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REACTION OF $PHMBH^+Cl^-$ WITH ACIDIC POLYSACCHARIDES, AND ITS APPLICATION TO THE PURIFICATION OF XANTHAN GUM

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ABSTRACT

Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl hydrochloride) [$PHMBH^+Cl^-$] reacts with acidic polysaccharides to form white, insoluble salts. The $PHMBH^+$ salts of sulphated polysaccharides can only be dissociated at or below pH 0·2. The salts of polysaccharides containing only carboxylate groups as their acidic functions are dissociated at or below pH 1·6, and by strong electrolytes above a critical electrolyte concentration.

The acidic polysaccharide xanthan may be recovered from a dispersion of its $PHMBH^+$ salt in aqueous potassium chloride by treatment with 2-propanol. This forms the basis of a method for the recovery of xanthan, in purified form, from Xanthomonas campestris *fermentation broths. The reaction of $PHMBH^+Cl^-$ with nucleic acids and proteins is also discussed.*

1. INTRODUCTION

Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl hydrochloride) (abbreviated to $PHMBH^+Cl^-$, a mnemonic for the trivial name poly(hexamethylene-biguanide hydrochloride) is a low molecular wieght polymer prepared by the condensation of 1,6-hexanediamine with a metallic salt of cyanocyanamide. The main structural repeat unit is shown in structure 1. The average molecular weight of the polymer is 1200, the value of n being 4–6. Compounds of this type are moderately strong bases and form well-defined salts.

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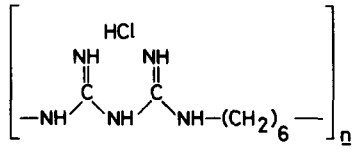

Structure 1.

The reactions of a number of cationic precipitants with acidic polysaccharides have been studied. Deuel *et al.* (1955) studied the interaction of polyethyleneimine with poly(D-galacturonic acid) in aqueous solution and determined the optimum conditions for the precipitation reaction. Jones (1953) found that the quaternary ammonium salt 1-hexadecanamium-N,N,N-trimethyl bromide, used for the isolation of bacterial nucleic acids, also precipitated acidic polysaccharides; the precipitates obtained from both nucleic acids and polysaccharides were soluble in molar sodium chloride solution. Scott (1955), in subsequent studies, found that proteins were precipitated from solution on addition of 1-hexadecanamium-N,N,N-trimethyl bromide but redissolved when the pH was adjusted to below their isoelectric points (pI). He also demonstrated that the 1-hexadecanamium-N,N,N-trimethyl bromide precipitates of nonsulphated anionic polysaccharides were soluble in a 30% aqueous sodium sulphate solution or at pH values below 1·8, whereas sulphated polysaccharides remained insoluble.

Albrecht *et al.* (1962, 1963) precipitated the acidic polysaccharide xanthan, which is of industrial importance, by treatment with long-chain quaternary ammonium chlorides, and this method was subsequently applied to the recovery of xanthan from fermentation broths. Xanthan has also been recovered, in purified form, from its fermentation broths by precipitation methods utilising 1-hexadecanamium-N,N,N-trimethyl bromide (Kennedy *et al.*, 1981) and polyethyoxylated quaternary ammonium salts (Gill & Lim, 1969). No work has previously been done on the interaction of $PHMBH^+Cl^-$ with acidic polysaccharides, a reagent which we predicted would interact/complex with ionic polysaccharides.

We now report a study of the interaction of $PHMBH^+Cl^-$ with biopolyanions (acidic polysaccharides, nucleic acids and proteins), and of the potential of the reagent in the purification of xanthan gum.

2. EXPERIMENTAL AND RESULTS

2.1. *General Methods*

IR spectra (KBr disc) were obtained with a Perkin-Elmer model 180 spectrometer. UV/visible spectra were recorded with a Pye-Unicam SP800 spectrometer and fixed wavelength measurements were made using a Pye-Unicam SP500 manual spectrometer. $PHMBH^+Cl^-$ was routinely estimated by direct absorbance measurement at 235 nm; the UV spectrum of $PHMBH^+Cl^-$ is shown in Fig. 1. Protein and nucleic acid were

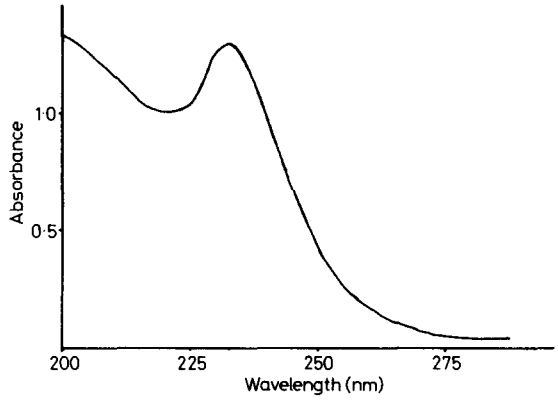

Fig. 1. Absorption spectrum of aqueous PHMBH$^+$Cl$^-$.

determined by their UV absorptions at 278 and 260 nm respectively. The xanthan samples used were either Keltrol (obtained from A.B.M. Chemicals Ltd, Stockport, Cheshire) or prepared in house from cultures of *X. campestris*.

