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Thermal Stability of Xanthan Preparations

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ABSTRACT

Xanthan, the exopolysaccharide from Xanthomonas campestris *has been evaluated in respect of its long-term thermostability. By following the decrease in viscosity and in measurable carbohydrate, it is clear that considerable differences are found in certain xanthan preparations. All xanthans were destroyed at 100°C and above under the conditions tested. At 80–90°C, in the presence of salts, a number of xanthans retained much of their viscosity and carbohydrate structure for periods up to 800 days. For maximum stability, salts and protective and sacrificial agents were necessary, as was the total absence of oxygen. Analysis of recovered polysaccharide indicated that whilst the carbohydrate structure was maintained, acyl groups were very rapidly lost.*

INTRODUCTION

Xanthan is produced commercially and widely used for food and non-food applications. It has a number of actual and potential applications in the oil industry (Sutherland & Kierulf, 1987). Its viscous and viscoelastic properties make it a potentially valuable polymer for use in water floods in Enhanced Oil Recovery (EOR). For such an application for use in the UK–Norwegian offshore areas, water-soluble polymers must withstand temperatures of 80°C and above, high salt concentrations and high pressures. Several studies on xanthan have indicated good viscosity retention in seawater at temperatures up to 90°C (Davison & Mentzer, 1980; Ash *et al.*, 1983). Xanthan solutions are much more stable in the presence of salts, probably due to the maintenance of some degree of order even at high temperatures. Liu *et al.* (1987) have indeed suggested that salt concentrations $< 0{\cdot}01$ M are necessary if the entire transition from ordered to disordered conformation is to occur at temperatures of 80°C or less.

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The importance of ensuring the absence of oxygen was noted by Wellington (1980). The presence of 1·56 μmol litre^{-1} dissolved oxygen was sufficient to reduce stability of viscous solutions of xanthan by a factor of five or more (Seright & Henrici, 1986). Such studies have used various commercially available xanthan preparations. The aim of the present study was to compare the stability of a number of *Xanthomonas campestris* polysaccharides of known acyl composition using viscosity, carbohydrate and acyl measurements.

MATERIALS AND METHODS

Total and residual carbohydrate

Polymer samples, after exposure to high temperature, were assayed directly to determine total carbohydrate using a micro-modification of the phenol–sulphuric acid procedure of Dubois *et al.* (1956). The same technique was used after dialysis of the sample to provide a measure of the residual carbohydrate. The ratio of glucose to mannose was determined using an HPLC procedure (Kennedy & Sutherland, 1987).

Acyl content

Ester-linked acetyl groups and pyruvate ketal were determined using the methods of Cain (1961) and Sloneker & Orentas (1962) respectively.

Chromatography

To determine the extent of polymer breakdown, samples of polymers were applied to a column of acrylamide–agarose (60 × 1 cm) (AcA34, LKB, Stockholm) and eluted with water at a flow rate of 3·4 ml h^{-1}.

Viscosity

All measurements were made on a Brookfield LVT instrument equilibrated to 25°C unless otherwise indicated, using a CP41 cone giving shear rates in the range 0·6–120 s^{-1}.

Polysaccharides

Several commercial samples of xanthan were kindly provided by the manufacturers. The remainder were prepared in this laboratory using

nitrogen-limited growth medium essentially as described by Tait *et al.* (1986), with culture conditions optimised to yield high viscosity and acylation of the polysaccharide. Bacterial cells were removed by prolonged high-speed centrifugation. After dialysis of the cell-free supernatant fluids to remove residual medium and other low molecular weight material, the polysaccharide solutions were concentrated in a Millipore Pellicon tangential flow filtration system and held at −40°C until required. Some samples were also dialysed against distilled water and lyophilised. The composition of the xanthans used is shown in Table 1.

