqns (1b) and (3) yields

$$\left(\frac{1}{[\eta]_t}\right)^{\left(\frac{1}{a}\right)} = \left(\frac{1}{[\eta]_0}\right)^{\left(\frac{1}{a}\right)} + k't \qquad (4)$$

where $k = k/2M_0K^{1/a}$, k is the rate of bond cleavage, M_0 is the monomer molecular weight, and K and a are MHS parameters. At low concentrations of polymer $[\eta] \approx \eta_{sp}/c$, and eqn (4) can be rewritten as

$$\left(\frac{1}{\eta_{sp,t}}\right)^{\left(\frac{1}{a}\right)} = \left(\frac{1}{\eta_{sp,0}}\right)^{\left(\frac{1}{a}\right)} + k''t \qquad (5)$$

where $k = k/2M_0cK^{1/a}$. Hence, a plot of $(1/\eta_{sp})^{(1/a)}$ versus the degradation time for a random depolymerisation of a single-stranded polymer should be linear.

In addition, the power law of eqn (2) can be expressed as

$$\left(\frac{[\eta]_t}{[\eta]_0}\right)^{\left(\frac{1}{a}\right)} \sim t^{-\nu} \qquad (6)$$

Since $[\eta]_0^{(1/a)}$ is constant we obtain by combining eqns (2) and (3) in addition to the approximation $[\eta] \approx \eta_{sp}/c$:

$$\eta_{sp} \sim t^{-\nu a} \qquad (7)$$

In this work a series of single-stranded polysaccharides has been degraded to confirm eqn (5) and the experimental conditions for random depolymerisation, before investigating known or assumed double- and triple-stranded polysaccharides. The two degradation mechanisms applied in these experiments were the acid catalysed hydrolysis, where H^+-ions catalyse the cleavage of glycosidic linkages with the addition of water, and the free radical degradation, where the ·OH radicals supposedly attack the polysaccharide chain and by unknown mechanisms cause chain cleavage (Smidsrød *et al.*, 1965; Herp, 1980). The ·OH radical may be generated from H_2O_2 with Fe^{2+} as the catalyst (Baxendale, 1952; Weiss, 1952):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- \qquad (8)$$

The ·OH radical is regenerated through a series of reactions which lead to a concentration of the ·OH radical, which is constant throughout the major part of the degradation (Smidsrød *et al.*, 1965). The degradation rate is practically independent of the amount of H_2O_2 added, but is very sensitive to the concentration of Fe ions (Smidsrød *et al.*,1965).

For polysaccharides, which may undergo an order–disorder transition, attempts were made to study the depolymerisation kinetics both in the ordered and disordered states. Conditions for regulating the conformational state have been identified either on the basis of separate optical rotation measurements, or by the use of literature data. Unfortunately, not all conditions that are necessary to invoke a particular conformation can be combined with the degradation methods used here. For example, scleroglucan may become single-stranded in dimethyl sulphoxide (DMSO) (Yanaki *et al.*, 1981) or in alkaline solutions at high temperature (Rinaudo & Vincendon, 1982). The latter precludes acid hydrolysis and should otherwise not be used in glass capillaries. DMSO decomposes in acidic solutions above 20 °C (Kolthoff & Reddy, 1962) and is, in addition, a powerful scavenger of free radicals (Wellington, 1983), precluding the use of H_2O_2/Fe^{2+} to induce degradation. For this reason, only triple-stranded scleroglucan has been investigated. In κ-carrageenan, the ordered conformation may be induced (without gelation) in the presence of iodide ion (Grasdalen & Smidsrød, 1981). Iodide is also a radical scavenger (Thomas, 1965; Rickards *et al.*, 1967), but is compatible with mild acids.

EXPERIMENTAL

Samples

Scleroglucan was purified from a fermentation broth (ScR 63, Statoil) by centrifugation at 16 000 *g* for 30 min and precipitation in isopropanol. The precipitate was suspended (1 mg/ml) in MQ-water and stirred overnight at 70°C for complete dissolution, followed by filtration through a 3·0 μm membrane filter (Versapor, Gelman Sciences) at 50°C. The scleroglucan solution was then further purified with activated charcoal as described by Truong and Gadioux (1987). The charcoal was removed by filtration through a coarse paper filter (Whatman) and a 3·0 μm filter at 50°C. The salt was then removed by dialysis against MQ-water. A fraction of the solution was sonicated in a Braun Labsonic 1500 sonicator at 300 W for 3 times 15 min, followed by centrifugation and dialysis against MQ-water. Finally, both samples were freeze dried.

A purified xanthan sample was obtained from a fermentation broth (Statoil Biosentrum) by centrifugation at 27 500 *g* for 30 min, followed by filtration through a 0·8 μm membrane filter (Sartorius) and precipitation in isopropanol. A pyruvate- and acetate-free xanthan sample was prepared from a food grade xanthan (Keltrol, Kelco) by hydrolysis of a 1 mg/ml solution in 0·1 mM HCl at 80 °C for 3 h (Christensen *et al.*, 1993*b*). The solution was then neutralised with NaOH, dialysed against 10 mM NaCl, then extensively against MQ-water and finally freeze dried.

