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Acid Hydrolysis and High-Performance Liquid Chromatography of Xanthan

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ABSTRACT

A method for the routine determination of the hexose and organic acid content of xanthan preparations based on acid hydrolysis of the exopolysaccharide followed by high-performance liquid chromatography (HPLC) of the hydrolysate on two cation-exchange columns was investigated. A kinetic model was fitted to the recoveries of these components over a range of hydrolysis conditions using non-linear regression, affording values for the rate constants and the initial concentration of each compotent in the unhydrolysed polysaccharide. The ratio of glucose to mannose was greater than the assumed 1:1 ratio in unhydrolysed xanthan, suggesting that the polysaccharide may contain fewer terminal mannosyl residues than previously proposed. Quantification of glucuronic acid was complicated by its lactonisation and consequent elution as two peaks and by interference from its degradation products. The possibility of determining this component as its aldobiouronic acid [β-D-GlcA p-(1→2)-D-Man p] precursor was investigated. Acetic acid recovery was not significantly affected by the hydrolysis conditions over the range studied. The difference between the maximum recoveries and

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the fitted initial concentrations of each component indicate that compositional analyses of xanthan samples based on single time and acid strength conditions should be treated with caution as they are unlikely to yield quantitatively accurate values.

INTRODUCTION

Xanthan, the extracellular polysaccharide synthesised by the bacterium *Xanthomonas campestris*, has found numerous industrial applications because of its unusual solution properties (Sutherland & Ellwood, 1979). The polymer has a cellulosic backbone with charged trisaccharide side-chains [β-D-Man*p*-(1→4)-β-D-GlcA*p*-(1→2)-α-D-Man*p*] attached by α-(1→3)-linkages to alternate β-D-glucopyranosyl residues; most or all of the internal mannosyl residues are substituted at O-6 with an acetyl group; and pyruvyl substituents are linked as cyclic ketals to the 4- and 6-positions of the terminal mannosyl residues in often less than stoichiometric amounts (Jansson *et al.*, 1975; Melton *et al.*, 1976). The distribution of the pyruvyl groups along the polymer chains has not been established, although xanthan preparations can be resolved into 'pyruvate-rich' and 'pyruvate-poor' fractions (Sandford *et al.*, 1978; Sutherland, 1981).

The extent of pyruvylation and acetylation of xanthan is thought to influence a number of its solution properties including its filterability (Wernau, 1978), its thermally-induced conformational transition (Smith *et al.*, 1981), and its viscosity (Sandford *et al.*, 1977). Because of the industrial importance of these properties, the effect of growth conditions on the composition of xanthan has been studied widely (e.g. Davidson, 1978; Tait *et al.*, 1986). The pyruvyl and acetyl substituents are the most variable of the components of xanthan although some mutant strains apparently synthesise exopolysaccharides lacking one or more of the side-chain hexose and hexuronic acid residues (Whitfield *et al.*, 1981; Tait, 1984; Betlach *et al.*, 1987).

At present, at least three separate assays are required to characterise the hexose, glucuronic acid, and pyruvic and acetic acid composition of a single xanthan sample, a range of chromatographic, colorimetric and enzymic techniques being used. Most of these methods involve a preliminary hydrolysis of the polysaccharide and therefore afford values for the recovery of an intermediate in a sequence of hydrolysis and degradation reactions rather than for the initial concentration of the component in the polysaccharide. Depending upon the hydrolysis conditions used,

the observed recovery and the true concentration of the component in the polysaccharide may differ considerably.

The aim of this study was therefore to develop a single, simpler method for the determination of the sugar and organic acid components of xanthan based on acid hydrolysis of the polymer followed by HPLC on Aminex HPX-85 (Pb^{2+}) and Aminex HPX-87 (H^+) cation exchange columns respectively. By fitting the observed recoveries of these components over a range of hydrolysis conditions to an equation describing the recovery of such intermediates in a two step hydrolysis and degradation reaction sequence, a more reliable means of estimating the true concentration of each component in the unhydrolysed polysaccharide was established.

MATERIALS AND METHODS

Xanthan production, isolation and purification

The xanthan preparation used was isolated from a sulphur-limited chemostat culture of *X. campestris* S459, a streptomycin-resistant variant of *X. campestris* ATCC 13951, growing at a dilution rate of 0·03 h^{-1}, pH 6·6 and 30°C. After dilution of the culture broth with an equal volume of physiological saline and centrifugation at 10000*g* for 45 min, the supernatant was decanted and treated with 3 volumes of cold acetone to precipitate the polysaccharide. This precipitate was resuspended, dialysed against running tap water for 72 h, then lyophilised.

