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Note

The reaction of xanthan with cetyltrimethylammonium bromide

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The reactions of various cationic precipitants with biopolyanions (*e.g.*, acidic polysaccharides, nucleic acids, and proteins) in aqueous solution have been reported[1–6]. These reagents have been used principally in the isolation and purification of biopolyanions. Deuel *et al.*[1] optimised the conditions for the precipitation of D-galacturonan from aqueous solution with poly(ethylene imine). Jones[2] reported the use of cetyltrimethylammonium bromide (CTAB) in the isolation of bacterial nucleic acids, and later that insoluble complexes were formed with acidic polysaccharides. Scott[3–5] showed that carboxyl- and sulphate-containing polysaccharides could be separated by virtue of their differential affinity for quaternary ammonium salts, and introduced the concept of critical electrolyte concentrations (CEC).

Recently, we reported[6] a comparison of CTAB with Cetavlon, a commercial mixture of quaternary ammonium salts, in the isolation of xanthan, the acidic polysaccharide produced by the micro-organism *Xanthomonas campestris*. The remarkable solution properties[7–9] of this biopolymer have led to its extensive use[9,10] in foods and non-foods as a stabiliser, emulsifier, and thickener. However, xanthan is difficult to recover in a purified form from the fermentation broth (due to problems with viscosity and cellular debris). We observed[6] that CTAB was a more efficient precipitant than Cetavlon for the isolation of xanthan. A higher molar ratio of Cetavlon–xanthan was required for quantitative precipitation of the polysaccharide, and larger quantities of residual precipitant were present in the final supernatant solution. The lower efficiency[3,4] of Cetavlon was due to its being a mixture of homologues (C_{12}, C_{14}, and C_{16}) with an average chain-length less than that of CTAB. However, when CTAB or Cetavlon was used to precipitate xanthan from *X. campestris* fermentation-broth, large proportions of precipitant were left in the supernatant solution just prior to the end-point.

There has been no report on the mode of formation of any biopolyanion–cationic precipitant complex. Using radio-labelled CTAB, we have studied the complexation reaction with xanthan.

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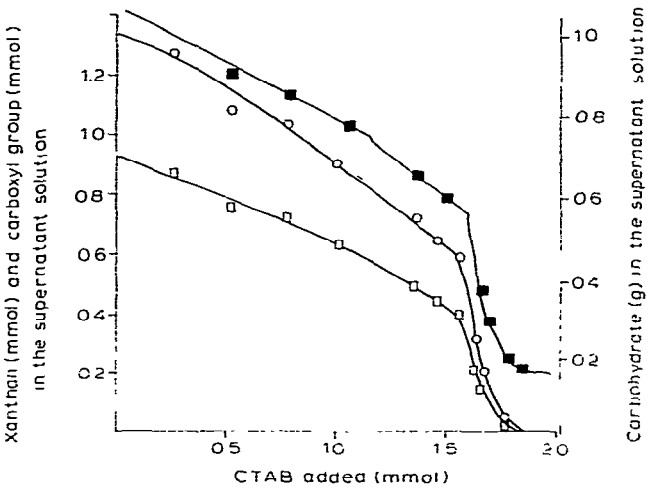

Fig. 1. Variation of carbohydrate (—■—), xanthan (—□—), and carboxyl group (—○—) in the supernatant solution with variation of CTAB addition to the *X. campestris* fermentation-broth.

A molar CTAB:xanthan ratio of 2 was required[6] to precipitate the polysaccharide quantitatively from *X. campestris* fermentation-broth. In this way, it was shown that 50 g of fermentation broth used in this study contained 0.917 mmol of xanthan. Stepwise addition of CTAB to the fermentation broth revealed a characteristic, sharp end-point for the precipitation (Fig. 1). The carbohydrate content of the supernatant solution decreased slowly during the initial stages of reaction. After the addition of 85% (1.566 mmol) of the CTAB end-point requirement, ~52% (0.56 g) of carbohydrate was still present in solution. However, the ~15% (0.16 g) of carbohydrate still present in solution at the apparent end-point may not have been xanthan. Of this material, ~75% was removed by dialysis and the remainder may have been neutral polysaccharides.