Gellan and welan were provided by Kelco. The samples were dissolved in MQ-water, and further purified with active charcoal at 40 °C (Troung & Gadioux, 1987) and filtered through a 0·8 μm filter. The solutions were then dialysed extensively against MQ-water to remove salt, and finally freeze dried.

Hydroxyethyl cellulose (HEC) and carboxymethyl cellulose (CMC) were obtained from Fluka. Alginate isolated from *Laminaria hyperborea* stipe (LF 10/60) was provided by Pronova Biopolymer; κ-carrageenan from *Eucheuma cottonii* was obtained from Sigma.

All samples were dissolved in MQ-water and filtered through a 0·8 μm membrane filter before use in degradation experiments.

Procedures for degradation of polysaccharides

Degradation of polysaccharides was performed by acid hydrolysis in 0·1 M HCl at 50°C. Free-radical depolymerisation was performed with 10 mM H_2O_2, 1 mM

$FeSO_4$, 10 mM Na_2EDTA, 10 mM Na acetate buffer (pH 5·0) and 0·1 M NaCl at 20 and 37°C, or 0·2 mM NaFeEDTA, 10 mM H_2O_2, 10 mM Na acetate buffer (pH 5·0) and 5, 15 or 300 mM NaCl at 60°C. The polysaccharide concentrations were in the range 0·1–2 mg/ml. All glass equipment, used to contain stock solutions and the capillary viscometer, was acid washed (1–2 M HCl, 1–2 h) prior to the degradation experiments.

Viscosity measurements

The degradation experiments were performed in a Schott-Geräte Ubbelohde capillary viscometer (type 531 01/0a, flow-through time for water 200 s at 20°C) immersed in a thermostated water bath. The flow-through times were measured with an AVS-310 (Schott-Geräte) control unit, and a computer was used for automatic data acquisition. The accuracy and reproducibility of the measurements at very low relative viscosities were examined, due to the temperature sensitivity of the viscosity of water (Lide, 1990). This was examined by regularly measuring the flow-through time of water for a period of 24 h at 20 and 50°C. The average flow-through times were 200·62 s (20°C) and 113·99 s (50°C), with standard deviations ($n = 60$) of 0·06 and 0·05 s, respectively. Linear regression analysis further showed that the average drift in the flow-through time (t_f), expressed as the slope $(dt_f/dt)_f$, was not significantly different from zero, which ensured sufficient stability, permitting measurements of relative viscosities below 1·01.

Optical rotation

Optical rotation was measured at 365 nm in a Perkin–Elmer (type 241) polarimeter. The 10 cm cell was thermostated by a circulating-water bath (Haake D8-G). A computer was used for automatic data acquisition and temperature control. Data were calculated as the specific rotation, corrected for minor changes in sample volume at high temperatures.

Low angle laser light scattering (LALLS)

LALLS measurements were performed on a Chromatic KMX-6 light scattering photometer as described by Christensen *et al.* (1993*a*). The intact and sonicated scleroglucan samples were filtered prior to light scattering measurements, using pore sizes of 0·8 and 0·22 μm, respectively.

Chemical analyses

Iodine was extracted with toluene (Custer & Nathelson, 1949). The absorbance of the organic phase was measured at 500 nm in a Shimadzu UV–150–01 spectrophotometer.

The total polysaccharide content was determined by the phenol–sulphuric acid method (Dubois *et al.*, 1956).

RESULTS AND DISCUSSION

Single-stranded polysaccharides (alginate, cellulose derivatives, κ-carrageenan)

The first aim of this work was to confirm that random degradation of single-stranded polysaccharides should give a linear increase in the inverse molecular weight or specific viscosity (eqn (1b) or (5)), as a function of degradation time. This has previously been shown for alginate by free radical degradation with H_2O_2/Fe^{2+} and L-ascorbic acid (Smidsrød *et al.*, 1965, 1967) and alkaline hydrolysis (Haug *et al.*, 1967), and for degradation of hyaluronic acid with L-ascorbic acid (Rickards *et al.*, 1967). However, in these investigations only the initial stages of the degradation were monitored.

The single-stranded polysaccharides used in these preliminary experiments were alginate, CMC, HEC and κ-carrageenan. Alginate is composed of two uronic acids, 4-linked β-D-mannuronic acid and 4-linked α-L-guluronic acid. The monomers are linked blockwise or alternating. Its ordered conformation is obtained by crosslinking blocks of L-guluronate segments by di- or tri-valent cations (Haug, 1964; Grant *et al.*, 1973). The two semisynthetic cellulose derivatives are both presumably single-stranded. κ-Carrageenan is built up by alternating 3-linked β-D-galactose, substituted with a sulphate hemiester in the C4-position, and 4-linked 3,6-anhydro-α-D-galactose. It is supposed to have a single-stranded conformation in its disordered state, induced by low ionic strength or high temperature and/or the absence of cations, which induce gelation.