The dried polymer was purified by resuspension in distilled water, centrifugation at 100000*g* for 45 min, dialysis against distilled water (changed daily) for 96 h at 4°C, conversion to its sodium form using a Bio-Rad AG 50W X8 cation-exchange resin, (Bio-Rad Ltd, Hemel Hempstead, UK) and finally lyophilisation. After drying this product to constant weight in a vacuum oven (50°C, 2–3 days), the extent of its contamination with protein, nucleic acid and water was estimated by carbon, hydrogen and nitrogen elemental analysis.

Hexose release and determination

Aliquots of a xanthan solution were dispensed into ampoules then evaporated to dryness, leaving approximately 5 mg exopolysaccharide in each tube. After the addition of 1·5 ml trifluoroacetic acid (TFA) at the appropriate concentration, each ampoule was sealed and heated at

110°C for the required time. The hydrolysates were then evaporated to dryness, washed in distilled water, resuspended in 1 ml distilled water, and finally chromatographed.

The results obtained (Table 1) were fitted using non-linear regression to a surface described by the equation:

$$y = \frac{e^{-(k_3 + Ak_4)T}(k_1 + Ak_2)\,x_0[e^{(k_3 + Ak_4 - k_1 - Ak_2)T} - 1]}{k_3 + Ak_4 - k_1 - Ak_2} \quad (1)$$

where T is the hydrolysis time (h), A is the acid concentration (M H^+), x_0 is the initial hexose content of xanthan (per cent), and k_1, k_2,... k_4 are the fitted parameters.

Equation (1) is derived from an equation (Moore, 1962) describing the concentration of an intermediate in two consecutive first-order reactions. The terms $k_1 + Ak_2$ and $k_3 + Ak_4$ correspond to the simple rate constants for the first and second reactions in Moore's equation and have been expanded to incorporate the known effect of acidity on the rate of reaction. For 0–2 M TFA, the molar concentration approximates to h_0, the effective hydrogen ion concentration, derived from the equation $H_0 = -\log_{10} h_0$ where H_0 is the Hammett acidity function (Spitzer *et al.*, 1976). For a range of polysaccharides including cellulose, the rate of hydrolysis is proportional to h_0 (Rochester, 1970).

Organic acid release and determination

The procedure was similar to that used for hexose release and determination: 5 mg aliquots of the same xanthan sample were hydrolysed at 95°C in 1 ml dilute sulphuric acid under the conditions required. Cooled hydrolysates were injected directly into the HPLC column. The results obtained (Table 2) were, where appropriate, fitted to eqn (1), the variable A in these analyses corresponding to the effective hydrogen ion concentration, h_0, and x_0 to the initial organic acid content of xanthan. The difference between h_0 and the molar concentration of sulphuric acid (Rochester, 1970) is shown in Table 2.

High-performance liquid chromatography

Varian liquid chromatographs equipped with autosamplers and injection valves and controlled by a Varian Vista 401 integrator (Varian Ltd, Warrington, UK) were used. The hexoses were separated at 85°C on an Aminex HPX-85 (Pb^{2+}) heavy metal cation-exchange column (300 mm × 7·8 mm i.d.) supplied by Bio-Rad Labs Ltd, Hemel Hempstead,

TABLE 1
Effect of Acid Strength and Hydrolysis Time on the Recovery of Glucose and Mannose (% of Xanthan Dry Weight) After Hydrolysis of 5 mg Aliquots of a Xanthan Preparation at 110°C in TFA