Carbohydrate determinations were made, as follows, using the resorcinol-sulphuric acid method which is a modification of the resorcinol-4,6-disulphonic acid method of Hunt & Sutcliffe (1953). To the sample (1·0 ml), aqueous resorcinol (4% w/v, 1·0 ml) and then sulphuric acid (98%, 6·0 ml) were added. The sample was cooled at 0°C, left to stand at room temperature for 15 min and the absorbance measured at 494 nm. Keltrol (food grade xanthan gum) was employed as the carbohydrate standard.

2.2. *Precipitation of Polysaccharides*

Homogeneous solutions of a range of polysaccharides were made up by dissolution of the required quantity of polysaccharide in distilled water (25 ml). Each solution was stirred magnetically and aqueous PHMBH$^+$Cl$^-$ (20% w/v, 1·5 ml) was added. The results are shown in Table 1.

2.3. *Precipitation of Nucleic Acids*

Aliquots of aqueous PHMBH$^+$Cl$^-$ (2% w/v, 0·2 ml) were added to solutions of yeast RNA (0·058% w/v, 10 ml) which had previously been adjusted to the required pH values. The solutions were agitated and the precipitates which formed were removed by centrifugation. The quantity of RNA remaining in the supernatant was determined spectrophotometrically. The experiment was repeated using distilled water in place of the aqueous PHMBH$^+$Cl$^-$. The results are presented in Fig. 2. The entire procedure was repeated using salmon sperm DNA at a concentration of 0·083%; the results are summarised in Fig. 3.

TABLE 1
The Effect of Aqueous $PHMBH^+Cl^-$ on a Range of Polysaccharide Solutions

Polysaccharide	*Concentration (% w/v)*	*Observation of precipitation*	*Comments*
Agar (Hypnea weed gum)	3·0	+	White, fibrous precipitate formed from solution at 90°C
Chondroitin 4/6-sulphate	2·0	+	White, partly conglomerated, amorphous precipitate
Dextran	2·0	–	–
Gum tragacanth	2·0	+	White, gelatinous precipitate
Heparin	2·0	+	White, partly conglomerated, amorphous precipitate
ι-Carrageenan	2·0	+	White, gelatinous precipitate
κ-Carrageenan	2·0	+	White, gelatinous precipitate
λ-Carrageenan	2·0	+	White, gelatinous precipitate
Locust bean gum	1·0	–	–
Starch	2·0	–	–
Uncut pectin	1·5	+	White, gelatinous precipitate
Xanthan (Keltrol)	2·0	+	White, gelatinous precipitate
Sodium alginate	1·0	+	White, gelatinous precipitate

–, No dissolution; +, degree of dissolution.

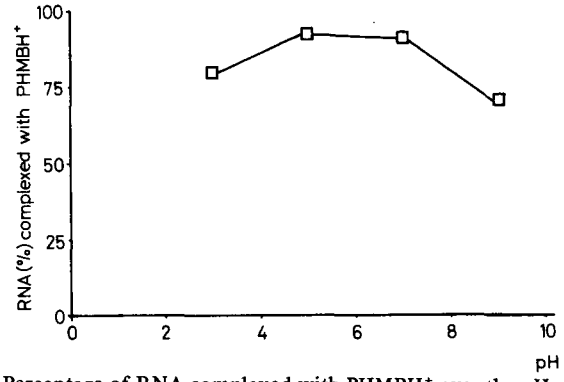

Fig. 2. Percentage of RNA complexed with $PHMBH^+$ over the pH range 3–9.

2.4. *Precipitation of Albumins*

Aliquots of aqueous $PHMBH^+Cl^-$ (2% w/v, 0·1 ml) were added to solutions of human (0·1% w/v, 10 ml) and bovine (0·1% w/v, 10 ml) serum albumin which had previously been adjusted to the required pH values. The procedure used was identical to that used for yeast RNA. The results for bovine and human albumin are summarised in Figs 4 and 5 respectively.