Figure 1 shows results from the degradation of alginate, CMC, HEC and κ-carrageenan, using free radical

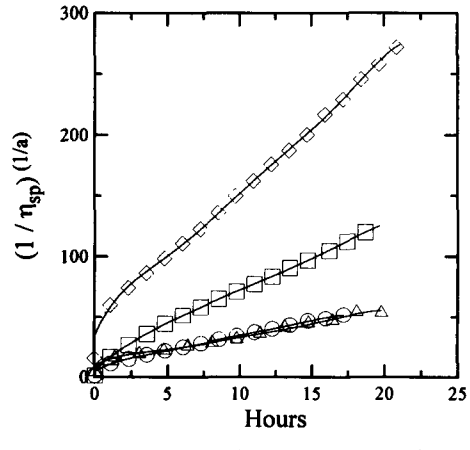

Fig. 1. Free radical depolymerisation at 20°C of (○) alginate ($a = 1·0$), (◇) κ-carrageenan ($a = 0·82$), (□) CMC ($a = 0·74$) and (△) HEC ($a = 0·87$). Polysaccharide concentrations were 1 mg/ml.

depolymerisation with H_2O_2 in the presence of Fe^{2+} ions at 20°C. The chosen values of the MHS exponent (a) were 1·0 for alginate (Smidsrød, 1970), 0·74 for CMC (Brown *et al.*, 1964), 0·87 for HEC (Brown *et al.*, 1963) and 0·82 for κ-carrageenan (Vreeman *et al.*, 1980). The plots of $(1/\eta_{sp})^{1/a}$ are linear in all cases, in accordance with a random depolymerisation process of single-stranded polymers. Of particular importance is that the linearity of the curves, as compared to the nonlinear curves discussed below, does not depend on a very accurate assignment of the MHS exponent. It can readily be demonstrated that an error of 20 to 30% will not change the basically linear shape. For practical purposes, a value of 1 may be used for polymers where the MHS exponent lies between 0·7 and 1·3, as is the case with the polysaccharides studied here. However, if these data should be used to calculate absolute rather than relative rate constants (e.g. k in eqn (1)), correct values for both MHS parameters must be used. A slight downward curvature, reflecting more rapid degradation, is observed for the first two to four measurements (Fig. 1). This is ascribed to an initial change in the relative distribution between ferrous and ferric ions. When, as in this case, iron is added as a ferrous salt the ability to catalyse free radical formation from H_2O_2 will be decreased as some of the ferrous ions are oxidised into ferric ions. The decrease continues until an equilibrium state is obtained (Smidsrød *et al.*, 1965), only after which the polymer degradation data should be analysed.

According to Fig. 1, the degradation has proceeded to very low relative viscosities (1·01 for κ-carrageenan). This illustrates the large range of viscosities which is accessible with sufficient accuracy in the case of this experimental procedure.

Xanthan

Xanthan is a bacterial polysaccharide from *Xanthomonas campestris*, and is a substituted β(1 → 4)-D-glucan. The trisaccharide side-chain [β-D-Man*p*-(1→4)-β-D-GlcA*p*-(1→2)-α-D-Man*p*] is attached to every second glucose of the backbone with (1 → 3) linkages. The α-mannose is partly substituted at O-6 with an acetyl group, and the β-mannose is partly substituted with a pyruvyl group as a cyclic ketal linked to the 4- and 6-positions. There is increasing evidence that the ordered conformation of xanthan is double-stranded (Sato *et al.*, 1984; Coviello *et al.*, 1986; Stokke *et al.*, 1986). Above the melting temperature, T_m, the strands partly separate into single chains (Liu *et al.*, 1987; Stokke *et al.*, 1987; Liu & Norisuye, 1988), although complete strand separation cannot be obtained even in pure water at 95°C (Kawakami *et al.*, 1991). The latter precludes in practice the possibility of performing degradation experiments on fully single-stranded xanthan.

The conformational state (or degree of order) of xanthan can be varied by varying the temperature and/or the ionic strength (Holzwarth, 1976; Morris *et al.*, 1977; Norton *et al.*, 1984; Liu *et al.*, 1987; Liu & Norisuye, 1988). Optical rotation measurements were, therefore, performed to identify suitable conditions for the degradation experiments. From these data (not shown), estimation of the degree of conformational order (Liu & Norisuye, 1988) shows that at 60°C, which was the upper practical limit in the present experiments, we obtain 20% order in 5 mM NaCl, 50% order in 15 mM NaCl and 100% order in 300 mM NaCl.

Figure 2 shows the results obtained by free radical degradation of xanthan under the conditions given above. A MHS exponent of 1·0 is used here, although literature values range from 0·95 in 10 mM NaCl at 80°C (Liu & Norisuye, 1988) to 1·2 to 1·5 (depending on M_w) in 0·1 M NaCl at 25°C (Liu *et al.*, 1987). For the sample in 300 mM NaCl an initially slow degradation is observed, which gradually becomes more rapid with time. The plot of $1/\eta_{sp}^{(1/a)}$ versus time deviates significantly from the straight line expected for single-stranded polymers, but is in accordance with results obtained by acid hydrolysis (Christensen & Smidsrød, 1991). For the sample in 15 mM NaCl the same curve shape is observed, but the rate of degradation is much higher than in 300 mM NaCl. This tendency is even more pronounced at 5 mM NaCl, where a very rapid degradation is observed, although the plot of $1/\eta_{sp}^{(1/a)}$ versus time is clearly not linear. As in the previous case, uncertainties in the assignment of the MHS exponent (within the actual range) have a minor influence on the *shape* of the curve (not shown). Also included in Fig. 2 are results from duplicate runs of these samples, demonstrating the reproducibility of the method, which is only obtained by using acid-washed glassware.