Acid strength (M)	*Hydrolysis time (h)*	*Glucose (%)*	*Mannose (%)*
0·1	8·0	20·6	16·8
	32·0	16·9	16·2
	48·0	10·6	9·7
0·25	4·0	28·0	19·7
	16·0	23·0	19·7
	24·2	15·6	14·6
	32·0	14·4	13·6
	48·0	8·6	7·5
	72·0	5·5	4·9
0·5	2·0	13·6	16·6
	4·0	28·9	22·6
	8·0	27·7	23·2
	16·0	16·9	15·7
	24·2	13·6	13·4
	32·0	10·6	10·0
	110·3	2·1	0·5
0·75	1·0	3·0	14·1
	8·0	30·1	24·5
	16·0	17·7	15·8
	32·0	8·1	7·3
1·0	0·5	0·6	6·2
	2·0	17·7	19·2
	4·0	29·4	25·7
	8·0	28·1	23·7
	16·0	20·0	18·9
	24·2	9·8	8·8
1·25	1·0	4·1	16·9
	2·0	23·9	18·3
	4·0	29·0	24·9
	8·0	26·9	24·2
	16·0	17·6	16·5
	24·2	7·5	6·3
1·5	0·5	4·2	4·2
	1·0	6·8	20·2
	2·0	26·1	22·5
	4·0	30·0	26·5
	8·0	28·3	26·4
	16·0	17·0	16·5
2·0	0·5	0·2	7·6
	2·0	23·7	17·5
	8·0	20·3	19·7

TABLE 2

Effect of Acid Strength and Hydrolysis Time on the Recovery of Pyruvic, Acetic, Glucuronic and Aldobiouronic acids (% of Xanthan Dry Weight) After Hydrolysis of 5 mg Aliquots of a Xanthan Preparation at 95°C in H_2SO_4

Acid strength (M)	(h_0)[a]	*Hydrolysis time* (h)	*Pyruvic acid* (%)	*Acetic acid* (%)	*Glucuronic acid* (%)	*Aldobiouronic acid*[b] (%)
0·25	0·37	1·0	5·2	6·3	—	—
		4·0	6·9	7·1	9·0	7·8
		5·0	7·2	6·9	9·4	—
		8·0	7·4	7·1	13·8	13·8
		24·1	7·3	7·3	—	31·0
0·5	0·74	0·5	4·7	5·6	—	—
		1·5	6·8	6·6	—	21·0
		3·0	7·2	6·5	—	—
		5·0	7·5	6·8	16·4	19·8
		6·0	7·5	6·8	18·2	24·5
		16·0	7·4	7·1	—	32·0
0·75	1·22	2·0	7·2	6·7	10·5	8·5
		4·0	7·3	6·8	16·9	25·1
		6·0	7·4	7·0	—	34·6
1·0	1·82	0·5	5·1	6·3	—	—
		1·5	7·3	6·8	10·8	12·1
		3·0	7·2	6·6	16·1	22·7
		4·0	7·2	6·6	22·0	28·3
		5·0	7·1	6·5	25·7	30·4
		8·0	6·9	6·5	40·3	29·4
		24·1	6·7	—	—	—
1·25	2·50	1·0	6·8	6·3	7·7	—
		2·0	6·8	6·8	11·1	21·8
		3·0	6·9	6·8	12·4	29·9
		4·0	7·0	7·0	18·0	33·4
		5·0	6·8	6·7	33·9	25·7
		16·0	6·5	—	—	25·5
1·5	3·52	1·5	6·7	6·6	12·2	15·8
		3·0	6·4	6·5	22·7	24·5
		4·0	6·4	6·8	32·5	23·7
		6·0	6·3	7·2	47·0	21·1
1·75	4·93	1·0	6·3	7·5	10·6	12·1
		2·0	6·2	6·5	18·8	22·1
		3·0	6·3	6·9	27·9	22·8
		5·0	5·9	7·3	42·7	18·1
		16·0	5·7	—	—	—
2·0	6·92	0·5	4·5	—	—	—
		1·5	5·9	6·3	15·7	17·8
		2·0	5·9	6·4	22·9	19·4
		3·0	5·8	6·7	31·5	18·9
		4·0	5·6	6·9	40·4	14·6

UK, using deionised water at a flow rate of 0·6 ml min^{-1} as eluent and a 100 μl injection loop. Peaks were detected using an Altex refractive index detector (Beckman-RIIC Ltd, High Wycombe, UK) maintained at 40°C by a Haake circulating water bath (Gebrüder Haake, Berlin, FRG). Organic acids were resolved at 40°C on an Aminex HPX-87 (H^+) ion exclusion column (300 mm × 7·8 mm i.d.) supplied by Bio-Rad Labs Ltd, Hemel Hempstead, UK, using 6·25 mM H_2SO_4 at a flow rate of 0·6 ml min^{-1} as eluent and a 50 μl sample size. Peaks were detected using a Varian UV-50 ultraviolet spectrophotometer (Varian Ltd, Warrington, UK) at 210 nm and 40°C. Hexose standards were prepared in distilled water and organic acid standards in 6·25 mM H_2SO_4.