To enable calculation of the number of moles of carboxyl group in the supernatant solution with variation of CTAB addition, it was necessary to determine the carboxyl group:xanthan molar ratio; the pyruvic acid content of the polysaccharide depends on the fermentation conditions[11]. However, there is no satisfactory method for accurate determination of the acidic groups in xanthan. Titration of the acid form of xanthan with alkali gave a carboxyl group:xanthan molar ratio of 1.439, which was used to calculate the carboxyl-group contents shown in Fig. 1. However, formation of D-glucurono-6,3-lactone occurs during the conversion of xanthan into the acidic form, thus leading to low carboxyl values as previously found[12]. Extensive formation of lactone has been reported for other polysaccharides containing 1,4-linked D-glucuronic acid or D-mannuronic acid residues when isolated from an acidic medium[13].

A carboxyl group:xanthan molar ratio of 1.381 was obtained by assay for pyruvic acid and D-glucuronic acid. However, neutral sugars in the xanthan interfere in the carbazole assay[14] for hexuronic acid.

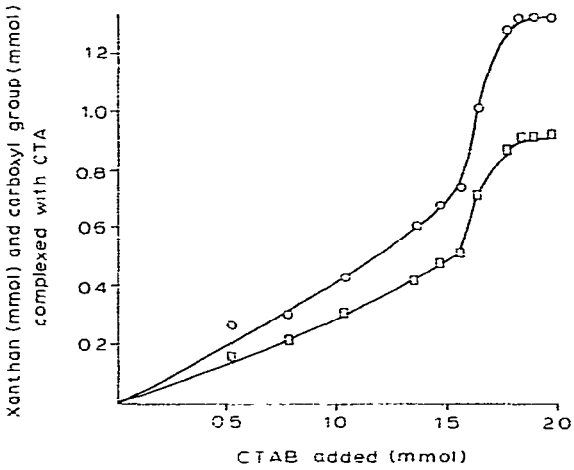

Fig. 2. Variation of xanthan (—□—) and carboxyl group (—○—) complexed to CTA with variation of CTAB addition to the fermentation broth.

The amount of xanthan complexed to the cetyltrimethylammonium (CTA) ion, with variation of CTAB addition (Fig. 2), was obtained by subtraction of the supernatant data in Fig. 1 from the amount (0.917 mmol) of polysaccharide originally present. The carboxyl group complexed to CTA was obtained similarly. The characteristic, sharp end-point is again emphasised.

The variation of CTAB in the supernatant solution with stepwise addition of precipitant was investigated by using ^{14}C-labelled CTAB. Liquid scintillation counting of [^{14}C]CTAB in aqueous solution necessitated the use of a toluene–Triton X-100 emulsion; 1.7 mg of polysaccharide/10 ml of emulsion causes no phase separation. The results showed that large proportions of precipitant were present in solution during the complexation reaction. Initially, only a small percentage of the added [^{14}C]CTAB was complexed to xanthan. However, at the end-point, only 3.2% of the residual precipitant remained in solution. Although this value was slightly higher than that found[6] using the methyl orange method, the results support the conclusion[6] that large proportions of quaternary ammonium salt are present in the supernatant solution prior to the complexation end-point. These data were used to calculate the amounts of unlabelled CTAB in solution, and the results are shown in Fig. 3. The build-up of CTAB in the supernatant solution during the initial stages of the complexation reaction, as predicted by the assessment of the xanthan in the supernatant solution (Fig. 1), is clearly shown. This build-up reached a peak of 0.607 mmol just before the mid-point addition of CTAB. Fig. 3 also suggests that no increase of CTAB present in the supernatant solution took place when quantities in slight excess of the complexation end-point requirement were added.

