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Thermal Stability and Chain Conformational Studies of Xanthan at Different Ionic Strengths

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SUMMARY

The aim of the present study was to determine the influence of the ionic strength on the thermal stability of xanthan, i.e. xanthan resistance to chain breaking at high temperatures. Xanthan solutions of various ionic strengths were kept at 80, 90 and 95°C for periods up to 95 h. The thermal stability was determined by measuring the intrinsic viscosity after the heating periods. The experiments showed a critical ionic strength for the thermal stability of xanthan between 10 and 100 mM NaCl or KCl in this temperature range. Below the critical ionic strength the intrinsic viscosity was rapidly reduced, whereas above the critical ionic strength the intrinsic viscosity was virtually unaffected by heating.

We then looked for a possible correlation between thermal stability and secondary structure of xanthan. The transition ionic strength (I_m) of xanthan solutions, i.e. where xanthan is midway between an ordered and a disordered structure, was determined by NMR at constant temperatures. I_m was found to be in the range of 24 mM at 80°C to 60 mM NaCl at 95°C, thus lying in the range of the critical ionic strength of the thermal stability.

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This suggests a close relationship between thermal stability and secondary structure of xanthan, indicated by the enhanced thermal stability in the ordered state. We believe this enhanced thermostability arises from a double-stranded conformation in the ordered state, as in DNA. The presence of double-stranded xanthan is also indicated by electron micrographs taken at both high and low ionic strengths.

The transition temperature (T_m) of xanthan was determined by NMR and optical rotation measurements. At the ionic strength of 7·5 mM the two methods resulted in T_m values of 67 and 52°C respectively. This difference in T_m can possibly be due to the fact that the observed NMR and optical rotation (OR) effects are caused by different molecular phenomena.

INTRODUCTION

The physical and chemical properties of xanthan, the extracellular polysaccharide of *Xanthomonas campestris* have been extensively studied. Possible uses of xanthan in mobility control in Enhanced Oil Recovery (EOR) is probably one reason for this interest.

An important parameter of xanthan for high temperature oilfield applications is its ability to retain the viscosity at elevated temperatures. Several authors have studied the thermal stability of xanthan by measuring the apparent viscosity as a function of temperature and incubation time (Lambert *et al.*, 1982; Ash *et al.*, 1983; Wellington, 1983; Lambert & Rinaudo, 1985; Seright & Henrici, 1986). They all reported less viscosity reduction of xanthan solutions kept at high temperatures in high compared with low salinity solutions.

In this study the thermal stability of xanthan is determined at various ionic strengths by determining the intrinsic viscosity. Since this quantity is directly related to the molecular weight for a given chain conformation, information on degradation of polymer molecules is thus obtained.

The primary structure of xanthan consists of a cellulosic β-(1→4)-linked D-glucose backbone substituted by a trisaccharide on every second glucose moiety. The trisaccharide: (α-1,3-D-mannose, β-1,2-D-glucuronic acid, β-1,4-D-mannose) contains various amounts of acetate at C-6 of the α-mannose and puruvate as a ketal at C-4 and C-6 on the terminal β-mannose (Jansson *et al.*, 1975).

Even though the primary structure of xanthan is known, the secondary structure is not fully understood. To study the secondary structure of xanthan several methods like viscometry, light scattering, optical rotation measurements (OR), circular dichroism (CD), nuclear magnetic resonance (NMR) and electron microscopy (EM) have been used (Holzwarth & Prestridge, 1977; Morris *et al.*, 1977; Holzwarth, 1978; Rinaudo & Milas, 1978; Milas & Rinaudo, 1979; Norton *et al.*, 1980; Frangou *et al.*, 1982; Paradossi & Brant, 1982; Sato *et al.*, 1984*a*, *b*); Stokke *et al.*, 1986, 1987).

