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THE ISOLATION OF XANTHAN GUM FROM FERMENTATIONS OF *XANTHOMONAS CAMPESTRIS* BY COMPLEXATION WITH QUATERNARY AMMONIUM SALTS

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ABSTRACT

A comparison of the use of the quaternary ammonium salts, cetyltrimethylammonium bromide (CTAB) and the commercial mixture Cetavlon, for the isolation of xanthan gum from fermentations of Xanthomonas campestris *indicated that the former was the more efficient complexating agent. Although in both cases more than the stoichiometric requirement was necessary to achieve quantitative recovery of the polysaccharide, CTAB left only 1·7% material in the supernatant from the precipitation of xanthan gum compared to 15% left by Cetavlon. This is congruent with the view that the efficiency of quaternary ammonium salts increases with increased paraffin chain length.*

An assessment of the use of Cetavlon for the isolation of xanthan gum in a recycle procedure showed that an 11·5% loss of precipitant per cycle occurred. In the procedure, the xanthan gum was precipitated as the purified K^+ salt from a dispersion of its quaternary ammonium complex in 2-propanol. Concentration of the 2-propanol wash permitted recovery of the quaternary ammonium salt.

1. INTRODUCTION

Xanthan gum, the extracellular heteropolysaccharide produced commercially by fermentation with the plant pathogen *Xanthomonas campestris*, has found widespread technological use as a viscosity enhancing agent due to the remarkable and sometimes unique properties it displays in solution. The primary structure consists (Jansson

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et al., 1975; Melton *et al.*, 1976) of a cellulose-like backbone, with a side-chain attached at every other D-glucose residue consisting of the 6-*O*-acetyl derivative of α-D-mannose, β-D-glucuronic acid and β-D-mannose. Pyruvic acid is attached to approximately one half of the terminal β-D-mannose residues through the four and six positions in non-stoichiometric proportions. Although the biopolymer is known to possess an ordered secondary structure, conflicting views exist regarding the exact nature of the ordering. Morris *et al.* (1977) suggest that the secondary structure is built up of single, rod-like molecules, while Holzwarth (1976, 1978) claims that the polysaccharide adopts a double-stranded, right-handed helix. Nevertheless, it is logical to assume that the secondary structure plays some role in determining the properties of xanthan gum in solution. Ironically it is the remarkable solution properties of xanthan gum which give rise to the problems involved in the isolation of the polysaccharide. The *X. campestris* fermentation broths produced commercially, containing 3–4% xanthan gum, are extremely viscous and difficult to handle.

The most popular method for the isolation of xanthan gum is repeated precipitation with 2-propanol (Colegrove, 1970). Although this procedure ultimately produces material suitable for food use, several drawbacks are involved. Large volumes of the solvent are required and considerable losses occur on recycling. Moreover, the precipitation process with an organic solvent is non-specific. Various isolation procedures reported, such as precipitation with Ca^{2+} (McNeely & O'Connell, 1966) and Al^{3+} (Towle, 1977) ions, have utilised the presence of the carboxy groups which occur in both the pyruvic acid and glucuronic acid units of the xanthan gum molecule.

Recently, we reported the use of a new polymeric cationic precipitant, poly-(hexamethylenebiguanide hydrochloride) (Kennedy *et al.*, 1981b), for the isolation of xanthan gum. Scott (1960, 1961) carried out a great deal of work on the isolation of acidic polysaccharides with another class of cationic precipitants, quaternary ammonium salts. An innovation of this work was the ability to separate carboxylated and sulphated polysaccharides according to their differing affinity of binding to a particular quaternary ammonium salt. The use of a certain commercial mixture of quaternary ammonium salts, Arquad, was reported by Albrecht *et al.* (1962). In this paper we compare the complexation of xanthan gum with cetyltrimethylammonium bromide (CTAB) and the commercial mixture, Cetavlon. While CTAB is a pure salt consisting entirely of a C_{16} aliphatic chain, Cetavlon (Anon., 1971) consists of mainly C_{14} along with smaller amounts of C_{12} and C_{16}. This study was devised not only to show the effect of chain length, but also the effect of a mixture of quaternary ammonium salts on complexation. To assess the suitability of Cetavlon for the isolation of xanthan gum, a recycle procedure involving reuse of the precipitant was devised.

