Carbohydrate Polymers 3 (1983) 23–38

Modified Xanthan – Its Preparation and Viscosity

I. J. Bradshaw, B. A. Nisbet, M. H. Kerr & I. W. Sutherland

Department of Microbiology, Edinburgh University, West Mains Road, Edinburgh EH9 3JG, UK

(Received: 5 May 1982)

SUMMARY

Xanthan with various pyruvic acid and acetate contents has been prepared from a single commercial polysaccharide sample using optimised chemical conditions (acid and alkali hydrolysis, respectively) for removal of acetal and acyl groups. The only significant change found on analysis of the modified xanthans was loss of pyruvic acid and/or acetate; no low moleculur weight carbohydrate-containing material was released. Contrary to some previous reports, evidence is presented to show that the pyruvic acid acetal and o-acetyl contents of xanthan do not affect solution viscosity. The viscosities of native, pyruvate-free and pyruvate/acetate-free xanthan solutions (0·3% w/v) were similar at shear rates 8·8–88·3 s^{-1} in both distilled water and 1% KCl. Over the concentration range 0·2–1·5%, the viscosities of native and pyruvate-free xanthan at 10 s^{-1} were similar. The viscosity increase on addition of 1% KCl to salt-free xanthan solutions was independent of pyruvic acid acetal substitution. Our results suggest that xanthan samples with various pyruvic acid acetal and o-acetal contents, prepared under different fermentation conditions of Xanthomonas campestri *should not normally be used for assessing the contribution of these groups to solution viscosity.*

1. INTRODUCTION

The extracellular polysaccharide (xanthan) formed by *Xanthomonas campestris* is commercially important (Sandford, 1979; Lawson &

Carbohydrate Polymers 0144-8617/83/0003-0023/$03.00

Sutherland, 1979) because of its unusual solution properties. The primary structure of the polymer consists of a cellulose backbone to which trisaccharide side-chains are attached on alternate β-D-glycopyranose residues (Jansson *et al.*, 1975; Melton *et al.*, 1976). The β-D-mannopyranosyl terminal non-reducing group of each side-chain may carry a 4,6 linked pyruvate acetal, the amount depending on the bacteria strain and culture conditions (Cadmus *et al.*, 1976, 1978). Although it is not certain whether the pyruvic acid acetal groups are uniformly distributed along the polymer chains or are present on adjacent β-D-mannopyranosyl residues, it is possible to resolve xanthan preparations into 'pyruvate rich' and 'pyruvate poor' fractions by ethanol fractionation (Sandford *et al.*, 1978) or on a micro-scale by affinity chromatography (Sutherland, 1981*a*).

Aqueous solutions of xanthan are highly viscous at low concentrations and very pseudoplastic (e.g. Jeanes *et al.*, 1961; Whitcomb *et al.*, 1977) showing good compatibility with salts over a wide pH range. Xanthan has found a wide range of applications in foods and in industry as a result of these properties (Cottrell *et al.*, 1980). However, the potential use of the biopolymer as a mobility control agent in enhanced oil recovery, would involve production and utilisation on a very much larger scale than is currently practised (Sandvik & Maerker, 1977; Wellington, 1980). Xanthan is not ideal for this role and the product from a mutant strain yielding pyruvate-free polysaccharide reportedly showed improved filtration properties in the presence of high brine concentrations likely to be encountered in oil reservoirs (Wernau, 1979).

Although it is clear that the high molecular weight (2×10^6 (Dintzis *et al.*, 1970; Kabir & Stanislav, 1980) or $14{\cdot}8 \times 10^6$ (Holzwarth, 1978)) of the polysaccharide and rigid nature of the molecular backbone (Morris, 1977) are important in determining the physical properties of xanthan solutions, it has also been suggested that pyruvic acid acetal (Sandford *et al.*, 1977; Smith *et al.*, 1981) and *o*-acetyl (Jeanes *et al.*, 1961) groups contribute to the viscosity characteristics of xanthan solutions. Sandford *et al.* (1977) on the basis of measurements at low shear rate (approximately $4\ s^{-1}$) suggested that xanthan preparations with high pyruvate acetal content possessed higher viscosity than low pyruvate acetal material. Further, Smith *et al.* (1981) suggested that the fractional change in viscosity on addition of KCl to salt-free xanthan solutions is positive only when the fraction of side-chains which carry

pyruvic acid acetal groups exceeds 0·31. Jeanes *et al.* (1961) reported that removal of acetate from xanthan resulted in increased solution viscosity.