Long-term storage

All polysaccharide samples were dispersed in a laboratory blender when using lyophilised material, or allowed to hydrate overnight at room temperature. The final solution was made in seawater by adding the following to the polysaccharide: (i) Concentrated seawater solution (seawater reduced in volume by rotary evaporation, such that the final polymer solution with all additions was effectively dissolved in normal seawater); (ii) thiourea; (iii) isopropanol; and (iv) sodium sulphite. The final concentrations of the last three chemicals were 0·1% (w/v), 0·2% (w/v) and 0·2% (w/v) respectively. The polysaccharide concentration of 0·2% (w/v) was determined by total carbohydrate analysis. All solutions were degassed under vacuum and thoroughly flushed with oxygen-free nitrogen before mixing. Hard glass ampoules of 30 ml total capacity were washed in distilled water and pre-flushed with nitrogen. The final polysaccharide solutions were added and further flushed with nitrogen, then sealed under the same atmosphere. Tests using the Chemet system

TABLE 1
Xanthan Preparations

Polymer preparation	*Source*	*Moles per repeat unit*		*Ratio Glucose:Mannose*
		Pyruvate	*Acetate*	
1	Commercial	0·72	0·31	1·00:0·98
2	Commercial	0·72	0·68	1·00:0·78
1128	Laboratory	0·13	0·78	1·00:0·95
556	Laboratory	0·64	0·19	1·00:0·93
646	Laboratory	0·57	0·79	1·00:0·95

(Chemetrics Inc., Warrenton, Virginia, USA) failed to reveal any oxygen in the ampoules. These were then held at temperatures of 60, 80, 90, 100, 110 and 120°C until tested. Some samples were similarly dissolved in distilled water and sealed under oxygen-free conditions.

RESULTS

Xanthan stability

At temperatures above 100°C, the xanthans tested showed rapid loss of viscosity, together with degradation of the polymer. Total and residual carbohydrate levels fell rapidly, indicating destruction of the carbohydrates and formation of products unreactive in the test systems used. At 90 and 100°C, marked differences were observed between the different preparations. After only four weeks at 90°C one of the non-commercial samples showed a 60% destruction of carbohydrate, whereas two commercial xanthans showed virtually no diminution in recoverable carbohydrate or in viscosity. A series of long-term experiments was therefore set up to determine the effect of prolonged storage at high temperature. The xanthans used comprised a range of commercial samples together with two laboratory preparations low in pyruvate and in acetate, respectively, and others which had normal acyl content (Table 1).

The best preparations retained much of their carbohydrate and were still viscous at 80°C after experiments lasting 800 days. Occasionally aberrant results were obtained because of apparent failure to remove all oxygen prior to sealing the ampoules. The very marked difference between holding at 80 and 100°C or over for a commercial polymer can be seen in Figs 1 and 2. Similar results over the same time-scale were obtained using several of the polymers listed in Table 1, including the laboratory preparation containing pyruvate but virtually no acetate. This particular polymer also showed good stability and viscosity retention at 90°C for periods up to 550 days. Even without the anti-oxidant and sacrificial agents normally included in the ampoules, the xanthan 556 retained 90% of its original viscosity for 615 days at 80°C.

As the critical temperature in terms of stability appeared to be between 80 and 100°C, a further series of ampoules was prepared and tested over a range of temperatures up to 100°C. The polymer used in Fig. 1 when retested at these temperatures was stable in terms of its viscosity at 60°C but showed increased loss of viscosity as the temperature increased (Fig. 3). Surprisingly, when a laboratory polymer with very