2. MATERIALS AND GENERAL METHODS

The *Xanthomonas campestris* fermentation broths used in the study were prepared in

house with a strain of *X. campestris* bacterium identical to NRRL B-1459. A commercial sample of xanthan gum (Keltol) obtained from A.B.M. Chemicals, Stockport, Cheshire, was used as a standard for carbohydrate determinations, which were carried out by the resorcinol-sulphuric acid assay as indicated previously (Kennedy *et al.*, 1981b). A molecular weight of 958, based on the presently accepted repeating structure (Jansson *et al.*, 1975), was used for the calculation of moles of xanthan gum. The pyruvic acid content of xanthan gum samples was determined using the 2,4-dinitrophenylhydrazine method of Sloneker & Orentas (1962). Uronic acid was determined by the carbazole assay using the method of Bitter & Muir (1962). The amino acid analysis of xanthan gum samples was carried out using a Locarte automatic analyser in combination with a Nova 1220 computer.

2.1. *Determination of Quaternary Ammonium Salts in Aqueous Solution with Methyl Orange*

A modified method using the general conditions outlined by Das Gupta (1973) in a review of the assay of quaternary ammonium salts with anionic dyes was used.

Procedure: Solutions of quaternary ammonium salts (1 ml) were pipetted into a separating funnel (50 ml) followed by citric acid-sodium citrate solution (0·1 M (pH 5); 3·5 ml) and methyl orange solution (0·2% w/v; 0·4 ml). After shaking to obtain a homogeneous mixture, the quaternary ammonium-methyl orange complex was selectively extracted with chloroform (6 ml x 3). The combined extracts were made up to 25 ml with further solvent and centrifuged to break up any emulsion which had formed in the chloroform layer. The absorbances of the yellow-orange chloroform solutions were measured at 420 nm after 25 min. Aqueous solutions (1 ml; 50–200 μg) of Cetavlon and CTAB were used to calibrate the procedure.

2.2. *Precipitation of Xanthan Gum with Quaternary Ammonium Salts*

Determination of optimum dilution of fermentation broth: To aliquots (50 g) of *X. campestris* fermentation broth, various volumes (100–300 ml) of water were added and a homogeneous solution was obtained by magnetic stirring. A 2% w/v CTAB solution (35 ml) was added, with further stirring, to each crude xanthan gum solution. The formation of a fibrous xanthan gum-CTA precipitate took place in all cases. The settling times observed for the precipitates were recorded.

Variation of polysaccharide precipitated with variation of quaternary ammonium salt added: To aliquots (50 g) of *X. campestris* fermentation broth, diluted with 250 ml of H_2O, various volumes (30, 35, 40 ml) of 2% w/v CTAB solution were added. After allowing 15 min for complex formation, the xanthan gum-CTA precipitates were recovered by centrifugation at 3000 rpm for 15 min. Samples of the supernatants obtained were subsequently examined for both xanthan gum and CTAB content. The experiment was repeated in a similar manner using Cetavlon.

2.3. *Recovery of the Polysaccharide and Quaternary Ammonium Salt from the Complex*

A centrifuged sample of xanthan gum-CTA precipitate, obtained from 50 g of *X. campestris* fermentation broth, was dispersed in 2-propanol (50 ml) by means of magnetic stirring. KCl (0·1 g) was added to the alcoholic solution of the dissolved precipitate with rapid stirring. Ion-exchange of K^+ for CTA^+ took place to precipitate xanthan gum (K^+ salt). When handling larger quantities of precipitate (from 300 g of fermentation broth), the ion-exchange step was performed with the use of a macerator.

The xanthan gum precipitate was centrifuged at 3000 rpm for 15 min and then allowed to dry in air. Polysaccharide samples requiring a higher degree of purity were given an additional wash in 2-propanol, dialysed and freeze-dried.

Recovery of the quaternary ammonium salt was achieved by rotary evaporation of the above supernatants (2-propanol/water mixture) to leave an aqueous solution of the precipitant.