The pyruvic acid acetal content probably influences the characteristic temperature induced order–disorder transition of xanthan. The mid-point temperature of transition was greater for pyruvate-free polysaccharide (Holzwarth & Ogletree, 1979) and *increases* with *decreasing* pyruvate acetal content (Smith *et al.*, 1981).

We now report an investigation of the role of acetal and acyl groups in determining the solution viscosity of xanthan. Previous studies have used xanthan samples with various acetal and acyl group contents prepared under different fermentation conditions. To eliminate any possible microbiological variations from our studies, we have prepared (from a single polysaccharide sample) xanthan with various pyruvate and acetate contents using optimised chemical conditions for removal of acetal and acyl groups, respectively, with essentially unaltered molecular weight.

2. MATERIALS AND METHODS

2.1 Polysaccharide

A commercial xanthan preparation (Keltrol) was kindly provided by Kelco Inc., San Diego, USA. Other microbial polysaccharides were prepared in the laboratory by standard procedures. The fungal polysaccharide scleroglucan was the generous gift of CECA, SA, France.

Total carbohydrate was measured by the phenol–sulphuric acid assay (Dubois *et al.*, 1965). Neutral sugar ratios were determined as their alditol acetates by gas liquid chromatography on columns of Supelco 3% OV 225 using a Pye Series 104 chromatograph. Uronic acid was determined on unhydrolysed polymer using the metahydroxydiphenyl method of Blumenkranz & Asboe-Hansen (1973). Pyruvate and acetate were determined by the 2,4-dinitrophenylhydrazine (Sloneker & Orentas, 1962) and hydroxamic acid (McComb & McCready, 1957) methods, respectively.

Viscosity was measured using a Ferranti-Shirley (Manchester) cone-plate viscometer at shear rates of 8·8–88·3 s^{-1}.

Molecular weight was determined by gel permeation chromatography on columns of Ultragel 4A (LKB, Bromma, Sweden) with Hepes buffer (1 mM, pH 7·1) as eluant using a flow of 15 ml h^{-1}.

2.4 Paper chromatography

Sugars in the diffusates after hydrolysis were examined by descending paper chromatography in ethyl acetate/acetic acid/formic acid/water (18 : 3 : 1 : 4, by vol.) and butanol/pyridine/water (6 : 4 : 3, by vol.). Chromatograms were developed in alkaline silver nitrate reagent (Trevelyan *et al.*, 1950).

2.3 Optimisation of conditions for modification of xanthan

Xanthan solutions (0·5%; 10 ml) were hydrolysed in sealed tubes under various conditions (described below). Aliquots (8 ml) were removed, dialysed against running water (24 h), then distilled water (24 h). The solutions were made up to 10 ml and used for viscosity measurements and analyses. All analytical results were related to samples which were dialysed only.

Xanthan was initially hydrolysed in 10–150 mM trifluoracetic acid (TFA) at 100°C for 90 min (Fig. 1). Optimisation of acid concentration was achieved by hydrolysis in 1–10 mM TFA (Fig. 2). Using 5 mM TFA at 100°C, the effect of time on pyruvic acid acetal group removal was next determined (Fig. 3). Finally, the optimal temperature for depyruvylation was tested using 5 mM TFA for 90 min (Fig. 4). During modification of larger volumes of polysaccharide solutions (1·5 litre) a temperature of 95–96°C was normally achieved. The time of heating was, therefore, extended to ensure that this temperature was maintained for the total time required.

For removal of *o*-acetyl groups, xanthan solutions (0·5% in 0·1 M KCl, 10 ml) were allowed to stand for 3 h at room temperature under nitrogen, using 5–25 mM KOH. Following neutralisation to pH 7 with dilute HCl, an aliquot (8 ml) of each was dialysed and analysed for acetate and pyruvate as well as viscosity.