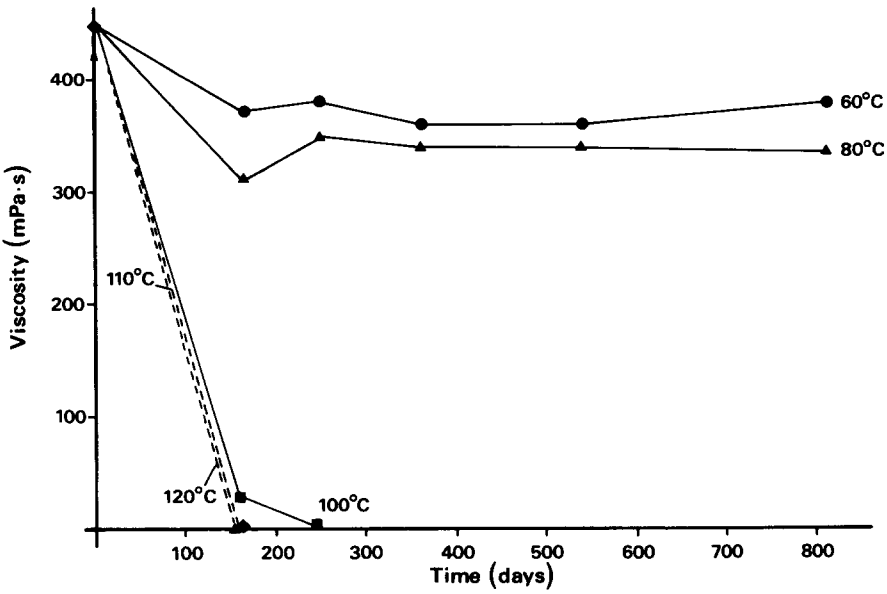

Fig. 1. Residual visosity of polymer 1. Viscosity was measured at 30°C at a shear rate of 1 s^{-1}.

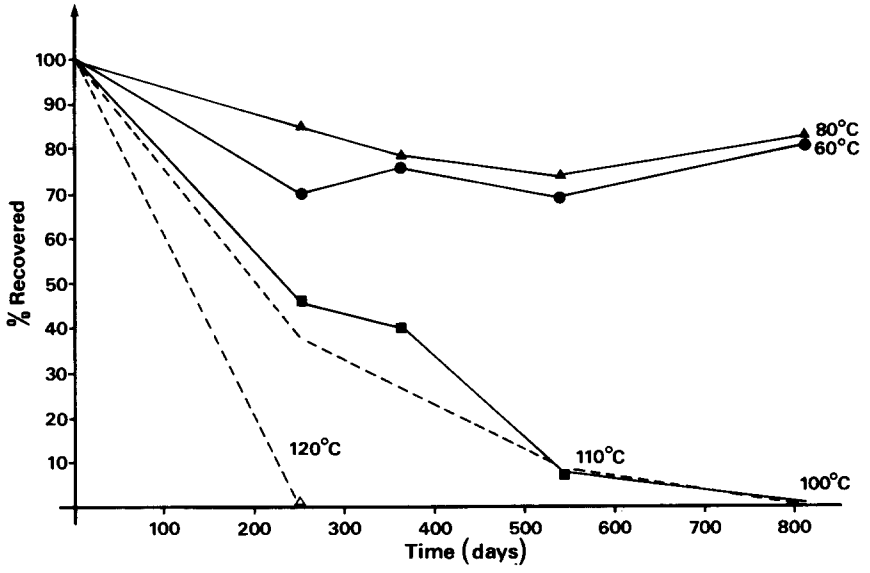

Fig. 2. Residual carbohydrate of polymer 1. Aliquots were removed from samples after dialysis and the residual carbohydrate measured by the phenol–sulphuric acid assay.