2.5. *Solubility of $PHMBH^+$-polysaccharide Salts*

Aliquots of xanthan (0·2 g) were dissolved in water (10 ml) and aqueous $PHMBH^+Cl^-$ (20% w/v, 0·6 ml) was added to the solutions. The precipitates of

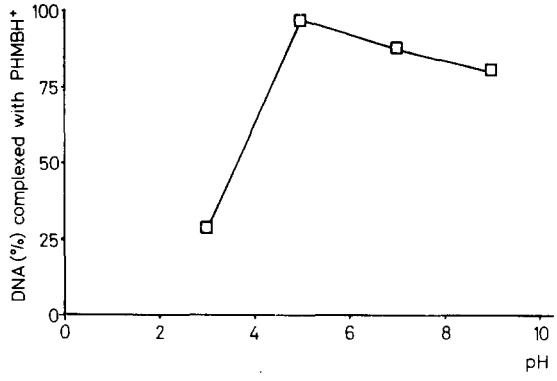

Fig. 3. Percentage of DNA complexed with PHMBH$^+$ over the pH range 3–9.

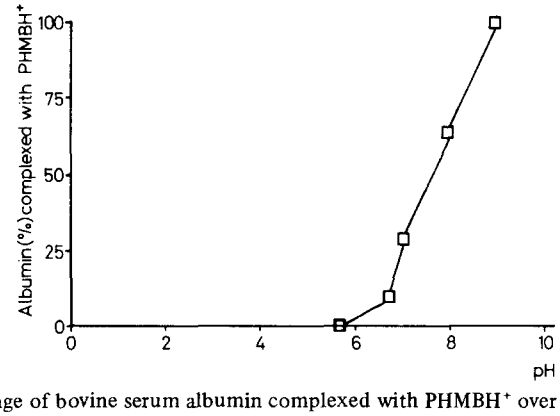

Fig. 4. Percentage of bovine serum albumin complexed with PHMBH$^+$ over the pH range 0–10.

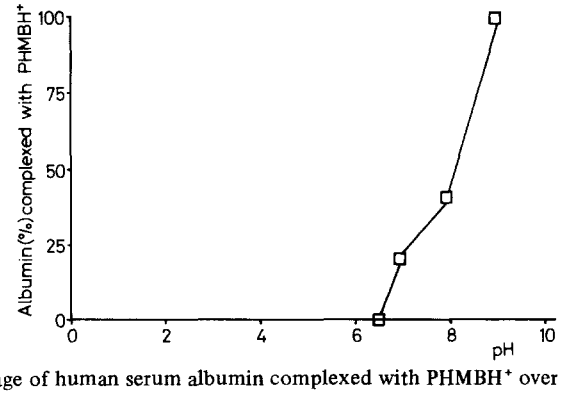

Fig. 5. Percentage of human serum albumin complexed with PHMBH$^+$ over the pH range 0–10.

$PHMBH^+$-xanthan were recovered by centrifugation, washed with water and dispersed in aliquots (10 ml) of water which had been adjusted with hydrochloric acid to produce a range of pH values (Table 2). Observations of degrees of dissolution were recorded.

The experiment was repeated using chondroitin 4/6-sulphate; the relevant results are presented in Table 3.

The solubilities of the $PHMBH^+$ salts of xanthan and chondroitin 4/6-sulphate in solutions of potassium chloride, sodium chloride, guanidine hydrochloride and urea were assessed. The relevant data and results obtained for the xanthan salt are given in Table 4. The $PHMBH^+$ salt of chondroitin 4/6-sulphate was found to be insoluble in all media studied.

TABLE 2
Variation in Solubility of $PHMBH^+$–Xanthan Complex in the pH Range 0·6–3·0

pH of fraction	*Observation of dissolution*	*Comments*
3·0	–	–
2·8	–	–
2·6	–	–
2·4	–	–
2·2	±	Upper limit of dissolution
2·0	+	Some precipitate dissolved
1·8	++	Most of precipitate dissolved
1·6	+++	Viscous liquid formed
1·4	+++	Viscous liquid formed
1·2	+++	Viscous liquid formed
1·0	+++	Viscous liquid formed
0·8	+++	Viscous liquid formed
0·6	+++	Viscous liquid formed

–, No dissolution; +, degree of dissolution.

TABLE 3
Variation in the Solubility of the $PHMBH^+$–Chondroitin 4/6- Sulphate Complex with pH

pH of fraction	*Observation of dissolution*	*Comments*
1·0	–	–
0·9	–	–
0·8	–	–
0·7	–	–
0·6	–	–
0·5	±	Upper limit of dissolution
0·4	+	Some precipitate dissolved
0·3	++	Most of precipitate dissolved
0·2	+++	Total dissolution of precipitate occurred
0·1	+++	Total dissolution of precipitate occurred

–, No dissolution; +, degree of dissolution.