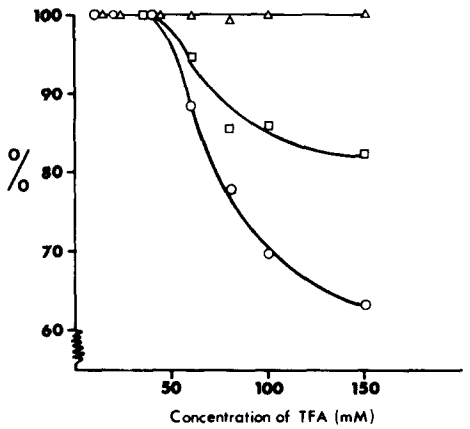

Fig. 1. Effect of high acid (TFA) concentrations (10–150 mM) on hydrolysis of xanthan at 100°C for 90 min. Results show the percentage of pyruvic acid acetyl groups (△) removed from xanthan with variation of acid concentration. Degradation of the polysaccharide was followed by monitoring the percentage of carbohydrate (□) and viscosity (○) retained after dialysis.

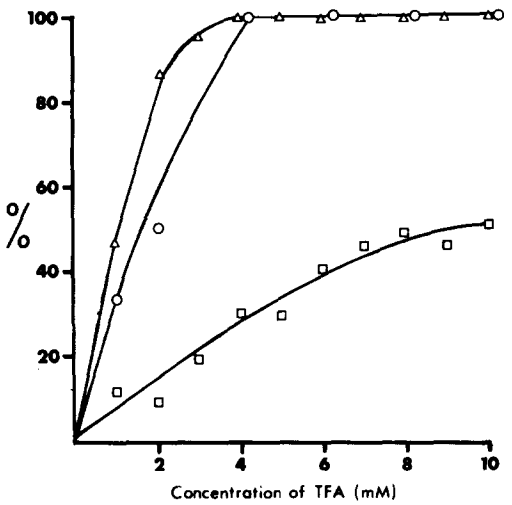

Fig. 2. Effect of low acid concentrations (1–10 mM) on TFA hydrolysis of xanthan at 100°C for 90 min. Data shown is for percentage of pyruvic acid acetal (△) and acetate (□) groups removed from the native xanthan with variation of acid concentration. Removal of pyruvic acid (○) from a xanthan sample containing a low acetal group content is also shown for comparison.

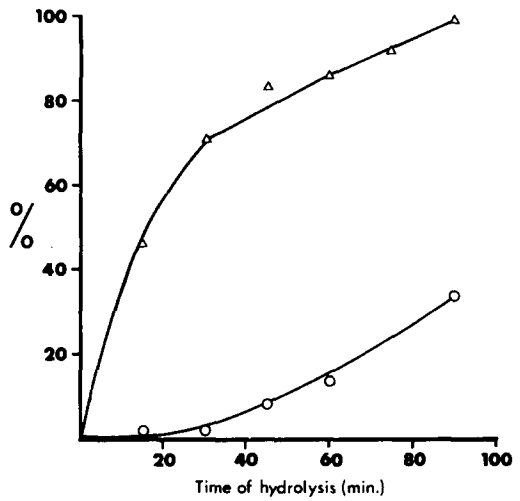

Fig. 3. The effect of time of hydrolysis on removal of pyruvic acid acetal (△) and acetate (○) groups from xanthan is shown. Each polymer sample was hydrolysed in 5 mM TFA at 100°C.

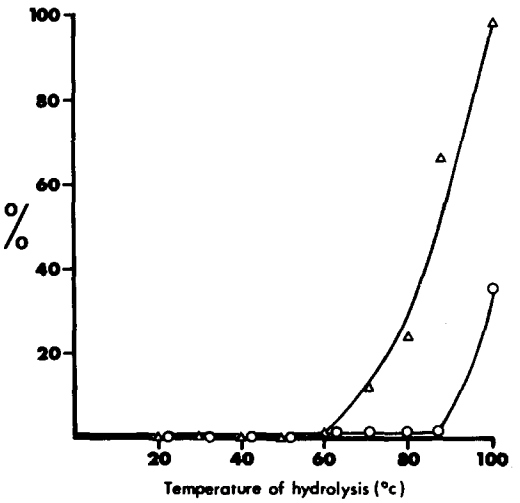

Fig. 4. The effect of temperature on hydrolysis of pyruvic acid acetal (△) and acetate (○) groups from xanthan is shown. Each polymer sample was hydrolysed in 5 mM TFA for 90 min.