RESULTS

Hexose release and determination

The amounts of glucose and mannose shown in Table 1 were recovered after hydrolysis of aliquots of xanthan at 110°C in 0·1–2·0 M TFA for 0·5–110 h. The highest levels of glucose and mannose were detected after hydrolysis in 0·75 M TFA for 8 h (30% of xanthan dry weight) and in 1·5 M TFA for 8 h (27% of xanthan dry weight) respectively.

Equation (1) was fitted to the observed glucose and mannose recoveries, giving the values for the parameters k_1, k_2, k_3, k_4 and x_0 shown in Table 3. The variance ratios (37·2 and 35·7 for the glucose and mannose analyses respectively) were both significantly greater than the equivalent calculated variance ratio (F) value [$F_{0.999}(5,30) = 5{\cdot}53$ (Fisher & Yates, 1963)], indicating that there was a good fit between the equation and the observed recoveries. Both sets of residuals were distributed randomly about their means when plotted against the fitted values, indicating that the model used was appropriate.

The fitted values for x_0 (Table 3) predict that the unhydrolysed xanthan preparation contained 42% glucose and 32% mannose, on a dry weight basis, giving a total hexose content of 74%. The predicted maximum recoveries, calculated by maximising y in eqn (1), are 29% glucose and 26% mannose (Table 3), a ratio of approximately 1·1:1. The corresponding times and acid concentrations for these maxima ($T_{y_{max}}$ and

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[a]Rochester (1970).

[b]Aldobiouronic acid values were calculated retrospectively from peak heights using a previously observed relationship between the response factors for pyruvic acid and aldobiouronic acid standards. The absolute but not relative values of aldobiouronic acid may therefore be in error.

TABLE 3
Regression of Hexose and Organic Acid Recoveries[a]

Parameter	*Glucose*	*Mannose*	*Pyruvic acid*	*Aldobiouronic acid*
k_1	0·196(±0·064)	0·163(±0·055)	1·063(±0·428)	0
k_2	0·128(±0·051)	0·348(±0·078)	1·136(±0·425)	0·264(±0·087)
k_3	0·035(±0·007)	0·029(±0·005)	0	0
k_4	0·027(±0·010)	0·017(±0·007)	0·003(±0·001)	0·019(±0·012)
x_0	41·9(±5·2)	31·9(±2·4)	6·98(±0·14)	32·12(±5·32)
$F_{(5,35)}$	37·2	35·7	—	—
$F_{(4,42)}$	—	—	1311	—
$F_{(3,30)}$	—	—	—	179·2
y_{max} (%)	28·8	25·9	6·98	26·1
$T_{y_{max}}$ (h)	10·8	3·3	20·3	5·5
$A_{y_{max}}$	0	1·98	0	1·96

[a]Equation 1 was fitted to the glucose, mannose, pyruvic acid and aldobiouronic acid recoveries in Tables 1 and 2, affording the values shown (±SE) for the parameters k_1, k_2, k_3, k_4, and x_0. The variance ratios (F), the predicted maximum recovery of each component (y_{max}) and the time ($T_{y_{max}}$) and acid strength ($A_{y_{max}}$) at these maxima are also shown.

$A_{y_{max}}$ respectively) are also shown in Table 3. Elemental analysis of the xanthan sample indicated that it contained 36% carbon, 6·1% hydrogen and 0·6% nitrogen.

The response surfaces fitted to the glucose and mannose recoveries are illustrated as contour plots in Fig. 1(a) and Fig. 1(b) respectively. Mannose is released initially more rapidly and latterly more slowly than glucose, reflecting perhaps the acid lability of the outer β-(1→4) glycosidic linkage and the acid stability of the β-(1→2) linkage in the aldobiouronic acid component. Although the acid strength has a broad optimum for the recovery of both hexoses, the two maxima (0 and 2 M TFA) differ considerably.