The scavenging effect of halogen ions on free radicals (Rickards *et al.*, 1967) could in principle contribute significantly to the decline in the apparent degradation

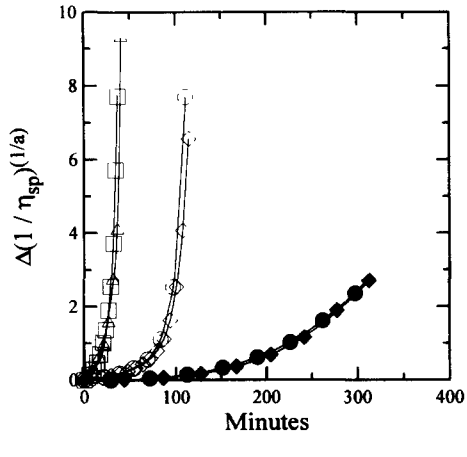

Fig. 2. Free radical depolymerisation at 60°C of xanthan (0·7 mg/ml) (a = 1·0). The concentration of NaCl was (□, △) 5, (○, ◇) 15 and (●, ◆) 300 mM.

rate upon increasing the concentration of NaCl. This possibility was examined by performing free radical degradation of HEC at various concentrations of NaCl. The calculated rates of degradation $(\Delta(1/\eta_{sp})^{(1/a)}/\Delta t)$ were found to be essentially independent of the concentration of NaCl up to 0·4 M (Fig. 3), suggesting that the decline of the degradation rate for xanthan upon increasing NaCl concentration must be associated with the transition from the disordered to the ordered conformation.

Free radical degradation of a partially hydrolysed xanthan was also performed. This xanthan sample had undergone mild acid hydrolysis, to remove the pyruvate and acetate groups from side-chains, to give a xanthan with sharper transition between the ordered and disordered conformation (Christensen *et al.*, 1993*b*). The degradation was performed at 20°C and the plot of $(1/\eta_{sp})^{(1/a)}$ versus time (Fig. 4) shows, in accordance with Fig. 2, deviation from linearity with an increasing degradation rate as a function of time. The calculated initial slope $(\Delta(1/\eta_{sp}/c)/\Delta t)$ of Fig. 4 was found to be

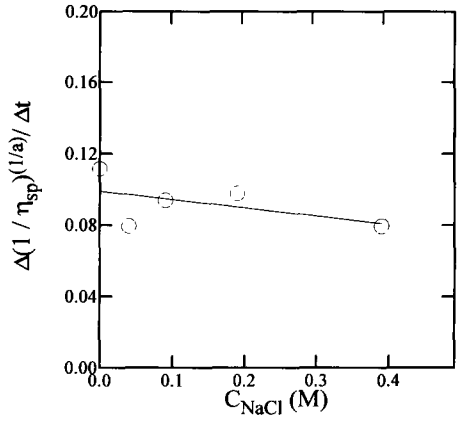

Fig. 3. The initial degradation rates $(\Delta(1/\eta_{sp})^{(1/a)}/\Delta t)$ for free radical depolymerisation at 37°C of HEC ($a = 0{\cdot}87$), at different concentrations of NaCl.

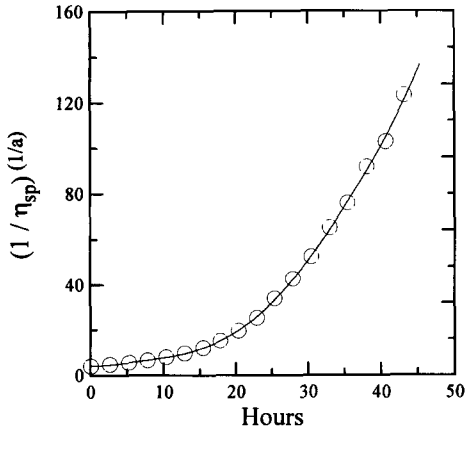

Fig. 4. Free radical depolymerisation at 20°C of partially hydrolysed xanthan (0·25 mg/ml) ($a = 1{\cdot}0$).

60 and 20 times lower than those of CMC and HEC, respectively (Fig. 1), under the same experimental conditions. In addition to the shape of the curve, this illustrates the stabilising effect of the double-stranded conformation of xanthan.

The deviation from single-stranded behaviour of xanthan may be explained qualitatively by a multiple-stranded structure (Christensen & Smidsrød, 1991; Stokke *et al.*, 1992). The gradual increase in the degradation rate is attributed to destabilisation of chain–chain interactions, which occurs between breaks which are separated by a number of polymer segments less than a certain critical value (DP_{min}) as a consequence of the cooperative character of the conformational transition (Stokke *et al.*, 1992). As shown by a Monte Carlo analysis (Stokke *et al.*, 1992) a time domain will be approached where a single break of a linkage on average leads to the formation of 2·7 (in double-stranded polymers) and 3·3 (in triple-stranded polymers) new species. An expected feature for multiple-stranded polymers is, therefore, that following the initially stable period they are subsequently more rapidly degraded, in terms of molecular weight decay, than single-stranded polymers as long as the rate of bond cleavage is constant. The deviation from single-stranded behaviour (Fig. 2) therefore indicates that xanthan is multiple-stranded under the conditions investigated. However, further analysis of the data using eqn (7) (Fig. 5) fails to give a ν value of 1·66 expected for double-stranded polymers. The slope νa was calculated at the time where $\eta_{sp} = 0{\cdot}1 \times \eta_{sp,t=0}$ (using $a = 1$), and ν was found to be 5·5, 12·5 and 2·7 for xanthan, in 5, 15 and 300 mM NaCl at 60°C, respectively, and 2·4 for partially hydrolysed xanthan at 20°C. It can be precluded that these discrepancies are caused by underestimation of the MHS parameter, since it has been shown that it actually decreases as a consequence of degradation (Christensen *et al.*, 1993*a*) in addition to the reduction caused by