3. RESULTS AND DISCUSSION

It is recognised that polysaccharide characteristics are sensitive to change in culture conditions (Evans *et al.*, 1979) and to the strain used (Sutherland, 1981*b*). The extent of pyruvylation and acetylation of a polysaccharide are two parameters which are likely to alter. Indeed, Davidson (1978) showed that different limiting nutrients for microbial growth yielded xanthan with various pyruvic acid acetal and *o*-acetal contents. Smith *et al.* (1981) found that available commercial xanthan samples show variation in substitution with pyruvic acid and acetate. Batch variation among products from a single source is also likely. Since control of pyruvylation and acetylation in xanthan by microbiological methods is less reliable than chemical methods, we have preferred to use acid and alkaline hydrolysis conditions for preparations of polymer with varying pyruvic acid acetal and *o*-acetyl constituents. Knowledge of the contribution of these groups to the solution properties of xanthan is clearly important, if reproducibility in commercial applications of the polysaccharide is to be maintained. Holzwarth & Ogletree (1979) used hydrolysis in 1 mM oxalic acid (0·1 M NaCl, pH 3 for 2 h at 95°C) to prepare pyruvate-free xanthan, claiming removal of approximately 90% of the pyruvic acid acetal groups. In our hands, this procedure resulted in higher residual pyruvic acid levels; consequently, we have optimised conditions with TFA for quantitative removal of the pyruvic acid acetal group with minimal release of carbohydrate.

Initially, a wide range of TFA concentrations (10–150 mM) were examined to determine the point at which significant breakdown of the xanthan molecule occurred (Fig. 1). The time (90 min) and the temperature (100°C) were chosen arbitrarily, but were later varied when the concentration of TFA had been optimised. Above 40 mM TFA, the viscosity and residual non-dialysable carbohydrate decreased with increasing acid concentration. Thus it was necessary to use concentrations of TFA < 40 mM to achieve pyruvic acid removal without significant cleavage of glycosidic linkages.

Further study with much lower concentrations (1–10 mM) indicated that 4–5 mM TFA was the optimum concentration for use at 100°C for 90 min (Fig. 2), although 1 mM acid did lead to removal of about 50% of the pyruvic acid acetal groups. Similar results were obtained for pyruvic acid removal from a xanthan preparation with low initial pyruvic acid content (2·2%). Surprisingly, only 30% of the *o*-acetyl

groups were removed under conditions where complete removal of pyruvic acid occurred. Using 5 mM TFA at 100°C, the effect of time on hydrolysis was tested (Fig. 3). The period of 90 min was optimal under these standard conditions. It is of interest that, although 50% of the pyruvic acid was removed after 15 min hydrolysis, a further 75 min was needed to achieve quantitative removal. The cleavage of *o*-acetyl groups was also time-dependent.

The temperature required for hydrolysis using 5 mM TFA for 90 min was also examined (Fig. 4) in an attempt to minimise hydrolysis of glycosidic linkages. No cleavage of pyruvic acid took place below 60°C and at 87°C only about 60% removal occurred under the conditions employed. Thus reduction of the temperature below 100°C would require longer hydrolysis times for quantitative removal of pyruvic acid acetal groups.

The optimum conditions for removal of pyruvic acid from xanthan were also applied to a number of other microbial polysaccharides carrying carboxyethylidene groups. These included colanic acid from *Escherichia coli* (Lawson *et al.*, 1969) and Klebsiella polymers K1, K8, K30, K56 and K58. In all these polymers, degradation of the polysaccharide took place *before* significant amounts of pyruvic acid could be removed. The xanthan molecule is thus much more stable to acid hydrolysis than many other microbial polysaccharides, probably due to its cellulose backbone. Scleroglucan, the neutral fungal polymer with a (1→3) β-D-glucan backbone, was also resistant to 5 mM TFA hydrolysis. Significantly, both xanthan (Wellington, 1980) and scleroglucan (Dawson & Mentzer, 1980) have been proposed for use in enhanced oil recovery because of their stability at temperatures of 90–95°C in high salt concentrations for periods of 300–400 days.

The *o*-acetyl groups in polysaccharides are relatively labile to alkali at room temperature and 25 mM KOH was used to deacetylate xanthan (Sloneker & Jeanes, 1962). Using a range of alkali concentrations (5–25 mM KOH) to determine the optimum for removal of the acetate groups in xanthan, we observed essentially quantitative loss with 15 mM KOH. The initial solution viscosity was retained and no pyruvic acid was removed.