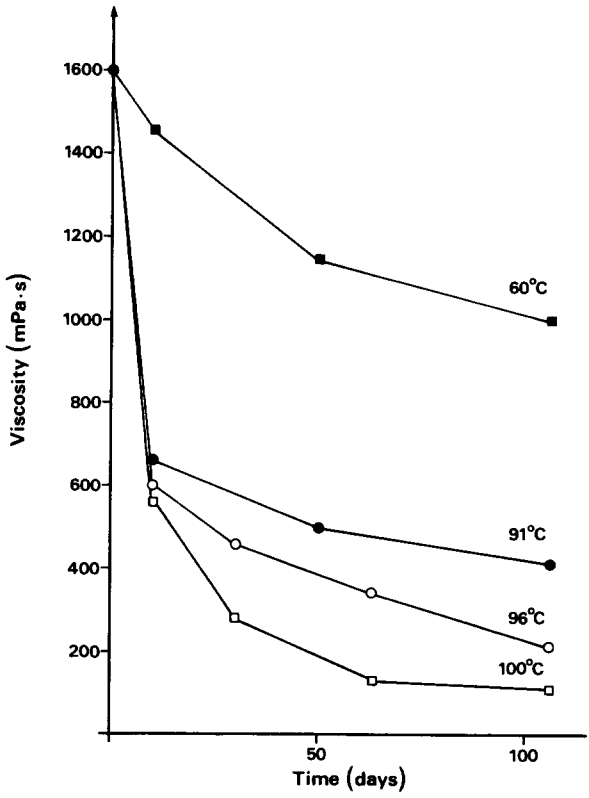

Fig. 3. Residual viscosity of polymer 1. Viscosity was measured at 25°C at a shear rate of 1 s^{-1}.

low pyruvate content was similarly tested, it first showed *increased* viscosity, then remained stable over the test period (Fig. 4). This feature was reproducible for the polymer and is assumed to be due to incomplete hydration when the material is first introduced into the ampoules. There may however be alternative explanations. In support of the present interpretation it can be seen that at 60°C the increase in viscosity is much more gradual than that observed at the higher temperatures.

All preparations required the presence of salts for their stability. When they were prepared in distilled water instead of seawater, even in the presence of the antioxidant and sacrificial agent, loss of carbohydrate and of viscosity were much more rapid at all temperatures.

Degradation of the xanthan molecule at high temperature

A typical preparation of xanthan exposed to 100°C under the standard conditions was examined by gel permeation chromatography (Fig. 5).

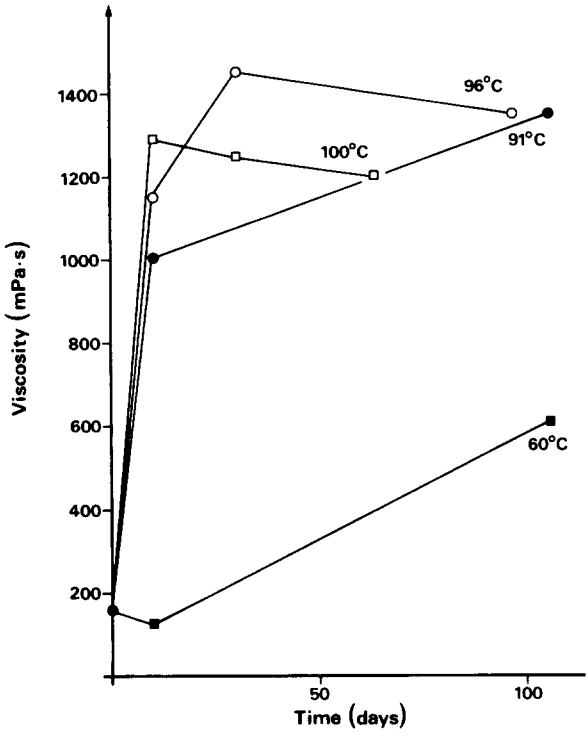

Fig. 4. Residual viscosity of polymer 1128. Viscosity was measured at 25°C at a shear rate of 1 s^{-1}.

The gel permeation column had an exclusion limit of *c.* 350 000 dalton and the relative amounts of smaller fragments can thus be seen. Interestingly, although viscosity at shear rate of 1 s^{-1} when measured at 30°C was rapidly reduced, much of the material showed no great diminution in molecular weight. However, after 12 months' exposure, no high molecular weight material remained. In an attempt to determine which parts of the xanthan molecule were degraded, the residual polysaccharides recovered after dialysis were analysed. In Fig. 6 the pyruvate content of a typical commercial preparation after exposure to temperatures in the range 60–100°C can be seen to decrease, except at 60°C. For the same polymer, acetate was removed more rapidly and even at 60°C had fallen from a value of 0·76 to 0·13 moles per repeat unit within 30 days. At temperatures between 91 and 100°C the level after 10 days was 0·1 mole and none was detectable after 30 days. The decrease in acyl groups was very much more rapid than loss of either carbohydrate or viscosity. Similar results were obtained with the other xanthan preparations.