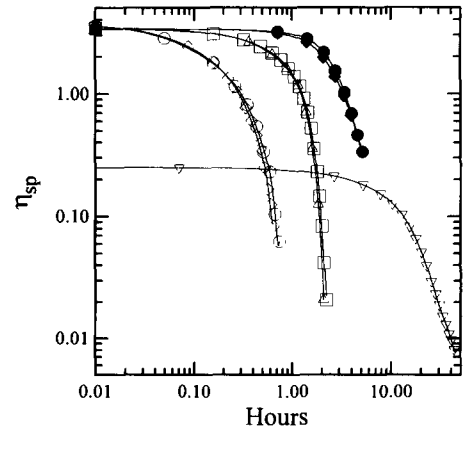

Fig. 5. Double-logarithmic plot of η_{sp} versus time for free radical depolymerisation of xanthan at 60°C in (○, ◇) 5, (□, △) 15 and (●, ◆) 300 mM NaCl and (▽) partially hydrolysed xanthan at 20°C (ionic strength ~ 0·15).

increased conformational disorder (Liu *et al.*, 1987). A more plausible explanation is that low M_w fragments are released from the xanthan backbone during the degradation (Christensen *et al.*, 1993*a*), leaving single-stranded regions in the double-stranded molecules. The rate of bond cleavage appears to be higher for such regions (Christensen *et al.*, 1993*a*), and v values higher than 1·66 will then be observed (Stokke *et al.*, 1993*a*).

A noticeable feature of Fig. 5 is that the xanthan sample, which was only 20% ordered at the beginning of the degradation, at no point approached single-stranded kinetics, i.e. a slope of −1 in Fig. 5, suggesting that the partly multiple-stranded structure prevails throughout the degradation.

It may thus be concluded that the degradation behaviour of xanthan deviates significantly from single-strand kinetics, but current models cannot give more accurate information regarding strandedness based on viscosity measurements alone.

Scleroglucan

Scleroglucan is a fungal polysaccharide from the *Sclerotium* spp., and is a $\beta(1 \rightarrow 3)$-D-glucan with a single D-glucose linked $\beta(1 \rightarrow 6)$ to every third glucose in the main chain. Polysaccharides with similar or related chemical structures include, among others, schizophyllan and lentinan, which differ from scleroglucan mainly in the degree of substitution and length of the side-chains. Otherwise these polysaccharides seem to have almost identical physical and chemical properties (Saito *et al.*, 1977; Yanaki *et al.*, 1981; Saito *et al.*, 1990; Stokke *et al.*, 1993*b*). The ordered conformation is accepted to be a triple helix (Yanaki *et al.*, 1981). The disordered conformation, a flexible single strand (Norisuye *et al.*, 1980), can be induced in aqueous solution either by increasing the pH above 11 (Rinaudo & Vincendon, 1982), by increasing the temperature above 135°C (Kitamura & Kuge, 1989), dissolving in DMSO (>88%) (Yanaki *et al.*, 1981) or at molecular weights less than 40 000 g/mol (Yanaki *et al.*, 1983). Another conformational transition, which apparently does not involve a change in strandedness, occurs in water at about 6°C (Itou *et al.*, 1986; Kitamura & Kuge, 1989).

The degradation course (plotted as $(1/\eta_{sp})^{(1/a)}$ versus time) for intact ($M_w \approx 6 \times 10^6$ g/mol) and sonicated ($M_w \approx 7 \times 10^5$ g/mol) scleroglucan, depolymerised in the ordered state with H_2O_2 in the presence of Fe^{2+} ions at 20°C, is shown in Fig. 6. A MHS exponent of 1·7 was used here except at molecular weights above 5×10^5, corresponding to $(1/\eta_{sp})^{(1/a)} < 5$, where a value of 1·2 was used (Norisuye *et al.*, 1980). As for the degradation of xanthan (Figs 2 and 4), both scleroglucan samples show the initially slow degradation followed by an accelerated regime. Again, the value of the MHS exponent does not influence the basic shape of the curve. Any changes during the degradation will most likely lead to a reduction in the exponent for the same reasons as in xanthan (see above). Such changes will generally lead to even more pronounced nonlinearity in plots of $(1/\eta_{sp})^{(1/a)}$ versus time. Because of the high shear rate in the capillary viscometer, shear thinning may occur at the beginning of the degradation of the high M_w sample. However, comparison of the two curves in Fig. 6 indicates that shear thinning plays a minor role regarding the shape of the curves.