A series of modified xanthans was prepared using the methods described above, scaled up to yield about 7·5 g material. In this way, acetate-free, pyruvate-free and (after initial deacetylation) pyruvate/acetate-free polysaccharides were produced. Since a direct comparison

of the solution viscosity between native and modified xanthans was intended, it was necessary to illustrate categorically that no chemical changes other than depyruvylation and deacetylation occurred to the polymer molecules. Comparison of the monosaccharide composition of the polymers is shown in Table 1, along with the respective pyruvic acid and acetate contents. No apparent change in chemical composition appears to have taken place during the modifications. As a further check, the hydrolysate from depyruvylated material was dialysed and the diffusible material recovered and evaporated to dryness. As a control, commercial xanthan was similarly treated. Neither product revealed identifiable monosaccharides on paper chromatography, indicating that glycosidic linkages had not been cleaved to yield free sugars in the depyruvylation procedure. To ascertain whether any cleavage of internal glycosidic linkages and thus reduction of molecular weight had occurred, the elution profile of the xanthan preparations was compared by gel chromatography. No material of lower molecular weight was found in the modified xanthans. However, a slight difference in the molecular weight profile was found in the range $2{\cdot}5 \times 10^6$–$4{\cdot}5 \times 10^6$, the amount of material in this fraction in the depyruvylated xanthan being lower. The deacetylated material resembled wild type preparations. The change represents an alteration to only a small proportion of the total molecular weight profile.

As chemical and molecular weight analysis revealed no significant breakdown of the polymer molecules during modification, this allowed a valid assessment of the contribution of pyruvic acid acetal and *o*-acetyl groups to the viscosity of xanthan solutions. The contribution of these

TABLE 1
Comparison of Analysis of Native and Modified Xanthans

Xanthan sample	*Pyruvate*[a] *(%)*	*Acetate*[a] *(%)*	*Sugar composition ratio glucose: mannose: glucuronic acid*
Native	4·3	4·1	1 : 0·81 : 0·51
Pyruvate-free	0·02	3·1	1 : 0·79 : 0·51
Acetate-free	4·3	0	1 : 0·79 : 0·51
Pyruvate/acetate-free	0·02	0	1 : 0·79 : 0·51

[a] Dry weight basis.

groups to solution properties of microbial polysaccharides is especially relevant to xanthan polymers as the pyruvic acid acetal and *o*-acetal constituents are located on the side-chain. Indeed, it has been suggested that the manner in which the side-chain aligns with the cellular backbone of the molecule is partly responsible for the solution properties of xanthan (Morris, 1977). Nevertheless, a comparison of the viscosity of native and modified xanthan in distilled H_2O (Fig. 5(a)) showed that depyruvylation and deacetylation had little effect on solution viscosity at shear rates between 8·8–88·3 s^{-1}. The similarity in the slope of the lines in Fig. 5(a) indicates that the pseudoplasticity of xanthan was unaltered after modification. The variation of log viscosity with log shear rate was almost unchanged for both native and modified xanthans in 1% KCl (Fig. 5(b)). Previous reports (Jeanes *et al.*, 1961; Smith *et al.*, 1981) also indicated that little change in viscosity of 0·3% xanthan solutions would be expected when salt was added.

Sandford *et al.* (1977) claimed that solution viscosity of xanthan was greater for polymers with high pyruvic acid content (4·0–4·8%) than for those with low pyruvic acid (2·5–3·0%). It was concluded that the high and low pyruvic acid samples interacted differently in solution, thus yielding the various viscosities when tested at low shear rate ($<4\ s^{-1}$). We have used higher shear rates, more appropriate to many commercial applications of xanthan. From our results, we conclude that any possible differing interactions of xanthan samples with various pyruvate acetal contents are not significant at higher shear rates. Further, when the viscosity of xanthan and pyruvate-free xanthan at $10\ s^{-1}$ was compared for solutions of 0·2–1·5% concentration in 1% KCl, the profiles were similar (Fig. 6).

Although our results do not compare directly with those of Sandford *et al.* (1977), we feel that the assessment of the contribution of pyruvate acetal groups to the viscosity of xanthan solutions can only be made when the polymer is all derived from the same batch. It thus appears probable that the results of Sandford *et al.* (1977) using polysaccharide of low pyruvic acid content derived from a variant strain of *X. campestris* could be attributed to changes in polymer characteristics as well as the acetal groups. The molecular weight of the polymer would be expected to contribute to the solution viscosity and could be altered by the differing culture conditions, but this does not appear to have been tested.

Recently, Smith *et al.* (1981) have presented evidence regarding the influence of the pyruvic acid content of xanthan on solution viscosity.