3. RESULTS AND DISCUSSION

The first stage in the isolation of xanthan gum using quaternary ammonium salts is the formation of the polysaccharide-quaternary ammonium ion (QN^+) complex. The formation of an insoluble complex arises from electrostatic interactions of oppositely charged groups present in the xanthan gum and CTAB. As already mentioned, xanthan gum contains carboxy groups, while the cetyltrimethylammonium ion (CTA) contains a positive charge at the hydrophilic end of the molecule.

$$R\text{-}COO^-K^+(aq) + QN^+Br^-(aq) \rightarrow R\text{-}COO^-QN^+(s)\downarrow + K^+Br^-(aq)$$

The strain of *X. campestris* used in this research was identical with NRRL B-1459, the one on which the majority of the research on xanthan gum has been directed. However, the extremely high viscosity of the original fermentation broth can prevent satisfactory formation of the xanthan gum-CTA complex. Therefore, various dilutions of the fermentation broth were assessed to formulate a satisfactory method for complex formation. The time for the xanthan-CTA precipitate to settle is shown in Table 1. The attainment of a flocculent xanthan gum-CTA precipitate indicates that the precipitation reaction is complete. At dilutions less than a factor of four observation of this precipitate is difficult. The optimum dilution factor was found to be six (Table 1), since it produced a fibrous, flocculent xanthan gum-CTA precipitate in the shortest time.

Scott (1960) suggests that the end-point of the reaction between acidic polysaccharides and quaternary ammonium salts should result in a flocculent precipitate. However, those studies were carried out using small volumes and dilute concentrations of purified polysaccharides. The high concentrations of crude xanthan gum present in

TABLE 1
Effect of Dilution of *X. campestris* Fermentation Broth on Precipitation of Xanthan Gum with CTAB

H_2O (ml) added to fermentation broth (50 g)	*Time (h) for xanthan gum-CTA precipitate to settle*
100	∞
150	∞
200	3
250	1
300	1

the fermentation broths were probably responsible for the initial problems with the undiluted fermentation broth. Although the pH of the fermentation broth did not affect the amount of CTAB required to precipitate the xanthan gum quantitatively, the properties of the complexes formed did vary. The xanthan gum-CTA complexes formed at pH >9 were very fine and did not settle on standing. Between pH 2-9, a satisfactory xanthan gum-CTA complex could be formed. Below pH 2, the ionisation of the carboxy groups is increasingly suppressed until the quaternary ammonium complex becomes soluble. Scott (1960) has outlined several methods for achieving recovery of the polysaccharide from its insoluble quaternary ammonium complex. In this study the xanthan gum was precipitated as its K^+ salt from a dispersion of the polysaccharide-QN^+ complex in 2-propanol, enabling the quaternary ammonium salt to be recovered from the 2-propanol supernatant (supernatant 2).

The recovery of the quaternary ammonium salt for reuse was an integral part of the recycling scheme used in the isolation of xanthan gum with Cetavlon. After recovery of the xanthan gum (K^+ salt), the polysaccharide was dialysed and lyophilised. The analysis of a typical xanthan gum sample purified by this method is shown in Table 2 along with the comparative data for crude material, obtained by lyophilisation of the fermentation broth. The carbohydrate content was increased by 35% and the protein content, as determined by amino acid analysis, decreased by 83·3% during purification of the xanthan gum. It would appear that a slight discrepancy generally occurs between the experimental and theoretical elemental analysis of xanthan gum samples.

TABLE 2
Effect of Purification by CTAB Treatment on Xanthan Gum Composition

	Elemental analysis[a]			*Carbohydrate*[b] *content (%)*	*Amino acid content (g/%)*
	C(%)	*H(%)*	*N(%)*		
Crude xanthan gum	33·1	5·3	1·4	61	0·078 (100%)
Purified xanthan gum (K^+ form)	38·7	5·4	0·6	96	0·013 (16·7%)

[a] Theoretical analysis for xanthan gum (K^+ form) is C(42%), H(5·3%).
[b] Relative to Keltrol.

In comparison, Holzwarth (1976) has reported Keltrol (food-grade xanthan gum) to consist of 38% carbon, 5·5% hydrogen and 1·2% nitrogen.

The scheme shown in Fig. 1 was used to assess the suitability of Cetavlon for the isolation of xanthan gum in a recycle procedure. The experiment was carried out for a total of six cycles and the relevant results are shown in Table 3.