TABLE 4
Solubility of the PHMBH$^+$-Xanthan Complex in Aqueous Media

Medium	*Observation of dissolution*	*Comments*
Distilled water	–	–
4 M Sodium chloride	+++	Forms viscous, opaque fluid
4 M Urea	+++	Forms clear, viscous solution
2 M Guanidine hydrochloride	+++	Forms clear, viscous solution
0·5 M Potassium chloride	–	–
0·7 M Potassium chloride	±	–
0·8 M Potassium chloride	+++	Forms viscous, opaque fluid
1·0 M Potassium chloride	+++	Forms viscous, opaque fluid
4 M Potassium chloride	+++	Forms viscous, opaque fluid

–, No dissolution; +, degree of dissolution.

2.6. *Determination of the Solubilities of PHMBH$^+$-albumin and PHMBH$^+$-nucleic Acid Complexes*

Aliquots of aqueous PHMBH$^+$Cl$^-$ (2%, 0·2 ml) were added to solutions of yeast RNA (0·058% w/v, pH 5·0, 10 ml) and salmon sperm DNA (0·083% w/v, pH 5·0, 10 ml). The precipitates obtained were recovered by centrifugation, washed with water then dispersed in aliquots of aqueous potassium chloride (10 ml) of various concentrations. The degree of dissolution was determined spectrophotometrically.

The experiment was repeated using solutions of human and bovine serum albumins (0·1% w/v, 10 ml). In all cases no evidence of dissolution was found.

2.7. *Recovery of Chondroitin 4/6-sulphate from its PHMBH$^+$ Salt*

Aqueous PHMBH$^+$Cl$^-$ (20% w/v, 3·0 ml) was added to a homogeneous solution of chondroitin 4/6-sulphate (1·0 g) in water (40 ml). The precipitate (of the complex) formed was washed with water and dispersed in hydrochloric acid (2 M, 50 ml). To the resulting solution, 2-propanol was added until precipitation occurred. The precipitate (of the complex) was recovered by centrifugation and dispersed in water (50 ml); no dissolution occurred. The precipitate was reisolated, dispersed and subsequently dissolved in hydrochloric acid (2 M, 50 ml).

2.8. *Recovery of Xanthan from its PHMBH$^+$ Salt*

Aqueous PHMBH$^+$Cl$^-$ (20% w/v, 3·0 ml) was added to a homogeneous dispersion of xanthan (1·0 g) in water (50 ml). The precipitate was recovered by centrifugation, washed with water then dispersed in hydrochloric acid (0·5 M, 50 ml). The addition of 2-propanol to the dispersion did not result in the separation of a xanthan precipitate.

The precipitation was repeated and the PHMBH$^+$-xanthan salt was dispersed in aqueous potassium chloride (1 M, 50 ml). The dispersion was stirred magnetically and 2-propanol was added until precipitation of the polysaccharide occurred. The volume of 2-propanol added was 100 ml. The isolated precipitate was dispersed in distilled water (50 ml); a viscous opalescent solution resulted. The above procedure was

repeated using 4 M potassium chloride; only 30 ml of 2-propanol was required to effect precipitation of the polysaccharide. The precipitate was recovered by centrifugation and dispersed in distilled water (50 ml). A viscous opalescent solution was formed.

2.9. *Optimisation of the Purification of Xanthan*

Aqueous $PHMBH^+Cl^-$ (20% w/v, 3·0 ml) was added to *X. campestris* fermentation broth I (50 ml) and the mixture was stirred magnetically. The resulting precipitate was recovered by centrifugation and the supernatant (A) was retained for analysis. The precipitate was dispersed in aqueous potassium chloride (4 M, 50 ml). The dispersion was treated with 2-propanol (30 ml). The precipitate was isolated by centrifugation and the supernatant (B) was retained for analysis. The precipitate was dissolved in distilled water; this solution and the two supernatants were analysed for carbohydrate and $PHMBH^+Cl^-$. The entire procedure was repeated using increasing volumes of 2-propanol. The relevant results are presented in Tables 5 and 6.

TABLE 5
Distribution of $PHMBH^+Cl^-$: Variation with Volume of 2-Propanol used

Volume of 2-propanol added (ml)	*Quantity of $PHMBH^+Cl^-$ (g)*		
	Supernatant A	*Supernatant B*	*Final solution of xanthan*
10	0·17	*a*	*a*
20	0·18	*a*	*a*
30	0·17	0·35	0·06
40	0·17	0·33	0·08
50	0·16	0·32	0·09
60	0·17	0·30	0·12

[a] Formation of a precipitate on 2-propanol addition seldom occurs.

TABLE 6
Distribution of Carbohydrate: Variation with Volume of 2-Propanol used

Volume of 2-propanol added (ml)	*Quantity of carbohydrate (g)*		
	Supernatant A	*Supernatant B*	*Final solution of xanthan*
10	0·13	*a*	*a*
20	0·13	*a*	*a*
30	0·11	0·07	0·83
40	0·14	0·08	0·82
50	0·12	0·07	0·83
60	0·10	0·07	0·88

[a] Formation of a precipitate on 2-propanol addition seldom occurs.