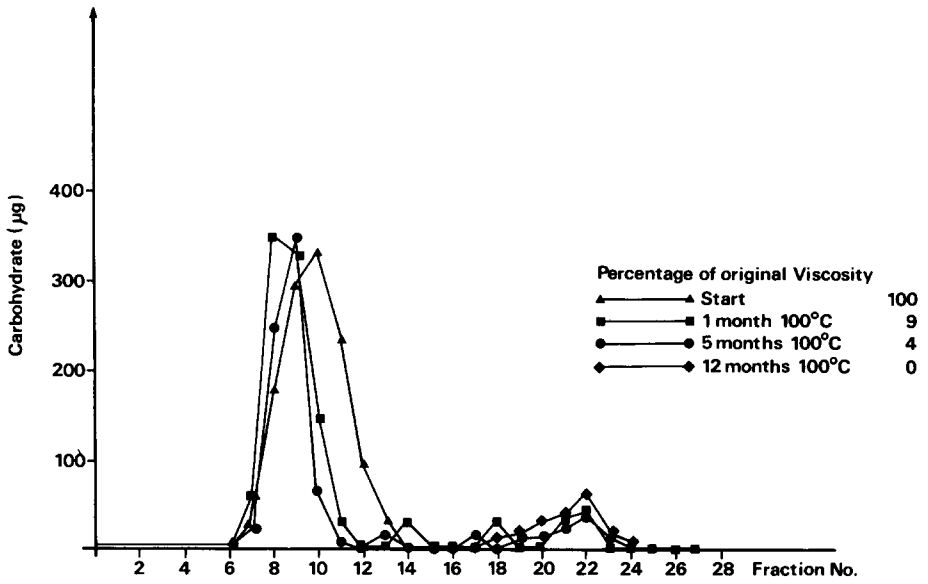

Fig. 5. Carbohydrate breakdown of polymer 1. Samples were run on an LKB AcA34 column. The polymer used was a commercial preparation but results were typical of those obtained for most polymers (viscosity measured at 30°C, shear rate 1 s^{-1}).

DISCUSSION

The biopolymer xanthan is widely used for industrial applications and has frequently been proposed as an agent for enhanced oil recovery. In this role, it must be both biologically and thermally stable to be effective. While the addition of antibacterial agents can be used to control biological stability, thermal stability is an inherent property of the polysaccharide molecule. It is perhaps of considerable value that xanthan does not undergo a sharp transition from the ordered to the disordered state, but rather, in the presence of salts, maintains some degree of order even at temperatures near 100°C, after a gradual transition from the totally-ordered form at lower temperatures. The present results are in broad agreement with similar studies (Davison & Mentzer, 1980; Wellington, 1980; Ash *et al.*, 1983). All these studies confirm the need for the presence of adequate salts and the absence of oxygen if xanthan solutions are to maintain their physical properties at high temperatures. These earlier studies were confined to commercial xanthan preparations and did not compare material differing from these in its acyl content. It was thus very surprising to find that a preparation almost devoid of acetate but containing pyruvate was so stable even without the additives normally present in the tests.

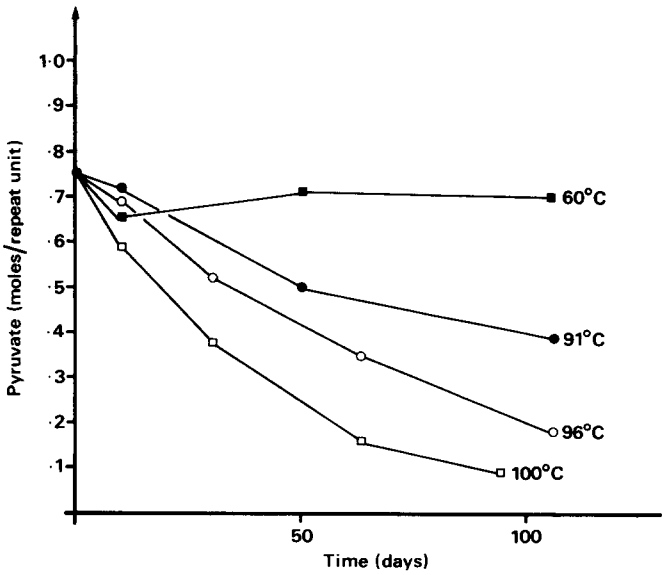

Fig. 6. Loss of pyruvate from polymer 1.