Initially, 17·12 mmol of Cetavlon was added to the 300 g of crude fermentation broth in the first cycle. If insufficient Cetavlon was recovered during the recycle procedure, 'fresh' precipitant was added to the xanthan during the formation of the insoluble complex in the next cycle. In fact, an additional 15·41 mmol of Cetavlon was required to complete precipitation of the polysaccharide in the subsequent five cycles. Taking into account the 4% non-quaternary ammonium salt impurities in Cetavlon and the material present in the first supernatant, the loss of Cetavlon per cycle can be calculated as 11·5%. The larger scale experiment used in the recycle of Cetavlon made observation of the precipitation end-point more difficult than normal. This may have been due to contamination build-up during the recycling procedure. A flocculent precipitate was not obtained in either cycle 3 or 6. However, no loss in the quantity of material isolated occurred during these cycles (Table 3).

The colour of supernatant 1 from the xanthan gum-Cetavlon precipitation accumulated a slight yellow colour from the fermentation broth during the experiment. Although 1500 ml of each supernatant 1 was recycled to dilute the *X. campestris* fermentation broth, about 60 ml was left as an excess. The reason for recycling supernatant 1 was to reuse any quaternary ammonium salt left from the xanthan gum-Cetavlon precipitation.

Residual Cetavlon in supernatant 1 was determined by a methyl orange assay procedure modified for such a purpose. Acid dyes such as methyl orange (MeO^-Na^+) can form 1:1 complexes with quaternary ammonium salts in aqueous solution. Further, a water immiscible solvent such as chloroform can extract the formed ion-pair (MeO^-QN^+) selectively. However, a small amount of uncomplexed methyl orange, dependent on pH, was extracted with the chloroform. Using a pH of 5 for the extraction, an acceptably low blank can be achieved for the assay method. The absorbance (420 nm) of the methyl orange-Cetavlon complex in chloroform was found to be completely stable between 15 and 30 min after the time of extraction. Outside this time limit, small variations in absorbance did occur. A suitable linear calibration curve and reproducible results were obtained using the above procedure.

Supernatant 1 from cycle 1 was found to contain 2·57 mmol (15%) of the Cetavlon added to the fermentation broth. Equally large amounts of precipitant were found in supernatant 1 from the subsequent cycles (Table 3). In cycle 2 no 'fresh' Cetavlon was added to the fermentation broth and a corresponding slight decrease of unused precipitant in supernatant 1 was observed. Supernatant 1 from cycle 6 was found to contain the largest amount of Cetavlon, despite the fact that no flocculent xanthan gum-Cetavlon precipitate was obtained. This suggested that the non-appearance of a flocculent precipitate in this cycle was not due to the presence of insufficient