Aqueous $PHMBH^+Cl^-$ (20% w/v, 2·2 ml) was added to *X. campestris* fermentation broth II (50 ml) and the mixture was stirred magnetically. The isolated precipitate was dispersed in aqueous potassium chloride (4 M, 50 ml). 2-Propanol (30 ml) was

added to the dispersion and the precipitate formed was recovered by centrifugation and dispersed in water (50 ml). This final solution and the two supernatants were analysed for PHMBH$^+$Cl$^-$ and carbohydrate, and were also microscopically examined for *X. campestris* cells (Table 7).

TABLE 7
Distribution of Carbohydrate, PHMBH$^+$Cl$^-$ and *Xanthomonas campestris* Cells on Recovery of Xanthan from Fermentation Broth

Fraction	*Quantity of carbohydrate present (g)* [a]	*Quantity of PHMBH$^+$Cl$^-$ present (g)*	*Cell count* [b]	*Situation of cells*
Supernatant A	0·08	0·05	30	–
Supernatant B	0·00	0·31	0	–
Final solution of xanthan	0·84	0·84	40	clumps

[a] By resorcinol assay; quoted in xanthan equivalent weights.
[b] Relative to the fermentation broth to which a value of 100 was assigned; the *X. campestris* cells in the fermentation broth were in a mobile situation.

The purification procedure which had been developed (Fig. 6) was applied to solutions of known xanthan content. The dialysed solutions of xanthan were lyophilised. The products were examined by IR and UV spectroscopy and their carbohydrate contents (Table 8) were determined.

3. DISCUSSION

The *X. campestris* fermentation broths produced commercially, containing 3–4% xanthan, are extremely viscous and difficult to handle. The most popular large-scale method for the isolation of xanthan is repeated precipitation with 2-propanol. However, the process requires large volumes of solvent and considerable losses occur on recycling. Further, the precipitation process is inherently nonspecific and insoluble impurities will be contained in the gelatinous xanthan material. In comparison, the PHMBH$^+$Cl$^-$ method uses only a small amount of 2-propanol and is specific for polymeric species containing acidic groups. Further, in the PHMBH$^+$Cl$^-$ method *X. campestris* cells are left behind in the supernatant from the precipitation reaction. The basis of the PHMBH$^+$Cl$^-$ method has some similarities to the isolation of acidic polysaccharides with quaternary ammonium salts (Scott, 1960).

PHMBH$^+$Cl$^-$ reacts with acidic polysaccharides to produce insoluble salts of the polycation-polyanion type. Monomeric carbohydrates (D-glucose, D-mannose, D-glucuronic acid, D-galacturonic acid) and neutral polysaccharides (dextran, starch, locust bean gum) are not precipitated (Table 1). We therefore conclude that precipitation occurs due to ionic crosslinking of the acidic polysaccharides by PHMBH$^+$Cl$^-$ to form an extended, insoluble system. There is a clear difference in the