Correlation between thermal stability and order – disorder conformational transition as detected by OR and NMR and indicated by EM is used to suggest a mechanism for the enhanced stability of xanthan in the ordered conformation. The present work was not designed to study thermal degradation under optimized conditions (oxygen free, free-radical scavenger added, buffered), and thus, the thermal stability *per se* is relatively poor compared to data for other xanthans (Wellington, 1983 Seright & Henrici, 1986).

MATERIALS AND METHODS

Xanthan obtained as a fermentation broth from Norsk Bioferm A/S was diluted with distilled water (2·5 mg xanthan ml^{-1}) over night. The solutions were filtered using 1·2, 0·8 and occasionally 0·45 μM filters. The polysaccharide was precipitated by adding two volumes of propan-2-ol to one volume of the xanthan solution. The precipitate was re-dissolved in distilled water and freeze-dried.

Nuclear magnetic resonance

Freeze-dried xanthan was dissolved in D_2O (Merck min. 99·75%) and solutions of NaCl in D_2O were added to a final xanthan concentration of 3·3 mg ml^{-1}. ^1H NMR spectra were recorded at 100 MHz on a Jeol JNM-FX 100 spectrometer at various temperatures, using a single pulse mode. The chemical shift of the acetate group at δ 2·09 was used as chemical shift reference (Rinaudo *et al.*, 1983). The integral of the acetate and pyruvate signals were measured using a planimeter and then related to the integral of an external standard of *p*-dichlorobenzene in CCl_4 (Morris *et al.*, 1977). When determining the transition temperature T_m, the NMR spectra were recorded with increasing temperatures, using the technique described by Morris *et al.* (1977). The samples were kept for 15 min at each temperature before recording the spectra. When determining the transition ionic strength I_m, NMR spectra of samples of various ionic strengths were recorded at constant temperatures. The T_m and I_m were taken as the temperature and ionic strength respectively where the area of the acetate or pyruvate ^{1}H NMR signal was 50% of the maximum value.

Optical rotation measurements

Freeze-dried xanthan was dissolved in distilled water overnight and the solution was clarified by ultracentrifugation at 35 000 rpm. A NaCl solution was added to a final xanthan concentration of 3.25 mg ml^{-1} and a NaCl concentration of 7·5 mM. Optical rotation was measured at the 365 nm mercury emission line using a Perkin Elmer 241 Polarimeter. The 100 mm measuring cell was thermostatically controlled by a Julabo 5 circulating waterbath (±0·2°C). The measurements started at low temperature and the samples were kept for 15 min at each temperature before measuring the optical rotation.

Thermal stability

Freeze-dried xanthan was dissolved in distilled water overnight. Solutions of NaCl or KCl were added to a final xanthan concentration of 1·0 mg ml^{-1}. The polysaccharide solutions of various salt concentrations were kept in sealed test tubes in an oven at 80, 90 or 95°C (±1.0°C) for various periods of time. The samples were then dialysed against 3×1000 ml of 0·1 M NaCl and the viscosity was determined using a Cartesian diver viscometer (Toll *et al.*, 1980) at a shear rate of 5 s^{-1} at 25·0°C using xanthan concentrations of 0·5 mg ml^{-1} or less. Intrinsic viscosities were determined as the intercept of reduced viscosity versus concentration.

Electron microscopy

Freeze-dried xanthan was dissolved in 10 mM ammonium acetate overnight. Aliquots of this solution were mixed with 100% glycerol, 1M ammonium acetate and distilled water yielding a 3–20 μg ml^{-1} xanthan concentration in 50% glycerol and the desired ionic strength. 50 μl aliquots of this solution were sprayed onto freshly cleaved mica, vacuum-dried, and rotary shadowed with Pt/C and C (0·7–1·2 nm) as described by Tyler and Branton (1980). Electron micrographs were obtained using a Philips EM 400 T electron microscope, and using a line grid of 1 200 lines mm^{-1} (S 102 Agar Aids) to calibrate the magnification.