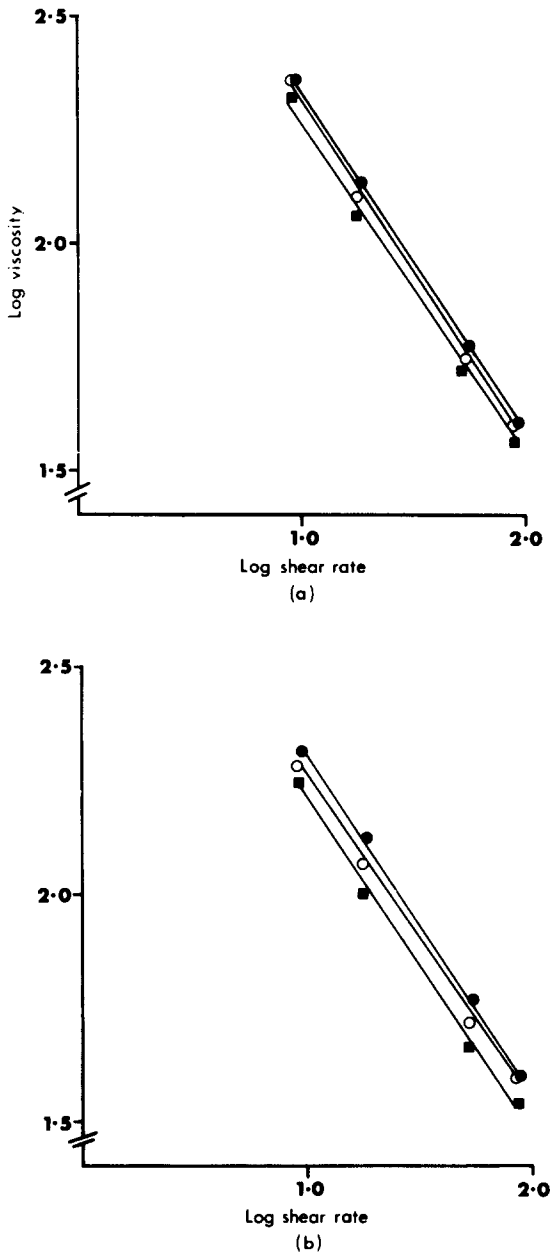

Fig. 5. Variation in the viscosity of various xanthan solutions (0·3%) with variation of shear rate. The results shown are for native xanthan (■), pyruvate-free xanthan (○) and pyruvate/acetate-free xanthan (●) in (a) distilled water and (b) 1% KCl. The data for acetate-free xanthan was very similar to the native polymer sample.

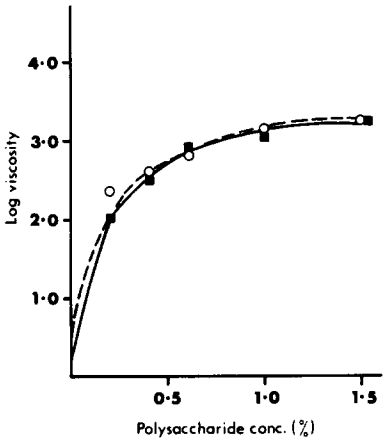

Fig. 6. Comparison of the variation in viscosity of native xanthan (■) and pyruvate-free xanthan (○) with variation of polysaccharide concentration. Viscosity measurements are shown for a shear rate of 10 s^{-1}.

They observed that the degree of pyruvic acid substitution affected the percentage increase in viscosity of salt-free xanthan solutions (1%) when KCl was added, a phenomenon typical of this polysaccharide. The xanthans used were different commercial preparations of the polysaccharide with various extents of substitution with pyruvic acid. Due to the inherent differences likely to be present in polymer from different strains produced in different laboratories, we have preferred to use xanthan from a single source, from which specified amounts of the pyruvic acid have been removed by variation of hydrolysis time under the optimum conditions of 5 mM TFA and 100°C. We thus hoped to provide a more defined polymer, known to be minimally different in molecular weight from native xanthan. When xanthan prepared in this way, with various pyruvic acid contents, was used for viscosity determination in distilled water and in 1% KCl the results shown in Fig. 7 were obtained. The variation in pyruvic acid acetal substitution had little effect on the viscosity of the polymer solution in either distilled water or 1% KCl at shear rates of 10 s^{-1} and 100 s^{-1}. It is thus concluded that the percentage increase in viscosity on the addition of 1% KCl to salt-free xanthan solution is *not* dependent on the degree of pyruvylation. These results indicate that the pyruvic acid acetal group may not have such a significant role in determining the viscosity of xanthan