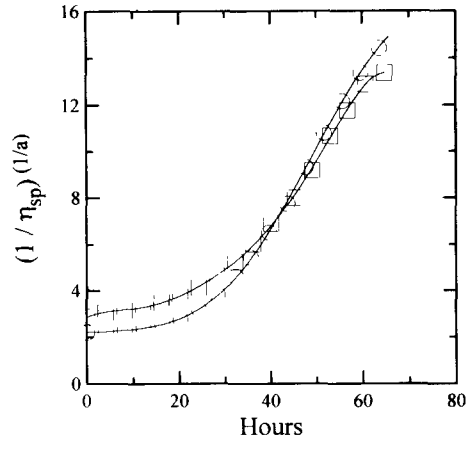

Fig. 6. Free radical depolymerisation at 20°C of (○) intact (0·1 mg/ml) and (□) sonicated (0·3 mg/ml) scleroglucan ($a = 1{\cdot}2$ for $M_w > 5 \times 10^5$ g/mol, otherwise $a = 1{\cdot}7$).

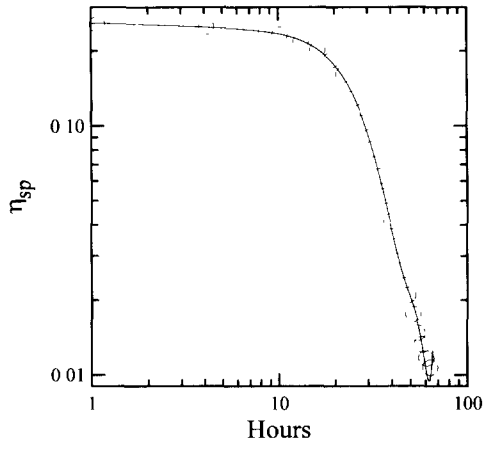

Fig. 7. Double-logarithmic plot of η_{sp} versus time for free radical depolymerisation of intact scleroglucan at 20 C.

The double-logarithmic plot of η_{sp} versus time for the degradation of scleroglucan is shown in Fig. 7. It emphasises the increased initial stability expected for triple-stranded polymers (Stokke *et al.*, 1992). However, the parameter v, which in this case was estimated to be 1·5 (for $a = 1{\cdot}7$), is actually lower than for the experimentally determined value for xanthan, and is closer to the value 1·66 calculated for a double-stranded polymer, than to 2·3, which might be expected for a triple-stranded polymer. This disagreement emphasises the conclusion given for xanthan regarding the possibilities of the current method to distinguish between double- and triple-stranded polymers.

κ-Carrageenan

It seems generally accepted that the disordered conformation of κ-carrageenan is a single-stranded coil, in agreement with the depolymerisation kinetics seen in Fig. 1. The nature of the ordered conformation of κ-carrageenan is somewhat more controversial. The two proposed conformations are the double helix (Anderson *et al.*, 1969; Morris *et al.*, 1980; Nerdal *et al.*, 1993) and the stiff, single-stranded coil (Smidsrød, 1980; Smidsrød *et al.*, 1980; Smidsrød & Grasdalen, 1984*a*). Conformational ordering of the κ-carrageenan molecules is induced by I^- ions at ambient temperature (Grasdalen & Smidsrød, 1981). Further ordering, which is accompanied by gelation, is achieved by potassium ions or di- and tri-valent cations (Smidsrød & Grasdalen, 1984*b*) at quite low concentrations.

Optical rotation measurements were applied to determine the conformational state of κ-carrageenan molecules at different ionic strengths, temperatures and salt types. The optical rotation curve obtained in 0·15 M LiCl (Fig. 8) shows that the midpoint of the transition (T_m) is 12·5°C, whereas the sample is fully disordered above 16 to 17°C. When free radical degradation of κ-carrageenan was performed at this ionic strength at 20°C, a linear relationship between $(1/\eta_{sp})^{(1/a)}$ and time was found (Fig. 1). This is in accordance with the assumed single-strandedness in the disordered state.

Figure 8 shows the optical rotation curves of κ-carrageenan in 0·3 M LiCl and LiI, with T_m values of 22 and 57°C, respectively. The figure also shows that the presence of 0·1 M HCl has no influence on the transition curve as long as the sample is exposed to high temperatures only for short periods, otherwise hydrolysis affects the curve, particularly above T_m. The independence of the conformational state of κ-carrageenan on pH, which contrasts with the behaviour of xanthan (Naksuga & Norisuye, 1988; Christensen & Smidsrød, 1991) can be ascribed to the strong acidity of the sulphate hemiesters of carrageenans.

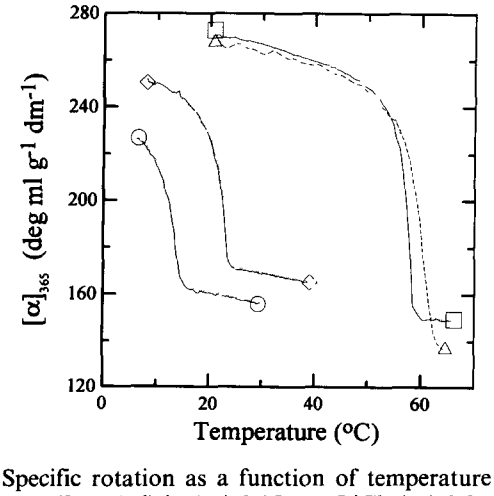

Fig. 8. Specific rotation as a function of temperature for κ-carrageenan (2 mg/ml) in (○) 0·15 mM LiCl, (◇) 0·3 M LiCl, (□) 0·3 M LiI, and (△) 0·3 M LiI and 0·1 M HCl. (——, — —) Heating curves: (- - - -) cooling curve.