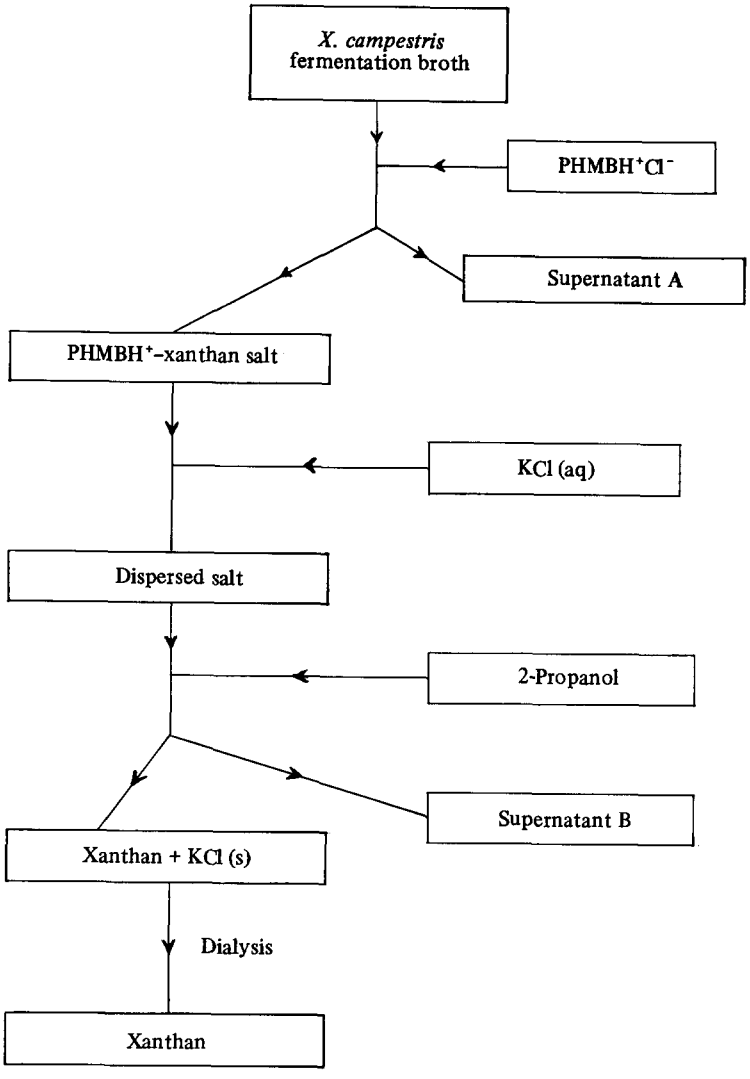

Fig. 6. Schematic representation of the purification procedure for xanthan gum.

form of the precipitates obtained from sulphated and nonsulphated polysaccharides (Table 1); the latter produce gelatinous precipitates under the conditions used, the former yield amorphous precipitates. Because of practical aspects, the precipitate formed from a solution of agar (a sulphated polysaccharide) cannot be fitted directly into this broad classification. Since agar solutions gel at room temperature, the reaction of agar with aqueous $PHMBH^+Cl^-$ had to be performed at an elevated

TABLE 8
Performance of the Purification Procedure

Sample code	*Xanthan (carbohydrate) content of product by resorcinol assay (% w/w)*	*Percentage recovery of xanthan (by weight)*
X	87	97
Y	89	96
Z	95	99

temperature (90°C). A white fibrous precipitate was formed when aqueous PHMBH$^+$Cl$^-$ was added to the agar solution at this temperature. On ceasing stirring after cooling, the precipitate settled and no sign of gelation was detectable.

Quantitative precipitation of nucleic acid did not occur on addition of aqueous PHMBH$^+$Cl$^-$ and both RNA and DNA showed a maximum at *c.* pH 5·0 (Figs 2 and 3), corresponding to the maximum precipitation by PHMBH$^+$Cl$^-$. Albumin was precipitated quantitatively by aqueous PHMBH$^+$Cl$^-$ at pH values $>9{\cdot}0$ (Figs 4 and 5). Below this value the quantity of albumin precipitated fell sharply. No precipitation occurred at pH values $<5{\cdot}0$. The pI of albumin is 4·9. At pH values below this value no precipitation would be expected since the molecule assumed a net positive charge. At pH values above the pI the albumin becomes increasingly negatively charged as pH increases until the maximum negative charge is developed on the protein. It is concluded that PHMBH$^+$Cl$^-$ precipitates proteins at pH values above their pI values. Precipitation is quantitative only when the protein develops its maximum negative charge; thus the pI value of the protein is lower than the pH at which quantitative precipitation occurs.

The salts formed by reaction of acidic polysaccharides with PHMBH$^+$Cl$^-$ are soluble at low pH values (Tables 2 and 3). Xanthan and chondroitin 4/6-sulphate were chosen as representative members of nonsulphated and sulphated anionic polysaccharides, respectively. It is assumed that other members of the same groups will behave similarly. Polysaccharides containing both carboxylate and sulphate groups (i.e. heparin) will behave as sulphated polysaccharides in their reaction with PHMBH$^+$Cl$^-$ and in the subsequent reactions of their PHMBH$^+$ salts. The PHMBH$^+$ salt of xanthan is soluble at pH values $<1{\cdot}6$ (Table 2) whereas the PHMBH$^+$ salt of chondroitin 4/6-sulphate is only soluble at pH values $<0{\cdot}2$ (Table 3). The sulphate group has a lower pK_a than the carboxylate group and there is a much stronger interaction between sulphated polysaccharides and PHMBH$^+$ than between carboxylated polysaccharides and PHMBH$^+$. This stronger interaction is demonstrated by the insolubility of PHMBH$^+$-(chondroitin 4/6-sulphate) in saturated solutions of alkali metal chlorides; the PHMBH$^+$-xanthan complex is soluble in sodium and potassium chloride solutions at salt concentrations greater than 0·8 M. The solubility of the PHMBH$^+$-xanthan complex in various media was assessed (Table 4). It was found that no dissociation of the PHMBH$^+$-(chondroitin 4/6-sulphate) complex occurred in any of these media. The properties of PHMBH$^+$ salts of sulphated and nonsulphated polysaccharides are conveniently summarised in Table 9.