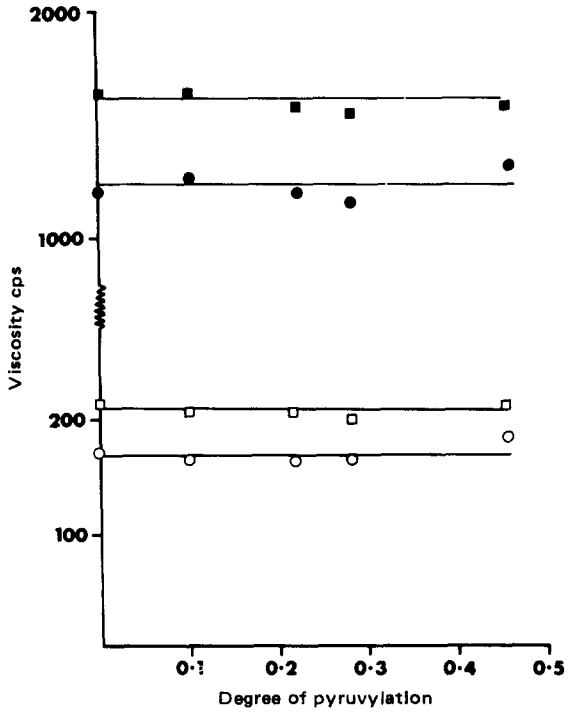

Fig. 7. The viscosities of xanthans with various pyruvate ketal contents in distilled water (○, ●) and 1% KCl (□, ■) at $10\ s^{-1}$ (○, □) and $100\ s^{-1}$ (●, ■). The degree of pyruvylation in each xanthan sample was calculated using the percentage of pyruvate ketal groups present and the primary structure of the polysaccharide depicted by Jansson *et al.* (1975).

solutions as has frequently been suggested. Comparative studies of biopolymers should probably be made where possible, when variation in the extent of acetal and acyl substitution has been achieved by gentle chemical procedures in preference to material from different fermentation conditions as greater heterogeneity of polymer molecules may then result.

Further evidence for this was seen when essentially pyruvate-free xanthan from a mutant strain of *X. campestris* (Wernau, 1979) was compared with our pyruvate-free material. Although both polysaccharides showed improved filtration properties through membrane filters (unpublished results) when compared to native polymer, the xanthan from the mutant strain showed significantly lower solution

viscosity and reduced pseudoplasticity. Thus, some change in addition to loss of pyruvic acid appears to have occurred in the polysaccharide prepared from the mutant strain. Both native and pyruvate-free xanthan (prepared by chemical modification) showed synergistic gelling interaction in solution with locust bean gum. Gelling action with the galactomannan requires xanthan to be in the ordered conformation (Dea & Morris, 1977). Thus, it appears that pyruvic acid acetal groups are not involved in the gelling mechanism and, further, that modification of xanthan does not disrupt the ordered conformation of the polymer in solution.

Chemical removal of the pyruvic acid acetal groups in xanthan by the methods we have described could lead to a polymer with improved filter ability. This could have particular value for use in enhanced oil recovery. Variation of the acid concentration, hydrolysis time and temperature, would allow production of xanthan with specified loss of pyruvate acetal groups. As fermentation broths are often heated to destroy cell viability and inactivate cellulase, the hydrolysis procedure for removal of pyruvic acid could be readily incorporated into the production process for xanthan.

ACKNOWLEDGEMENTS

This work formed part of a study under Department of Energy contract F443. The gift of polysaccharides from Kelco Inc. and CECA, SA is gratefully acknowledged.

REFERENCES

Blumenkrantz, N. & Asboe-Hansen, G. (1973). *Anal. Biochem.* **54**, 484.

Cadmus, M. C., Rogovin, S. P., Burton, K. A., Pittsley, J. E., Knutson, C. A. & Jeanes, A. (1976). *Can. J. Biochem.* **22**, 942.

Cadmus, M. C., Knutson, C. A., Lagoda, A. A., Pittsley, J. E. & Burton, K. A. (1978). *Biotech. Bioeng.* **20**, 1003.