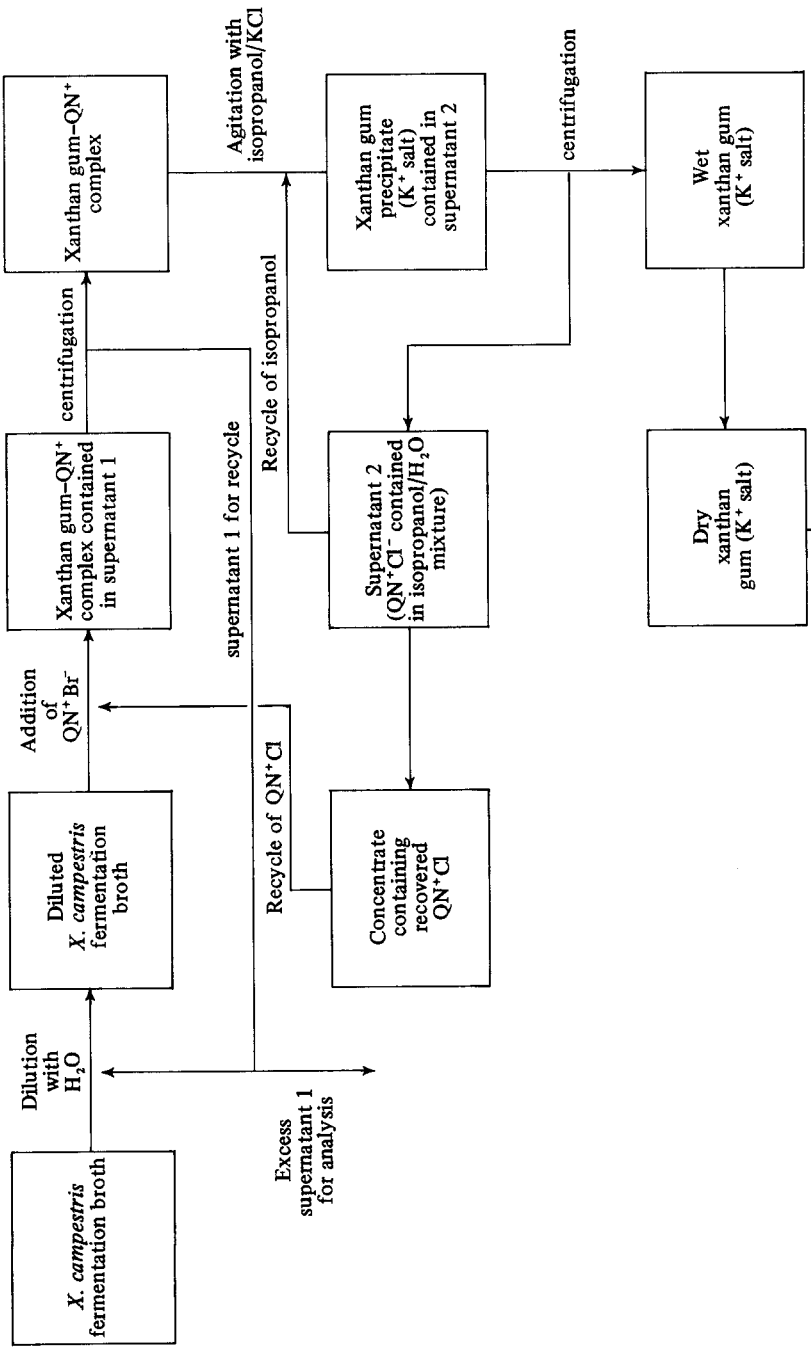

Fig. 1. Recycle procedure for isolation of xanthan gum with quaternary ammonium salts.

TABLE 3
Isolation of Xanthan Gum with Cetavlon

Cycle	*Cetavlon added (mmol)*	*Weight of material isolated (g)*	*Carbohydrate content of material isolated (%)*	*Xanthan gum isolated (mmol)*	*Cetavlon in supernatant 1 (mmol)*
1	17·12	7·63	82	6·53	2·57
2	0·00	7·68	80	6·41	2·26
3	2·85	7·75	79	6·39	2·00
4	2·82	7·61	82	6·51	2·88
5	0·00	7·47	81	6·31	2·48
6	4·28	7·90	79	6·51	3·56

Cetavlon. The determinations of unused Cetavlon suggested that a certain percentage (15%) of precipitant is continually recycled via supernatant 1 without ever complexing to the xanthan gum. Therefore, this would result in a 15% loss of Cetavlon per cycle if supernatant 1 was not recycled. However, if it were desirable to reduce a build-up of contamination resulting from the recycling of supernatant 1, the unused Cetavlon could be recovered with an ion-exchange column.

Various quantities of 2-propanol were assessed for the elution of quaternary ammonium salt from the xanthan gum–Cetavlon complex and the results are displayed in Table 4. In this table one volume refers to the equivalent volume of fermentation broth used. As can be seen, 0·42 volumes of 2-propanol was the lower limit to achieve successful elution of the Cetavlon. The concentrate containing the recovered precipitant, obtained after removal of the 2-propanol, became increasingly more brown coloured as the recycling progressed. This pigmentation, arising from the fermentation broth, did not stain the next batch of xanthan gum–Cetavlon precipitate to any large extent, although the polysaccharide isolated did tend to become slightly yellow coloured as the recycling procedure progressed.

The polysaccharide isolated in each of the six cycles was found to have a carbohydrate content of about 80% (Table 3). The corresponding number of moles of xanthan gum isolated is also shown in the same table. The impurities contained in the undialysed materials may have been quaternary ammonium salt or KCl. A purity of 90% can be achieved by treating the xanthan gum to an additional wash in 2-propanol. This also reduced the coloration of the final product.