RESULTS

Thermal stability

The intrinsic viscosity of xanthan was determined after heating the samples in various concentrations of NaCl or KCl to measure the thermal stablity. Figures 1 and 2 demonstrate the effect of adding NaCl to the xanthan solution. When heating the xanthan solutions at NaCl concentrations lower than 50–100 mM, the intrinsic viscosity rapidly decreased from 80 to 10–20 dl g^{-1} during 64 hours of heating. For NaCl concentrations of 100 mM or higher the intrinsic viscosity was more or less unchanged.

The thermal stability of xanthan was also studied with various concentrations of KCl. The results in Fig. 3 show that the temperature stability of xanthan is also increased by adding KCl. As for NaCl, the intrinsic viscosity was strongly reduced when heating xanthan in 10 mM KCl, but nearly constant for KCl concentrations of 100 mM or higher.

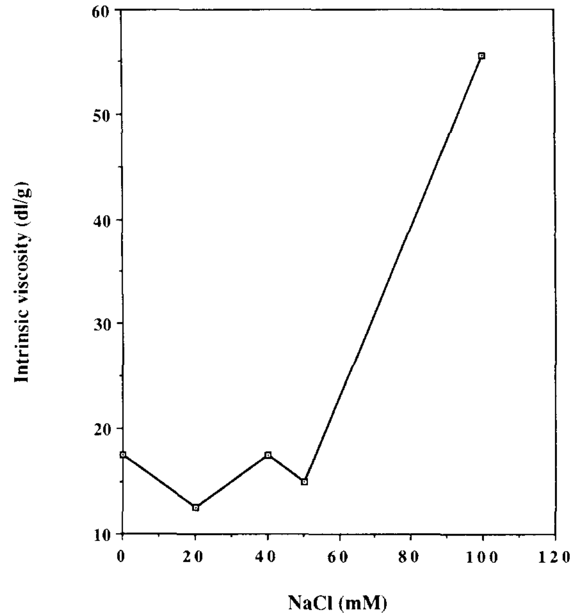

Fig. 1. Intrinsic viscosity of xanthan heated for 64 h at 95°C in different NaCl concentrations.

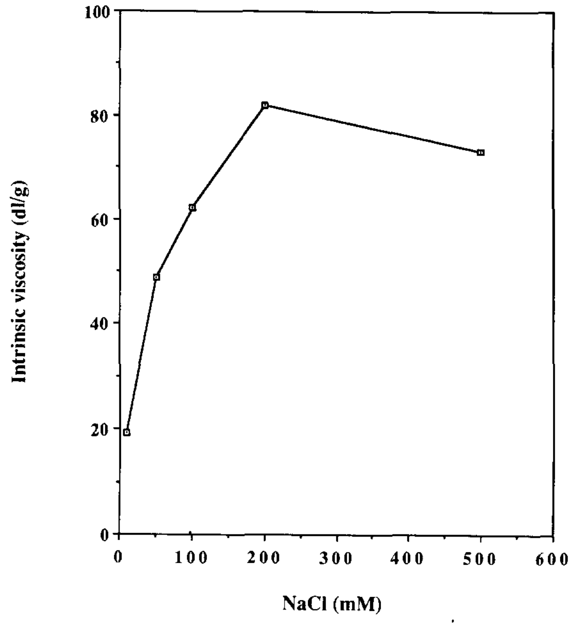

Fig. 2. Intrinsic viscosity of xanthan heated for 38 h at 80°C in different NaCl concentrations.