TABLE 9
Summary of the Characteristics of $PHMBH^+$–Anionic Polysaccharide Complexes

Polysaccharide precipitated	*Type of precipitate*	*Effect of pH*	*Effect of degree of saturation with potassium chloride*
Polysaccharide containing only carboxyl groups	White, gelatinous	Soluble at or below pH 1·6	Soluble above 0·8 M
Polysaccharides containing sulphate groups only, or in addition to carboxyl groups	White, conglomerates	Soluble at or below pH 0·2	Insoluble in a saturated solution

It was discovered that the $PHMBH^+$ salts of nucleic acids and proteins were insoluble in aqueous potassium chloride over the entire concentration range. The insolubility of the $PHMBH^+$-nucleic acid complexes can be explained by the strong interaction between $PHMBH^+$ and the nucleic acids' phosphate groups. The interaction is stronger between $PHMBH^+$ and $=PO_4^-$ than between $PHMBH^+$ and $-CO_2^-$ and the former cannot be dissociated by salt effects.

Albumin would be expected to contain only carboxylate groups as its acidic functions and thus its $PHMBH^+$ salt should dissociate at potassium chloride concentrations $>0{\cdot}8$ M. The observation that no dissolution occurred may suggest that denaturation of the protein had taken place or that the albumins contained phosphate impurities. However, a more subtle reason may be responsible for the strength of attraction observed between $PHMBH^+$ and albumin. Since the $PHMBH^+$ contains charged groups arranged in a regular manner, the spatial arrangement of the charged groups of the biopolyanion may be a critical factor in determining the strength of attraction. Therefore, a biopolyanion which gives a good 'fit' with $PHMBH^+$ may have a stronger affinity for the precipitant than one which does not.

It has been shown that solution of the $PHMBH^+$ salt of chondroitin 4/6-sulphate can reasonably be achieved by treatment with hydrochloric acid but the polysaccharide cannot then be recovered by alcohol precipitation. Purification of sulphated polysaccharides via their $PHMBH^+$ salts is extremely difficult due to the very strong interaction between $PHMBH^+$ and the polysaccharide. $PHMBH^+Cl^-$ cannot be removed by cation exchange chromatography since the (sulphated polysaccharide)-$PHMBH^+$ complex is only dissociated at pH values which render the ion-exchange resin inoperative. *The xanthan salt of $PHMBH^+$ will dissolve in dilute acid but attempts to precipitate the xanthan with 2-propanol were ineffective.*

Xanthan was recovered from a dispersion of its $PHMBH^+$ salt in aqueous potassium chloride. It was discovered that the volume of 2-propanol required to precipitate the xanthan decreased with increasing potassium chloride concentration. Some potassium chloride was coprecipitated with the xanthan. This method of recovery was applied to crude *Xanthomonas campestris* fermentation broth in an attempt to recover xanthan in purified form. A cream-coloured gelatinous precipitate was obtained on addition of aqueous $PHMBH^+Cl^-$ to the tan-coloured fermentation broth I, in contrast

to the white precipitates previously obtained using solutions of pure xanthan. The xanthan solution obtained at the end of the purification procedure (Fig. 6) was viscous and opalescent, characteristic of a solution of pure xanthan.

In order to optimise the purification procedure, the entire purification sequence was performed on aliquots of fermentation broth I using a different volume of 2-propanol on each occasion. The results are shown in Tables 5 and 6. It can be seen from Table 5 that excess PHMBH$^+$Cl$^-$ was added since all supernatants A show the presence of the same quantity of PHMBH$^+$Cl$^-$. The large values obtained for PHMBH$^+$Cl$^-$ in supernatants B indicate that very little PHMBH$^+$Cl$^-$ is coprecipitated with the xanthan on addition of 2-propanol to the PHMBH$^+$-xanthan dispersion in aqueous potassium chloride. The quantity of coprecipitated PHMBH$^+$Cl$^-$ increases with the use of increasing volumes of 2-propanol; a greater quantity of PHMBH$^+$Cl$^-$ is found in the final solution of xanthan (Table 5). A greater quantity of potassium chloride is also coprecipitated when large volumes of 2-propanol are used. On this basis, the smallest volume of 2-propanol which produced reproducible precipitation of the xanthan was selected as the optimum volume to use. This volume was 30 ml.

The use of an increased volume of 2-propanol did not, within the limits of experimental error, precipitate a greater quantity of xanthan (Table 6). Xanthan is quantitatively precipitated by aqueous PHMBH$^+$Cl$^-$. The carbohydrate present in supernatant A (Table 6) is unfermented glucose. The carbohydrate in supernatant B was ascribed to acidic, lower molecular weight degradation products. The *X. campestris* fermentation broth used (broth I) had been stored for several months at – 20°C prior to use and degradation had taken place during this period. When freshly fermented broth (broth II) was purified, supernatant B contained no carbohydrate (Table 7).

The use of a smaller quantity of PHMBH$^+$Cl$^-$ resulted in quantitative precipitation of xanthan from *X. campestris* fermentation broth II. Excess was still present (Table 7) since the quantity required was calculated on the assumption that some degradation products would be present in fermentation broth II.