TABLE 4
Effect of Isopropanol Level on the Elution of Quaternary Ammonium Salt from the Xanthan Gum–Cetavlon Complex

Volume of isopropanol	*Comments*
0·33	Separation of xanthan gum and Cetavlon could not be achieved
0·42	Limit for successful separation of xanthan gum and Cetavlon
0·50 0·66 1·00	Satisfactory separation of xanthan gum and Cetavlon

The large amount (15%) of Cetavlon which was found to be contained in supernatant 1, would be a prohibitive factor for this precipitant in a large scale isolation of xanthan gum. However, if this level of unused material in supernatant 1 could be reduced, the use of Cetavlon might be more favourable. The addition of less than the requirement of Cetavlon to precipitate the xanthan gum quantitatively was considered to be a possible method of reducing the unused precipitant in supernatant 1.

Various quantities (1·712, 1·998 and 2·283 mmol) of Cetavlon were reacted with 0·917 mmol of crude xanthan gum contained in 50 g of the *X. campestris* fermentation broth; 2·283 mmol of Cetavlon was observed to give quantitative precipitation (a flocculent precipitate) of the polysaccharide. The addition of 1·712 mmol and 1·998 mmol of Cetavlon did not produce quantitative precipitation of the xanthan gum. The quantity of Cetavlon in each supernatant was again assessed using the methyl orange dye method (Table 5). The results are expressed as moles of Cetavlon found in the supernatant and as a percentage of the precipitant added to the xanthan gum.

TABLE 5
Dependence of the Level of Xanthan Gum and Cetavlon in the Supernatant on the Amount of Cetavlon added to the Diluted Fermentation Broth

Cetavlon added (mmol)	*Cetavlon in the supernatant (mmol)/(%)*	*Xanthan gum in the supernatant (mmol)/(%)*
0·000	0·000 (0%)	0·917 (100%)
1·712	0·455 (26%)	0·173 (18%)
1·998	0·314 (16%)	0·042 (4%)
2·283	0·386 (17%)	0·000 (0%)

To allow the quantity of xanthan gum in the supernatants to be calculated, it was necessary to first of all determine the carbohydrate material present. The supernatant obtained from the precipitation with 2·283 mmol of Cetavlon was assumed to contain no xanthan gum. The carbohydrate content of this supernatant was taken as the blank and subtracted from the carbohydrate content of the other supernatants. The xanthan gum in each supernatant was then calculated by evaluating the following ratio: the corrected supernatant carbohydrate content times the number of moles of xanthan gum contained in 50 g of *X. campestris* fermentation broth over the corrected carbohydrate content of 50 g of the *X. campestris* fermentation broth. The xanthan gum in each supernatant is also expressed as a percentage of the polysaccharide originally present.

As expected, the xanthan gum present in the supernatant increased with a decrease in the amount of Cetavlon added to the *X. campestris* fermentation broth. Surprisingly, when 1·712 mmol of Cetavlon was reacted with 0·917 mmol of xanthan gum, 26·5% (0·455 mmol) of the precipitant was left in the supernatant. This was despite the fact that 0·173 mmol (18%) of the xanthan gum was also present in this

same supernatant. The results indicated clearly that it would not be possible to reduce the quantity of Cetavlon in the supernatant from the precipitation with xanthan gum, by adding less than 100% requirement of precipitant.

The large amount of Cetavlon left in the supernatant at the end-point suggested that this material was not efficient as a complexation agent for xanthan gum. According to Scott (1961) the efficiency of precipitation of a particular polysaccharide rises with an increase in the paraffin chain length of the quaternary ammonium salt. This efficiency is reflected in the quantity of quaternary ammonium salt which is required to produce a flocculent precipitate with the polysaccharide and also in the quantity of quaternary ammonium salt which is left in the supernatant.

It follows, therefore, that Cetavlon which consists of mainly the C_{14} homologue along with smaller quantities of C_{12} and C_{16}, would be less efficient in precipitating xanthan gum than CTAB which contains only the C_{16} homologue. Indeed this lower efficiency is reflected in the larger quantity of Cetavlon than CTAB left in the supernatant from the precipitation of xanthan gum (Tables 5 and 6).