There are clearly very marked differences between xanthan preparations which cannot be ascribed solely to differences in acyl content. The increase in viscosity of xanthan after exposure to high temperature was also observed by Ash *et al.* (1983) using a concentrated xanthan solution which apparently contained a normal level of both acyl groups. It may well be unconnected with the presence or absence of acyl groups. Callet *et al.* (1987) have recently presented results to show that acetate and pyruvate contents do not influence the dilute solution viscosity of xanthan, or its intrinsic viscosity at a given molecular weight. Thus the gradual loss of viscosity at higher temperatures would not be expected to correlate to loss of acyl groups. Loss of pyruvate was observed in the present study, whereas Milas *et al.* (1988) found no hydrolysis at 80°C. This is almost certainly due to slight differences in the storage conditions used, as this group also found conditions where pyruvate as well as acetate was totally lost at 80°C. The profile on gel permeation chromatography (Fig. 5) clearly shows that only a small proportion of the polysaccharide is degraded to low molecular weight material under the conditions used in this study. The lack of significant main-chain breakage to small fragments even at relatively high temperatures permits retention of high viscosity by the xanthan solutions. The elution profile shown in Fig. 5 perhaps indicates main-chain cleavage to material of $\geqslant 350\,000$ dalton, and would thus explain the observed loss in viscosity. Satisfactory resolution of high molecular weight material remains difficult, but will

hopefully become achievable with improved HPLC methods. The loss in carbohydrate material may well result initially from degradation of side-chain rather than main-chain components, but more detailed study of this aspect is necessary. The present results and those of other laboratories clearly indicate the potential value of xanthan for enhanced oil recovery. However, because of the variability of biopolymers and the marked differences in physical properties and stability of material which cannot be distinguished on the basis of chemical analyses, the material must be carefully chosen and extensively tested prior to use.

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REFERENCES

Ash, S. G., Clarke-Sturman, A. J., Calvert, R. & Nisbet, T. M. (1983). *Soc. Petrol. Eng.*, paper 12085.
Cain, R. B. (1961). *Biochem. J.*, **79**, 300.
Callet, F., Milas, M. & Rinaudo, M. (1987). *Int. J. Biol. Macromol.*, **9**, 291.
Davison, P. & Mentzer, E. (1980). *Soc. Petrol. Eng.*, paper 9300.
Dubois, M., Gilles, K. A., Hamilton, J. K, Rebbers, P. A. & Smith, F. (1956). *Anal. Chem.*, **28**, 350.
Kennedy, A. F. D. & Sutherland, I. W. (1987). *Biotechnol. Appl. Biochem.*, **9**, 12.
Liu, W., Sato, T., Norisuye, T. & Fujita, H. (1987). *Carbohydr. Res.*, **160**, 267.
Milas, M., Linossier, J. L. & Contat, F. (1988). *J. Appl. Polymer Sci.*, **35**(4) 1115.
Seright, R. S. & Henrici, B. J. (1986). *Soc. Petrol. Eng.*, paper 14946.
Sloneker, J. H. & Orentas, D. G. (1962). *Nature*, **194**, 478.
Sutherland, I. W. & Kierulf, C. (1987). In *Microbial Problems in the Offshore Oil Industry*, ed. E. C. Hill, J. L. Shennan & R. J. Watson. J. Wiley & Sons, Chichester, pp. 93–103.
Tait, M. I., Sutherland, I. W. & Clarke-Sturman, A. J. (1986). *J. Gen. Microbiol.*, **132**, 1483.
Wellington, S. L. (1980). *Soc. Petrol. Eng.*, paper 9296.