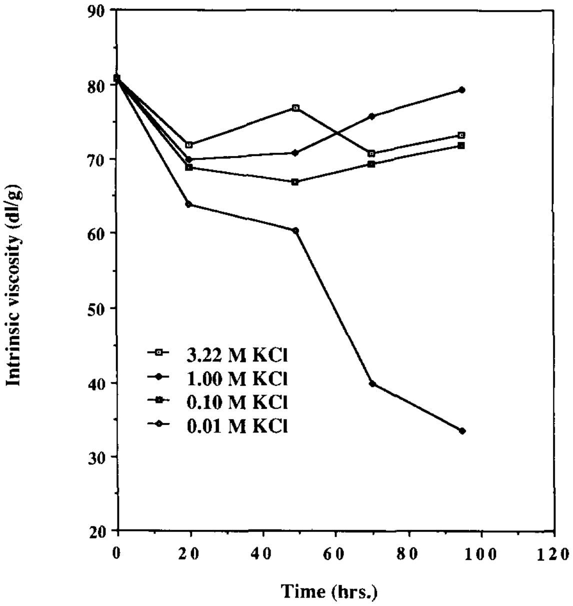

Fig. 3. Intrinsic viscosity of xanthan heated at 80°C in different KCl concentrations as a function of time.

Transition ionic strength I_m, at constant temperature

To determine the ionic strength at which xanthan in solution changes conformation at a given temperature, the transition ionic strength I_m of NaCl was determined by ^{1}H NMR. I_m increased with increasing temperatures as shown in Fig. 4. At 80°C an I_m value of 27 mM NaCl was determined whereas at 95°C the I_m was 60 mM NaCl.

The ^{1}H NMR spectra obtained at 90°C were in agreement with previously reported NMR data for xanthan (Morris *et al.*, 1977; Rinaudo *et al.*, 1983). The spectrum showed the presence of one acetate and one pyruvate group per repeating unit.

Transition temperature T_m, at constant ionic strength

The transition temperature T_m is a parameter used to describe changes in polysaccharide conformation. T_m is the temperature where the polysaccharide is halfway between the ordered and disordered state. In this study T_m was determined by ^{1}H NMR and OR measurements. The results obtained at the same xanthan and NaCl concentrations yielded $T_m = 52$°C (OR) and $T_m = 67$°C (NMR) (Fig. 5).

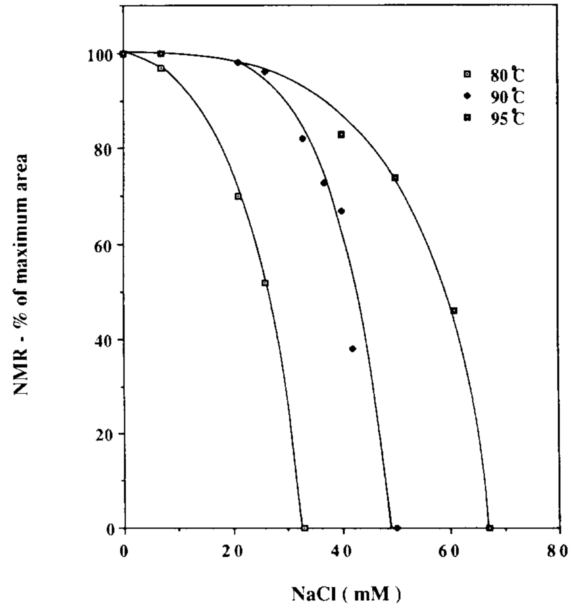

Fig. 4. Determination of transition ionic strength (I_m) at 80, 90 and 95°C.

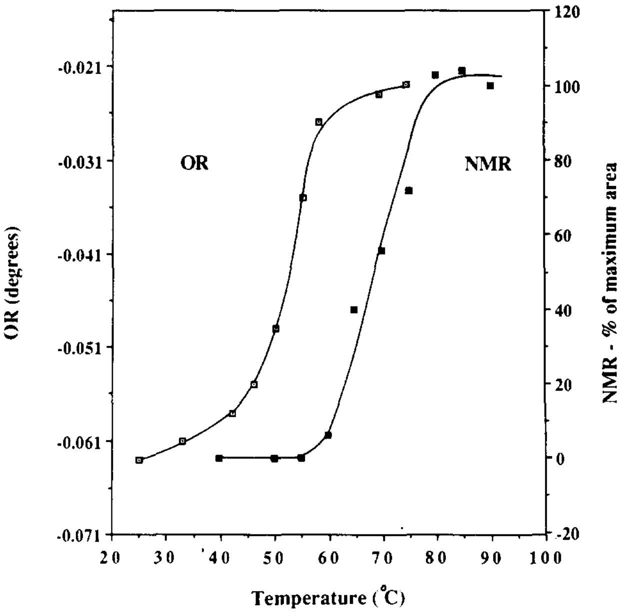

Fig. 5. Determination of transition temperature (T_m) by NMR and optical rotation measurements at 7·5 mM NaCl.