As mentioned earlier, deviation from stoichiometry was observed for the combination between the acidic groups of xanthan and the CTA ions. Since the study involved crude xanthan, proteins in the fermentation broth may have interfered. It

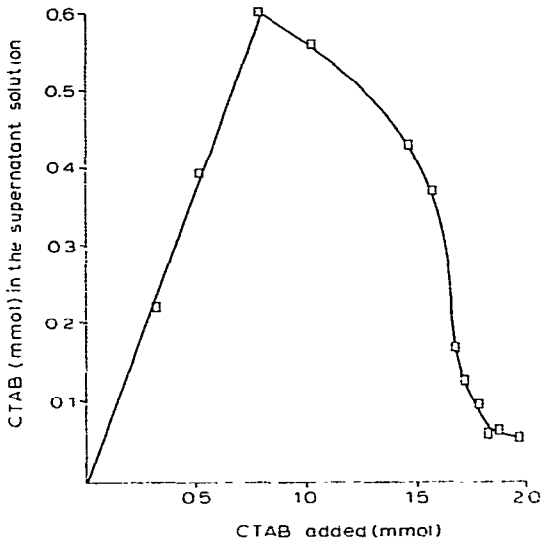

Fig. 3. Variation of CTAB in the supernatant solution with variation of CTAB addition to the fermentation broth.

was found that ~75% of the fermentation-broth protein was co-precipitated with the xanthan–CTA complex at the end-point; when 0.423 mmol of the xanthan had been precipitated, ~50% of the fermentation-broth proteins were present in the complex. At the end-point, the complex contained ~10% of protein.

EXPERIMENTAL

Materials and general methods. — *Xanthomonas campestris* fermentation-broths were prepared in house. Unlabelled CTAB was obtained from Hopkins and Williams Ltd., and [1-^{14}C]cetyltrimethylammonium bromide and [^{14}C]hexadecane from the Radiochemical Centre, Amersham. Radiochemical determinations were made with an Intertechnique ABAC SL 40 instrument using a toluene–Triton X-100 (2:1) counting-medium and butyl-PBD as the scintillator. Efficiencies were determined by the Channels Ratio method using [^{14}C]hexadecane as the standard.

Carbohydrate was determined by the resorcinol–4,6-disulphonic acid method[15], using Keltrol (food-grade xanthan, A.B.M. Chemicals, Stockport, Cheshire) as the standard.

Amounts of xanthan were calculated by assuming a mol. wt. of 958 for the repeating unit of the polysaccharide[16]. The pyruvate content of xanthan was determined by the 2,4-dinitrophenylhydrazine method[17], the hexuronic acid content by the carbazole assay[14], and the amino-acid content by using a Locarte automatic analyser in combination with an on-line Nova 1220 computer. Materials were dried at 61° *in vacuo* over P_2O_5 before analysis.

Variation of xanthan precipitated with variation of CTAB added. — Aliquots (50 g) of fermentation broth were diluted with distilled water (250 ml) and stirred

until dissolution was complete. Aqueous 2% CTAB (0.261–2.610 mmol) was added, with stirring for 30 min, to precipitate the xanthan complex, which was isolated by centrifugation at 3,000 r.p.m. for 15 min. The variation of carbohydrate content of the supernatant solutions with variation of CTAB added is shown in Fig. 1.

The experiment was also conducted with aqueous CTAB containing 0.15 μCi of [^{14}C]CTAB/ml. From the d.p.m. of the [^{14}C]CTAB solution, the percentage of added [^{14}C]CTAB present in the supernatant solution, and hence the actual quantity of residual precipitant, was calculated (Fig. 3).

Determination of carboxylic acid groups present in xanthan. — To an aliquot (50 g) of fermentation broth, diluted with distilled water (250 ml), was added 2% aqueous CTAB to precipitate the polysaccharide. The xanthan–CTA precipitate recovered from the supernatant by centrifugation (3,000 r.p.m., 15 min) was dissolved in 0.5M KCl. The purified polysaccharide was precipitated as the potassium salt by the addition of propan-2-ol (50 ml). An aqueous solution of the potassium salt was dialysed and freeze-dried. The residue (0.956 g) was dissolved in deionised water (500 ml) and an aliquot (25 ml) was found to contain 0.0439 g of xanthan, corresponding to a value of 0.917 mmol for the polysaccharide content of the fermentation broth.

A further aliquot (25 ml) was diluted ten-fold with deionised water and passed down a column of Zerolit SCR-225 (H^+) resin. The eluate was freeze-dried and the residue was dissolved in neutralised, deionised water (50 ml). From the titration of the xanthan solution with 0.01M NaOH, the molar ratio of carboxylic acid groups: polysaccharide repeating-unit was calculated to be 1.439.

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