Organic acid release and determination

The amounts of pyruvic, acetic, glucuronic and aldobiouronic acid in Table 2 were recovered after hydrolysis of aliquots of xanthan at 95°C in 0·25–2·0 M H_2SO_4 for 0·5–24 h. The maximum recoveries were 7·5% pyruvic acid after 5–6 h in 0·5 M H_2SO_4; 7·5% acetic acid after 1 h in 1·75 M H_2SO_4; 47% (apparently) glucuronic acid after 6 h in 1·5 M H_2SO_4; and 16·4% aldobiouronic acid after 6 h in 0·75 M H_2SO_4. The values for aldobiouronic acid were calculated retrospectively from peak

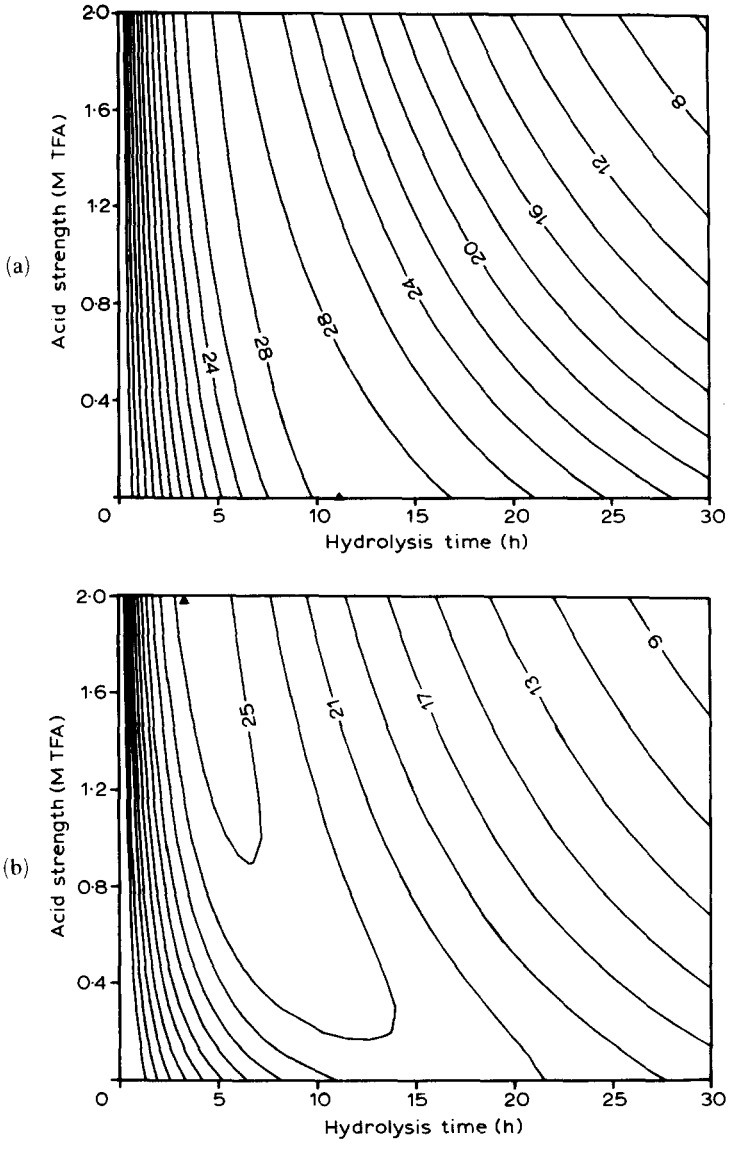

Fig. 1. Predicted effect of acid strength and hydrolysis time on (a) glucose and (b) mannose recovery (per cent of xanthan dry weight) after hydrolysis of xanthan at 110°C in TFA, calculated using eqn (1) and the fitted parameters in Table 3. The contours increase in steps of (a) 2% from 4% to 28%, to a peak (▲) of 28·8 at 11 h and 0 M TFA, and (b) 2% from 5% to 25%, to a peak (▲) of 25·9 at 3·3 h and 1·98 M TFA.

heights using a previously observed relationship between the response factors for pyruvic acid and an aldobiouronic acid standard derived by hydrolysis of xanthan. The absolute but not relative values of aldobiouronic acid may therefore be in error.

Equation (1) was fitted to the observed pyruvic and aldobiouronic acid recoveries using the effective hydrogen ion concentration, h_0, rather than the molarity. The values fitted to the parameters k_1, k_2, k_3, k_4 and x_0 are shown in Table 3. Zero values were assigned to k_1 (aldobiouronic acid analysis) and k_3 (both analyses) after preliminary runs gave nonsignificant values for these parameters. The variance ratios (1311 and 179 for the pyruvic and aldobiouronic acid analyses respectively) were both significantly greater than the equivalent calculated F-values [$F_{0\cdot999}(4,40) = 5\cdot70$; $F_{0\cdot999}(3,30) = 7\cdot05$ (Fisher & Yates, 1963), indicating that the fitted equation and observed recoveries were in good agreement. The variance ratio decreased from 1311 to 17 when the molarity rather than h_0 was used in fitting eqn (1) to the pyruvic acid data. The random distribution of the residuals about their means when plotted against the fitted values indicated that the model was again appropriate. Equation (1) was not fitted to the recoveries of either acetic acid, which was not significantly affected by the hydrolysis conditions within the range studied (mean 6·7%), or glucuronic acid, which proved difficult to quantify for the reasons described below.