Electron microscopy

Figure 6 shows an electron micrograph obtained from a replica prepared from a low ionic strength solution ($I \sim 0{\cdot}1$ mM) of xanthan. The electron micrograph shows single-stranded xanthan molecules (S) and double-stranded molecules (D) branching into single-stranded segments. Electron micrographs obtained from replicas of xanthan prepared from 100 mM ammonium acetate solutions show predominantly (>95%) molecules with the same thickness as those designated D. The appearance of this xanthan is similar to another xanthan (Stokke *et al.*, 1986) which was reported to be consistent with a double-stranded structure in high ionic strength.

DISCUSSION

Thermal stability — transition ionic strength I_m

The results presented in Figs 1–3 show that the thermal stability of aqueous solutions of xanthan is increased by raising the ionic strength of the solution. This result is in line with earlier reports:

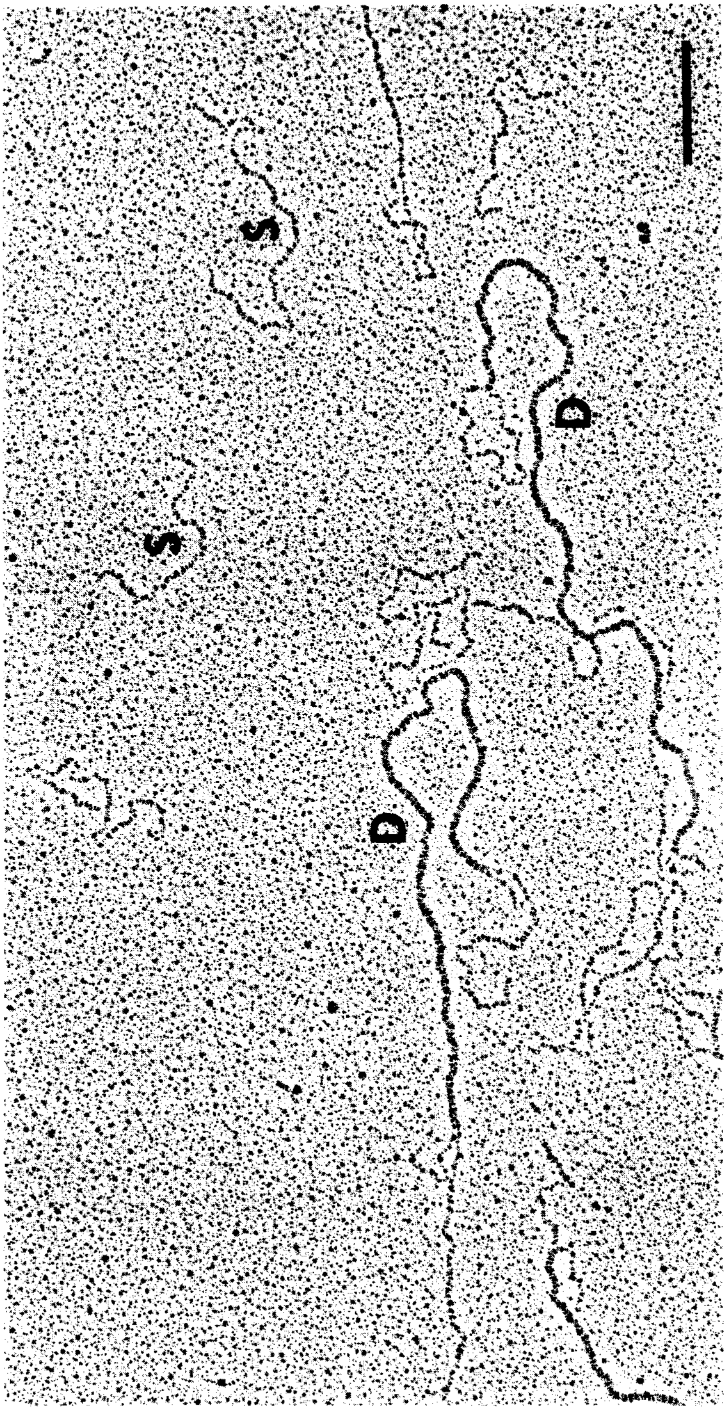

Fig. 6. Electron micrograph of xanthan in 50% glycerol, 0·1 mM ammonium acetate, pH 7, showing double-stranded xanthan (D) dividing into single-strands (S). Bar = 200 nm.

Ash *et al.* (1983) found that 1% NaCl plus 0·1% $CaCl_2$ or higher gave 100% retained viscosity of xanthan for a period of 42 days at 70°C, whereas 0.1% NaCl plus 0.01% $CaCl_2$ gave only 20% retained viscosity.

Wellington (1983) using a synthetic brine found that 1·2–2·4% total dissolved solids (80% NaCl) gave retained viscosity after 110 days at 97°C whereas using lower salt concentrations resulted in 80–90% loss of apparent viscosity.

Lambert & Rinaudo (1985) reported a large increase in thermal stability at 90°C measured as retained viscosity of xanthan solutions containing 1 M NaCl or 1 M $CaCl_2$ relative to a solution in pure water.

Seright & Henrici (1986) found that xanthan solutions kept at 120°C in 0·57 M NaCl or higher were significantly more stable than xanthan solutions in 0·28 M NaCl or less measured by apparent viscosity.