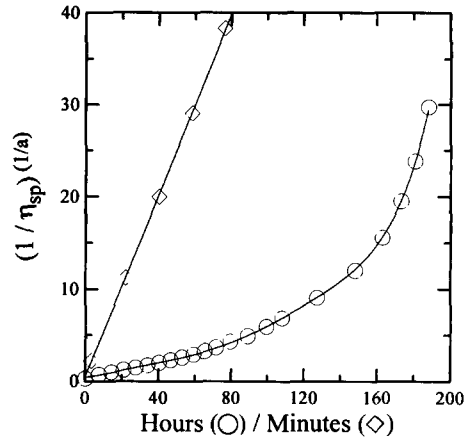

Fig. 9. Acid hydrolysis of κ-carrageenan (2 mg/ml) in 0·1 M HCl at 50°C and (◇) 0·3 M LiCl ($a = 0·78$) or (○) 0·3 M LiI ($a = 0·98$).

Results from the acid hydrolysis of κ-carrageenan in 0·1 M HCl in its disordered (0·3 M LiCl) and ordered state (0·3 M LiI), at 50°C, are shown in Fig. 9. In the case of degradation in LiI, the flow-through time of the solvent decreased (0·4% in 180 h) and a brown colour developed, which was ascribed to the oxidation of iodide to iodine. The concentration of iodine was found to increase linearly with time. The corrected flow-through times of the solvent could then be obtained by interpolation. After 180 h the I_2 concentration was 24 mM, and it was assumed that neither the conformation nor degradation rate was severely affected, since the concentration of iodide was essentially constant.

The degradation of κ-carrageenan in 0·3 M LiCl at 50°C results, as expected, in a linear relationship between $(1/\eta_{sp})^{(1/a)}$ and time, using $a = 0·78$ (Vreeman *et al.*, 1980). The effect of conformational ordering in (0·3 M LiI, using $a = 0·98$ (Smidsrød, 1974)) is pronounced, since the apparent degradation rate is initially about 600 times lower than in the disordered conformation. Further, the shape of the curve is changed from a straight line to a curve that bends upwards, somewhat similar to that of ordered xanthan or scleroglucan.

The double-logarithmic plot of η_{sp} versus degradation time for κ-carrageenan in the ordered state is shown in Fig. 10. The figure shows that the degradation curve differs from that of xanthan. The initial stable period is not as pronounced, and the shape is actually more similar to that of the disordered conformation of κ-carrageenan (not shown) or 80% disordered xanthan (Fig. 5). Another difference is that a region with constant slope, according to eqn (7), is not approached. Estimation of the exponent, *va*, was calculated for $\eta_{sp,t}/\eta_{sp,0} = 0·1$ to be 1·7. A MHS exponent (a) of 0·98 (Smidsrød, 1974) gives a v value of 1·7, which is near the expected value for a double-stranded polymer (1·66). However, the slope seems to increase further after this point. As with xanthan and scleroglucan, it seems that

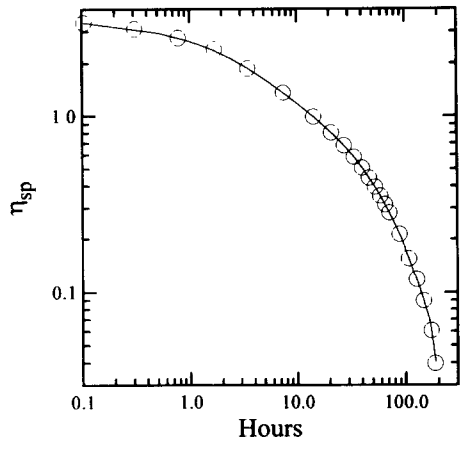

Fig. 10. Double-logarithmic plot of η_{sp} versus time for acid hydrolysis (50°C, 0·1 M HCl) of κ-carrageenan in its ordered state (0·3 M LiI).

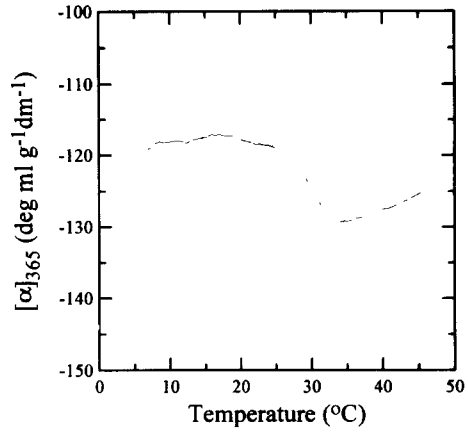

Fig. 11. Specific rotation as a function of temperature for gellan (1 mg/ml) in 0·01 M NaAc and 0·02 M Na_2EDTA. (——) Heating curve; (- - - -) cooling curve

viscosity decay data are not adequate to assess the number of strands which form the ordered conformation. Of particular importance will be the direct determination of the rate of bond cleavage, which for xanthan has been shown to be influenced by the degree of degradation (Christensen *et al.*, 1993*a*).