TABLE 6
Dependence of the Level of Xanthan Gum and CTAB in the Supernatant on the Amount of CTAB Added to the Diluted Fermentation Broth

CTAB added (mmol)	*CTAB in the supernatant (mmol)/(%)*	*Xanthan gum in the supernatant (mmol)/(%)*
0·000	0·000 (0%)	0·917 (100%)
1·556	0·339 (21%)	0·332 (36%)
1·837	0·029 (1·7%)	0·000 (0%)
2·088	0·029 (1·4%)	0·000 (0%)

Alternatively, the presence of a mixture of homologues in Cetavlon may also be responsible for this reduced efficiency. Consider the possible mechanism of the precipitation of xanthan gum with Cetavlon. At the start of the reaction, the C_{12}, C_{14} and C_{16} homologues of Cetavlon may bind indiscriminately to the anionic sites in the xanthan gum. However, as the reaction proceeds, the C_{12} homologue may be replaced by a C_{14} or C_{16} homologue due to the greater affinity which the latter pair have for the anionic sites. Finally, at such time when a flocculent precipitate is obtained all the C_{12} homologues may have been replaced from their initial binding to xanthan gum and are therefore present in solution. The exact composition of Cetavlon could not be obtained from the manufacturers (Imperial Chemical Industries) but it would be interesting to know the percentage of the C_{12} homologue present in the precipitant, since this might give some indication of how much of the Cetavlon left in the supernatant from the xanthan gum precipitation was due to the C_{12} homologue.

When the addition of varying quantities of quaternary ammonium salt to a constant concentration of xanthan gum was repeated with CTAB instead of Cetavlon, only a

small amount (1·7%) of precipitant was left unused in the supernatant at the end-point of the precipitation with the polysaccharide. This indicated that CTAB was more efficient than Cetavlon for the complexation of xanthan gum. However, the results in Table 6 show some similarity with those obtained using Cetavlon (Table 5). When 1·566 mmol of CTAB was reacted with 0·917 mmol of xanthan gum, 0·339 mmol (21·2%) of precipitant were left in the supernatant. Again, this was despite the fact that 0·332 mmol (36·2%) of the xanthan gum was also present in this same supernatant.

The discovery that xanthan gum and quaternary ammonium salt could be present in the same solution without forming an insoluble complex was surprising. Although a great deal of work on the precipitation of acidic polysaccharides with quaternary ammonium salts has been carried out by Scott, no investigation of the mechanism has been reported. A detailed study of the reaction of xanthan gum with [^{14}C]-CTAB has shown an interesting mechanism exists (Kennedy *et al.*, 1981a). Another surprising aspect of the results was that when 2·088 mmol of CTAB were reacted with 0·917 mmol of xanthan gum, only 0·029 mmol of the precipitant was found in the supernatant. Since 1·837 mmol of CTAB was just sufficient to cause quantitative precipitation of the xanthan gum, 0·251 mmol of this 2·088 mmol of CTAB was expected to be in the supernatant, this suggested that incorporation of the CTA ion into the xanthan gum–CTA complex took place after the end-point of precipitation.

The contrast between Cetavlon and CTAB for the complexation of xanthan gum is summarised in Table 7. Since the xanthan gum sample used was found to contain 1·381 moles of carboxy group/mole of xanthan gum repeating unit, this suggested that non-stoichiometric combination was occurring between the anionic groups of the polysaccharide with the quaternary ammonium salt. This deviation may have been due to the impurities such as protein contained in the fermentation broth.

TABLE 7
Comparison of Cetavlon with CTAB for the Complexation of Xanthan Gum

	Cetavlon	*CTAB*
Molar ratio of precipitant/xanthan gum required to achieve quantitative recovery of the polysaccharide	2·24	2·00
Percentage of precipitant left in the supernatant at the end-point	15·0	1·7

While Cetavlon could be used to obtain industrial grade xanthan gum, large losses of precipitant would occur if the supernatant 1 were not recycled. Therefore, in the isolation of xanthan gum it would be preferable to use a quaternary ammonium salt with an aliphatic chain of C_{16} or above such as CTAB. Presumably a mixture of quaternary ammonium salts with aliphatic chains at least C_{16} length would also be suitable.

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