Rinaudo & Milas (1980) studied enzymatic degradation of xanthan. They reported that the enzymatic cleavage of the xanthan β-1, 4-glucose chain, measured as the drop in reduced viscosity, stopped, going from the disordered to the ordered state, by adding 0·1 M NaCl.

The results shown in Figs 1–3 indicate that for the thermal stability of xanthan there is a certain critical ionic strength, in accordance with the results of Ash *et al.* (1983), Seright & Henrici (1986) and Wellington (1983). Below this ionic strength the viscosity of the solution is rapidly reduced at high temperatures. At ionic strengths above the critical value, the viscosity is more stable and apparently independent of the salt concentration.

The results in Figs 1 and 2 indicate a critical NaCl concentration of 50 mM to 100 mM for temperatures of 80 to 95°C. For KCl the critical concentration is between 10 and 100 mM at 80°C (Fig. 3).

Earlier results indicate critical concentrations of 50–100 mM NaCl (0·3–1·0%) at 70°C (Ash *et al.*, 1983), *ca* 1% total dissolved solids (*ca* 80 mM NaCl) at 97°C (Wellington, 1983) and 283–567 mM NaCl at 120°C (Seright & Henrici, 1986).

Rinaudo & Milas (1980) found a critical NaCl concentration of 7–10 mM for the enzymatic degradation of xanthan at 38°C.

In this experiment the transition ionic strength, I_m of the xanthan solutions was determined by ^1H NMR. I_m increased from 27 mM NaCl at 80°C to 60 mM at 95°C, thus being in the range of the critical ionic strength of the thermal stability (Fig. 4). This suggests a close relation between I_m and the critical ionic strength and hence a relation between xanthan conformation and thermal stability.

The reason for the poor thermal stability observed for the xanthan in the present study compared to that reported by others (Wellington, 1983; Seright & Henrici, 1986) is probably because of the oxygen-containing environment. The present data are therefore not an adequate indicator of high-temperature performance of xanthan from Norsk Bioferm relative to other xanthans. Although we have used non-optimized conditions, there is an enhanced stability above the critical ionic strength.