The fitted values for x_0 (Table 3) indicate that the unhydrolysed polysaccharide contained 7·0% pyruvic acid and 32% aldobiouronic acid (on a dry weight basis). The predicted maximum recoveries, calculated by maximising y in eqn (1), are 7·0% pyruvic acid and 26% aldobiouronic acid (Table 3). The response surfaces fitted to the recoveries of pyruvic and aldobiouronic acid are illustrated as contour plots in Fig. 2(a) and Fig. 2(b) respectively. The aldobiouronic acid surface illustrates the rapid release and slow subsequent hydrolysis of this component.

High-performance liquid chromatography of hydrolysates

The hexoses and organic acids in xanthan hydrolysates were determined by HPLC on Aminex HPX-85 (Pb^{2+}) heavy metal and Aminex HPX-87 (H^+) ion exclusion columns respectively. Retention times of standards on the former column were: cellobiose, 10·3 min; glucose, 12·6 min; galactose, 14·7 min; mannose, 16·9 min; pyruvic and acetic acids, approximately 40 min. Retention times on the latter column were: aldobiouronic acid, 7·7 min; glucuronic acid, 8·8 min; pyruvic acid, 10·7 min; and acetic acid, 16·5 min (Fig. 3). Glucuronic acid was in fact eluted as two peaks with retention times of 8·8 min and 12·6 min. The later,