Approximately 2·08 ml of a 20% w/v aqueous PHMBH$^+$Cl$^-$ is required to precipitate 1 g of xanthan. This represents a dry weight ratio PHMBH$^+$Cl$^-$/xanthan of 0·416 and a molar ratio PHMBH$^+$Cl$^-$/carboxylic acid groups of 0·221 (based on the average repeating unit of xanthan polysaccharide (Jansson *et al.*, 1975). In comparison a dry weight ratio PHMBH$^+$Cl$^-$/polysaccharide of 1·059 is required to precipitate sodium alginate. This corresponds to a molar ratio PHMBH$^+$Cl$^-$/carboxylic acid groups of 0·174. Thus PHMBH$^+$Cl$^-$ would appear to be more efficient at precipitating a more highly charged polysaccharide such as sodium alginate than xanthan.

The lack of degradation products is indicated by the zero value for the carbohydrate content of supernatant B (Table 7). This also indicates that the xanthan is quantitatively precipitated from the dispersion of PHMBH$^+$-xanthan in KCl (aqueous) by addition of 2-propanol. The quantity of *X. campestris* cells in the final product is substantially reduced compared with the quantity in the fermentation broth (Table 7). The clumped cells in the final product are rendered bacteriologically inactive by the cytoplasmic precipitant action of the PHMBH$^+$Cl$^-$.

It is possible to produce pale cream-coloured fibrous $PHMBH^+$-xanthan precipitates which are more tractable than the gelatinous precipitates produced by direct treatment of the magnetically stirred *X. campestris* fermentation broths with aqueous $PHMBH^+Cl^-$. One method of producing a fibrous precipitate is to dilute the fermentation broth with six volumes of water prior to treatment with aqueous $PHMBH^+Cl^-$; the other is to replace the inefficient magnetic stirring with efficient maceration. The fibrous precipitates may be recovered by filtration using coarse grids. The purification procedure modified by use of prior dilution of the crude solutions was carried out on solutions of known xanthan content. Recovery of the xanthan was almost quantitative and averaged out at 97%.

IR spectra of the solid samples of purified xanthan were compared with the spectra of Keltrol (food grade xanthan gum) and $PHMBH^+Cl^-$. The IR spectrum of $PHMBH^+Cl^-$ showed absorption peaks due to amine and imine N—H stretching (3310 and 3180 cm^{-1}); methylene C—H stretching (2930 and 2855 cm^{-1}); imine C=N stretching (2175 cm^{-1}); amine and imine N—H bending (1640 and 1550 cm^{-1}); and methylene CH_2 rocking (720 cm^{-1}). Keltrol showed absorption peaks due to hydroxyl O—H stretching (3200-3600 cm^{-1}); methyl and methylene C—H stretching (2920 cm^{-1}); carboxylic acid C=O stretching (1740 cm^{-1}) and ether and hydroxyl C—O stretching (1010-1090 cm^{-1}). The purified samples of xanthan showed the same absorptions as Keltrol. The peak at 1740 cm^{-1} varied in intensity between samples owing to their differing pyruvate contents. Trace impurities of $PHMBH^+Cl^-$ in the products derived from samples X and Y were detectable by the presence of an absorption peak at 1550 cm^{-1}.

The UV spectrum of $PHMBH^+Cl^-$ showed an intense maximum at 235 nm ($\epsilon = 7\,000\,000$). The UV spectrum of Keltrol showed increasing absorption below 260 nm. This was also exhibited by the purified samples and made quantification of their $PHMBH^+Cl^-$ contents difficult. This indicates that $PHMBH^+Cl^-$ contents of the final products obtained during the optimisation studies (Tables 5 and 7) may be too high due to combined xanthan UV absorption. The UV spectra of the purified products derived from samples X and Y showed shoulders due to the $PHMBH^+Cl^-$ absorption maximum. The product derived from Z gave no detectable absorption due to $PHMBH^+Cl^-$.

We therefore recommend the following procedure for the purification of xanthan. To *X. campestris* fermentation broth (1·7% w/v xanthan, 50 ml), add aqueous $PHMBH^+Cl^-$ (20% w/v, 2·0 ml) and macerate the mixture immediately. Recover the precipitate by centrifugation, wash with water and disperse in aqueous potassium chloride (4 M; 50 ml). On attainment of homogeneity add 2-propanol (30 ml). Recover the precipitate by centrifugation, disperse in distilled water (50 ml), dialyse and lyophilise.

It is also feasible that the method developed for the recovery of xanthan, in purified form, from *X. campestris* fermentation broths could be applied to the recovery/purification of other acidic polysaccharides containing only carboxylate groups as their acidic functions.

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