Gellan and welan

Gellan and welan are bacterial polysaccharides from *Pseudomonas elodea* and *Alicagenes* spp., respectively. They have identical repeating tetrasaccharide units in the main chain, consisting 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 →]$_n$. Gellan is unbranched while welan contains a single α-L-Man*p* (33%) or an α-L-Rha*p* (67%) linked in 3-position of the second D-glucose unit in the main chain repeating unit. Acetylc and L-glycerate groups originally present in gellan are removed, resulting in a polymer which may form strong gels in the presence of a wide range of cations. In addition, gellan displays a conformational transition in the presence of non-gelling cations, e.g. tetramethylammonium, by varying temperature or ionic strength (Crescenzi *et al.*, 1986, 1987; Milas *et al.*, 1990). No such transition has been observed for the nongelling welan in the temperature interval 10–70°C (Crescenzi *et al.*, 1986), and the solution properties are almost insensitive to ionic strength, reflecting a weak polyelectrolyte behaviour of welan despite the presence of one carboxylate group in the repeating unit (Campana *et al.*, 1990). A MHS exponent of 1·41 has been reported (Urbani & Brant, 1989), which is close to the values for rigid multiple-stranded polymers (e.g. scleroglucan). The ordered conformations of gellan and welan have been suggested to be double helices as for xanthan, based on X-ray diffraction (Chandrasekaran *et al.*, 1988; Lee & Chandrasekaran, 1991), light scattering (Dentini *et al.*, 1988; Milas *et al.*, 1990) and potentiometric investigations (Campana *et al.*, 1992).

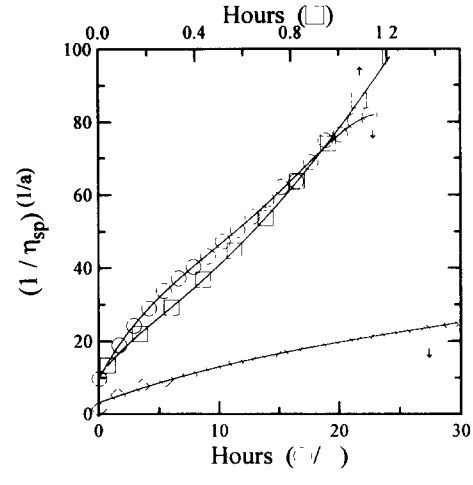

Fig. 12. Free radical depolymerisation of (○) gellan (1 mg/ml) (a = 1·0) and (◇) welan (0·25 mg/ml) (a = 1·41) at 20°C, and (□) gellan (1 mg/ml) (a = 1·0) at 50°C.

Optical rotation measurements were performed to determine the conformational state of gellan in 0·01 M NaAc and 0·02 M Na_2EDTA as a function of temperature. Figure 11 shows that gellan undergoes a conformational change at 30°C in accordance with the results of Milas *et al.* (1990), and free radical degradation was therefore performed at 20 and 50°C.

The degradation curves obtained upon free radical degradation of gellan (20 and 50°C) (a set equal to 1) and welan (a = 1·41 (Urbani & Brant, 1989)) are shown in Fig. 12. The curves are very different from those of xanthan and scleroglucan, and display the linearity observed for the single-stranded polysaccharides. The single-strandedness of gellan in its disordered state was expected, but these results also suggest that gellan in its ordered state and welan both are single-stranded. Gellan is depolymerised about 25 times faster at 50°C than at 20°C. This is approximately the same ratio as that observed for HEC and alginate for the same temperature interval (data not shown), indicating that the optically

detected conformational transition of gellan, in contrast to xanthan and χ-carrageenan, does not influence the degradation rate to any significant extent.

CONCLUSIONS

The conformational state in general, and the strandedness of the polysaccharides in particular, have pronounced effects on the behaviour towards random depolymerisation. It has been shown that single-stranded polysaccharides such as alginate, κ-carrageenan (disordered state), HEC and CMC display the expected linearity between $(1/\eta_{sp})^{(1/a)}$ versus time, and that the degradation can be monitored over a large range of specific viscosities by continuous measurements in a capillary viscometer.

Polysaccharides such as scleroglucan (in the ordered state), xanthan (20–100% order) and κ-carrageenan (in the iodide-induced, ordered state) show degradation patterns which deviate strongly from, e.g. alginate. The observed increases in the apparent degradation rates with time are in part attributed to the initially stabilising effect of the multiple-stranded structures, and in part to different rates of bond cleavage in ordered and disordered regions, respectively. Despite the difficulties in interpreting the data in terms of strandedness, the results obtained both with xanthan and κ-carrageenan illustrate that conformational ordering is accompanied by a pronounced increase in the stability towards loss of viscosity.

Degradation of gellan, in the ordered and disordered state, and welan, gave results which were quite similar to, e.g. alginate, which is in contrast to the double-helical conformations reported in the literature.

It therefore seems that simple analysis of the degradation kinetics presented here has limited applicability as an independent and conclusive method to identify the strandedness of a polysaccharide. However, strongly upward curved plots of $(1/\eta_{sp})^{(1/a)}$ versus time strongly suggest that the conformation is not single-stranded.

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