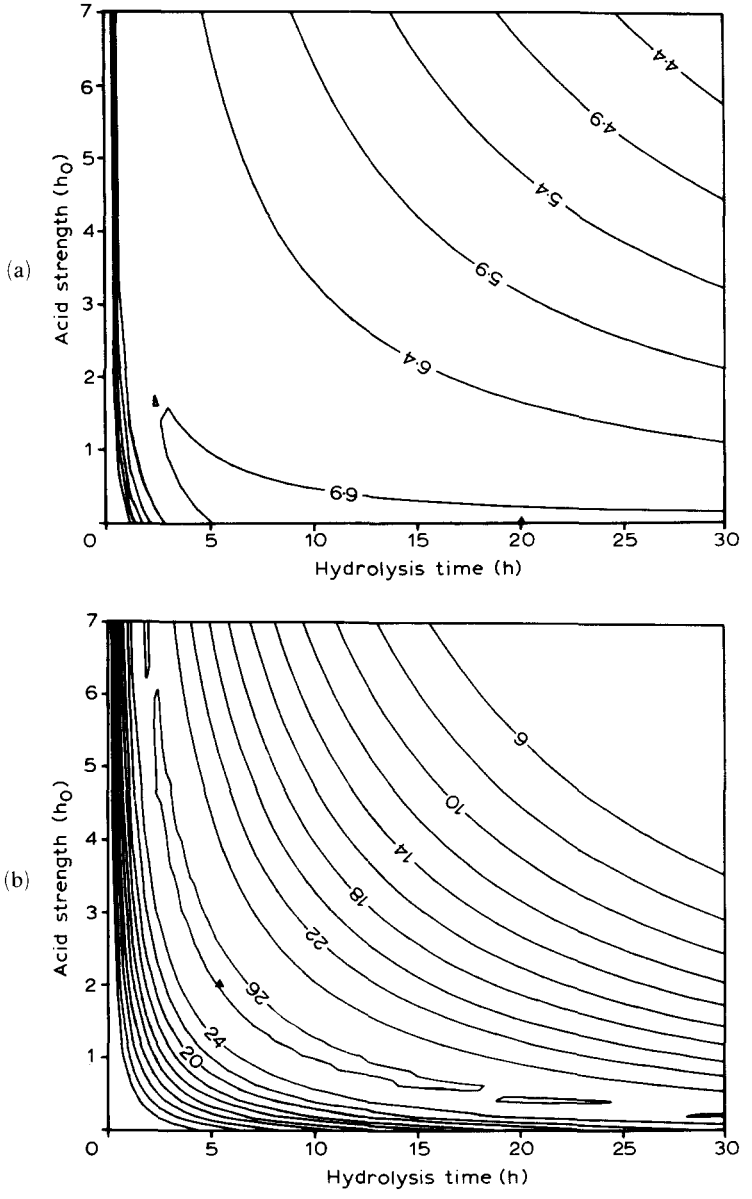

Fig. 2. Predicted effect of acid strength and hydrolysis time on (a) pyruvic acid and (b) aldobiouronic acid recovery (per cent of xanthan dry weight) after hydrolysis of xanthan at 95°C in H_2SO_4, calculated using eqn (1) and the fitted parameters in Table 3. The contours increase in steps of (a) 0·5% from 4·4% to 6·9%, to a peak (▲) of 6·98 at 20 h and 0 M H_2SO_4, and (b) 0·5% from 6% to 26%, to a peak (▲) of 26·1 at 5·5 h and an acid strength (h_0) of 1·96.

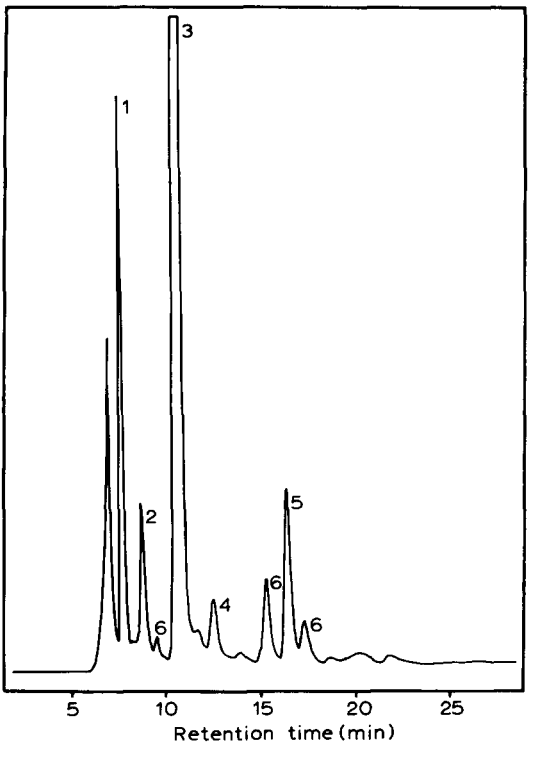

Fig. 3. Chromatogram of xanthan hydrolysate on an Aminex HPX-87 ion exclusion column, showing peaks corresponding to: 1, aldobiouronic acid (7·7 min); 2, glucuronic acid (8·8 min); 3, pyruvic acid (10·7 min); 4, glucuronolactone (12·6 min); 5, acetic acid (16·5 min); and, 6, glucuronic acid degradation products (8·3, 15·3, 17·4 min). The polysaccharide was hydrolysed in 0·5 M H_2SO_4 at 95°C for 16 h; the HPLC conditions are described in the Methods section.

smaller peak corresponds to glucuronolactone [D-glucofuranurono-(6→3)-lactone] formed by partial lactonisation of the uronic acid under the acid conditions used for hydrolysis and chromatography. The equilibrium mixture appeared to have a constant composition, the area of the larger peak (8·8 min) being proportional ($R = 0{\cdot}95$–$0{\cdot}99$) to the concentration of glucuronic acid in the range 0·1–1·5 mg ml^{-1}. The identity of the smaller peak was confirmed after a standard solution of the lactone was eluted as a peak with a retention time of 12·6 min.

After hydrolysis in TFA, some acid persisted despite repeated washing and re-evaporation, possibly due to formation of the TFA–water azeotrope [b.p. 105°C at 1 atm (Horsley, 1962)]. This residue appeared to be the cause of a large premature rise in the column pressure which could only partly be restored by regeneration. Following hydrolysis in

H_2SO_4 and neutralisation with $BaCO_3$, no column degradation was observed.

The effect of hydrolysis conditions on the chromatography of glucose and mannose was investigated by heating standards in 0·5 M TFA for 4–5 h at 110°C. Although this did not affect the baseline separation of the two sugars, untreated sugars were eluted as taller and narrower peaks than those of sugars heated in acid. A similar effect was observed on Aminex HPX-87 chromatograms after heating organic acid standards in 0·5 M H_2SO_4 for 4–5 h at 95°C, although on one occasion the pyruvic acid peak conversely increased in height and decreased in width. A heterogeneous mixture of degradation products, manifest as at least 10 different peaks on Aminex HPX-87 chromatograms, was formed when glucuronic acid was heated in sulphuric acid.