The mechanisms for break down of xanthan at high temperatures are probably complex. In this study the pH of the xanthan solutions was in the range of 5–8 and hence acid or base hydrolysis of the cellulosic backbone can probably be neglected. However, no antioxidant or radical scavengers were used, and oxygen plus transition metals like Fe may induce free radicals like singlet oxygen and hydroxyl radical which are reactive to polysaccharides (Smidsrød *et al.*, 1965; Harris *et al.*, 1971, Wellington, 1983; Gilbert *et al.*, 1984; Parsons *et al.*, 1985). The generated radicals can break the chemical bonds of xanthan in several ways (Lambert & Rinaudo, 1985). Enzymatic degradation of xanthan in the disordered state can also occur when cellulases are present (Rinaudo & Milas, 1980; Ash *et al.*, 1983). No precautions against enzymatic degradation were taken in this study.

The rapid loss of viscosity at high temperatures found for xanthan solutions at low salt concentrations comes from break down of the polysaccharide backbone (Lambert & Rinaudo, 1985). The ordered conformation must then be more resistant to this type of cleavage. The ordered state of xanthan is reported to be either a single- (Morris *et al.*, 1977; Rinaudo & Milas, 1978; Milas & Rinaudo, 1979; Norton *et al.*, 1980; Frangou *et al.*, 1982) or a double-stranded structure (Holzwarth & Prestridge, 1977; Holzwarth, 1978; Paradfossi & Brant, 1982; Sato *et al.*, 1984*b*; Stokke *et al.*, 1986, 1987) where the trisaccharide side chains are oriented parallel to the polysaccharide backbone due to intramolecular hydrogen bonds which stabilize the helical conformation of the main chain (Moorhouse *et al.*, 1977). The increased thermal stability of xanthan in the ordered state can be explained by a double-stranded conformation. A double-stranded polysaccharide backbone could tolerate several fractures in the polysaccharide chain and still keep its hydrodynamic volume, due to the intermolecular interactions between the two chains, as previously found for DNA (Thomas, 1956). The disordered xanthan being a single or partly dissociated double chain would probably have less or no such intermolecular stabilization to fractures in the backbone.

A double-strand conformation of xanthan in the ordered state is also supported by the electron micrographs. Figure 6 shows a xanthan solution at low ionic strength. The xanthan molecules exist predominantly as single-strands but partly dissociated double-strands can also be seen.

The electron micrographs taken at high ionic strengths showed mainly (> 95%) double-strands (not shown), in agreement with earlier results for xanthan (Stokke *et al.*, 1986, 1987).

Transition temperature T_m

Several authors have studied the order–disorder conformational change of xanthan, many by using optical rotation measurements (OR) (Holzwarth, 1976; Morris *et al.*, 1977; Holzwarth & Ogletree, 1979; Paoletti *et al.*, 1983; Norton *et al.*, 1984). In this experiment the transition temperature of xanthan was determined both by ^1H NMR and OR (Fig. 5). The difference of about 15°C between the T_m values found by the two methods may be explained by the fact that the two techniques are monitoring two different molecular phenomena. In ^1H NMR the area of the side chain pyruvate and acetate signals is measured. A major contribution to the observed reduction of these signals is possibly the ordering of the trisaccharide side chains parallel to the polysaccharide backbone, causing reduced flexibility (Morris *et al.*, 1977).

For optical rotation measurements of xanthan, on the other hand, it is postulated that the observed spectrum is a result of a helix-coil transition of the polysaccharide backbone (Morris *et al.*, 1977).

If the transition from the disordered to the ordered state starts with an ordering of side chains parallel to the xanthan backbone, this would cause a reduction of the pyruvate and acetate NMR signals giving the observed T_m of 67°C. A further ordering of the molecule, taking place as the polysaccharide backbone takes a helical conformation, would then result in the observed optical rotation with a T_m of 52°C.

However, because D_2O was used for the NMR spectra and H_2O was used for the OR measurements, more experiments are needed to establish if single events in the conformational change of xanthan can be differentiated using NMR and OR measurements.

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