Cottrell, I. W., Kang, K. S. & Kovacs, P. (1980). In *Handbook of water-soluble gums and resins*, ed. R. L. Davidson, New York, McGraw-Hill, pp. 24.1-24.31.

Davidson, I. W. (1978). *FEBS Letters* **3**, 347.

Dawson, P. & Mentzer, E. (1980). Polymer flooding in North Sea oil reservoirs, SPE 9300.

Dea, J. C. M. & Morris, E. R. (1977). In *Microbial extracellular polysaccharides*, eds. P. A. Sandford & A. Laskin, ACS Symposium 45, p. 174.

Dintzis, F. R., Babcock, G. E. & Tobin, R. (1970). *Carbohydr. Res.* **28**, 351.

Dubois, M., Gillies, K. A., Hamilton, J. K., Rebbers, P. A. & Smith, F. (1965). *Anal. Chem.* **28**, 350.

Evans, C. G. T., Yeo, R. G. & Ellwood, D. C. (1978). In *Microbial polysaccharides and polysaccharases*, eds. R. W. C. Berkeley, G. W. Gooday and D. C. Ellwood, London, New York and San Francisco, Academic Press, pp. 51–68.

Holzwarth, G. (1978). *Carbohydr. Res.* **66**, 173.

Holzwarth, G. & Ogletree, J. (1979). *Carbohydr. Res.* **76**, 277.

Jansson, P. E., Kenne, L. & Lindberg, B. (1975). *Carbohydr. Res.* **45**, 275.

Jeanes, A., Pittsley, J. E. & Senti, F. R. (1961). *J. Appl. Poly. Sci.* **5**, 591.

Kabir, C. S. & Stanislav, J. F. (1980). *Polymer* **21**, 564.

Lawson, C. J., McLeary, C. W., Nakada, H. I., Rees, D. A., Sutherland, I. W. & Wilkinson, J. F. (1969). *Biochem. J.* **115**, 947.

Lawson, C. J. & Sutherland, I. W. (1979). In *Economic microbiology*, ed. A. H. Rose, Vol. 2, London, New York and San Francisco, Academic Press, pp. 327–92.

McComb, F. A. & McCready, R. H. (1957). *Anal. Chem.* **29**, 819.

Melton, L. D., Mindt, L., Rees, D. A. & Sanderson, G. R. (1976). *Carbohydr. Res.* **46**, 245.

Morris, E. R. (1977). In *Microbial extracellular polysaccharides*, eds. P. A. Sandford & A. Laskin, ACS Symposium 45, p. 81.

Sandford, P. A. (1979). *Adv. Carbohydr. Chem. Biochem.* **36**, 265.

Sandford, P. A., Pittsley, J. E., Knutson, C. A., Watson, P. R., Cadmus, M. C. & Jeanes, A. (1977). In *Microbial extracellular polysaccharides*, eds. P. A. Sandford & R. H. Laskin, ACS Symposium 45, p. 192.

Sandford, P. A., Watson, P. R. & Knutson, C. A. (1978). *Carbohydr. Res.* **63**, 253.

Sandvik, E. I. & Maerker, J. M. (1977). In *Microbial extracellular polysaccharides*, eds. P. A. Sandford & A. Laskin, ACS Symposium 45, p. 242.

Sloneker, J. H. & Jeanes, A. (1962). *Can. J. Chem.* **40**, 2066.

Sloneker, J. H. & Orentas, D. G. (1962). *Nature* **194**, 478.

Smith, I. H., Symes, K. C., Lawson, C. J. & Morris, E. R. (1981). *Int. J. Biol. Macromol.* **3**, 129.

Sutherland, I. W. (1977). In *Microbial polysaccharides and polysaccharases*, eds. R. W. C. Berkeley, G. W. Gooday & D. C. Ellwood, London, New York and San Francisco, Academic Press, pp. 1–34.

Sutherland, I. W. (1981*a*). *J. Chromatogr.* **213**, 301.

Sutherland, I. W. (1981*b*). *Carbohydr. Polymers* **1**, 107.

Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature* **166**, 444.
Wellington, S. L. (1980). Biopolymer solution viscosity, stabilization, polymer degradation and antioxidant use, SPE 9296.
Wernau, W. C. (1979). British Patent 2008 600A.
Whitcomb, P. J., Elk, B. J. & Macosko, C. W. (1977). In *Microbial extracellular polysaccharides*, eds. P. A. Sandford & A. Laskin, ACS Symposium 45, p. 160.