DISCUSSION

By interpreting the recovery of the hexose and organic acid components of xanthan with respect to time as that of intermediates in two consecutive pseudo-first-order reactions (i.e. release from the polysaccharide and degradation), an equation (eqn (1)) describing the recovery of such intermediates and adapted to include the reported linear relationship between the acid strength, h_0, and the reaction rate (Rochester, 1970) can be fitted to the observed recoveries. Statistically, this equation appeared to be a good model for the recovery of these components, despite the simplifications involved, particularly with respect to the different rates of release of the terminal and internal mannosyl residues. A second 'binomial' model was also fitted to the glucose, mannose and pyruvic acid data (Tait, 1984). This afforded very similar surfaces to those obtained in this study but overestimated the maximum recoveries and generally gave lower variance ratios.

The values fitted to the reaction rates (Table 3) indicate that glucose and mannose have very similar degradation rates but different rates of release, k_2 for mannose being approximately three times that for glucose. This difference is a consequence of the slow release of the internal mannosyl residue from its aldobiouronic acid precursor, the acid-resistant nature of which is reflected in the low values fitted to k_3 and k_4 for this latter component.

Assuming a sodium-form xanthan molecule with an average of 1 acetyl and 0·5 pyruvyl substituents per repeating unit (Jansson *et al.*, 1975), the polysaccharide would contain 39% glucose, 39% mannose, 41% aldobiouronic acid, 6% acetic acid, 4% pyruvic acid and 43%

carbon. Since the actual carbon content of the sample used in this study was only 36%, these figures should be corrected by a factor of 0·84 to 33% glucose, 33% mannose, 34% aldobiouronic acid, 5% acetic acid and 3% pyruvic acid. These compare with the fitted x_0 values (Table 3) of 42% (±5%) glucose, 32% (±2%) mannose, 32% (±5%) aldobiouronic acid and 7% (±0·1%) pyruvic acid and an observed recovery of 6·7% (±0·4%) acetic acid. Whereas the figure of 7% pyruvic acid is not unusual given the reported variability of this component of xanthan, the high ratio of glucose to mannose suggests that the native polysaccharide may contain less than stoichiometric amounts of the terminal mannosyl residue. More marked structural alterations in the side-chain hexoses and hexuronic acid have been reported in a number of mutant strains of *X. campestris* (Whitfield *et al.*, 1981; Tait, 1984; Betlach *et al.*, 1987).

The quantification of glucuronic acid as its aldobiouronic acid precursor has the advantage that the shorter hydrolysis times required should reduce the amount of glucuronic acid degradation products in the hydrolysate and thus allow a more accurate determination of the other organic acids present. Additionally, the relative proportions of the internal and terminal mannosyl residues in the xanthan molecule could perhaps be determined since only the former forms part of the aldobiouronic acid component. Another method of xanthan analysis based on methanolysis and HPLC of the methyl glycosides on a reversed-phase column (Cheetham & Sirimanne, 1983) also gave low yields of glucuronic acid.

In conclusion, the full hexose and organic acid composition of xanthan preparations should be quantifiable by an acid hydrolysis/HPLC method. This would involve hydrolysis in sulphuric acid rather than TFA which apparently was incompatible with the Aminex HPX-85 column. Samples of the hydrolysate would be (a) neutralised with, for example, $BaCO_3$ then chromatographed on the Aminex HPX-85 column to determine its glucose and mannose content, and (b) injected directly into the Animex HPX-87 column to determine its acetic, pyruvic and aldobiouronic acid contents.

In principle, by using eqn (1) and the fitted values in Table 3, the hexose or organic acid content (x_0) for any xanthan sample can be calculated for any given hydrolysis time, acid strength and recovery. In practice, a series of samples measured at different times and at the 'optimum' acid strength and fitted to an equation such as that by Moore (1962) would see more appropriate. The difference between the maximum recoveries and the fitted initial concentrations of each component indicate that compositional analyses of xanthan samples based on single time and acid strength conditions should be treated with caution as they are unlikely to yield quantitatively accurate values.

Further work is in progress to confirm the utility of this approach and includes an examination of the effect of temperature, with the aim of reducing the overall analysis time. Preliminary observations are in line with those made here.

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