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Xanthan Precipitation from Solutions and Fermentation Broths

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ABSTRACT

Precipitation of xanthan from solutions and fermentation broths is studied. The effects of different precipitating agents, initial xanthan concentrations, and salt additions on xanthan solutions are examined. The effect of sodium and calcium chloride concentrations added to isopropanol (IPA) is studied for xanthan solutions. Xanthan precipitation from fermentation broths has been carried out, and the effect of salt addition to IPA is considered.

Key Words. Xanthan gum; Precipitation; Isopropanol; Salt addition

INTRODUCTION

Xanthan is an exopolysaccharide of high molecular weight, obtained from sugar solutions and the action of bacteria of genus *Xanthomonas*. *X. campestris* and *X. juglandis* are the two species commonly used for batch and continuous cultures, respectively (1).

Xanthan gum aqueous solutions have a high and stable viscosity. This is what makes this gum suitable for commercial uses, which are mainly in the food industry (2) and to enhance oil recovery (3), among others applications (1).

A typical fermentation broth—after 24 to 90 hours depending on the strain, the medium composition, and the experimental conditions—is composed of 1–4% (w/w) xanthan, 0.2–0.5% (w/w dried) cells, 0.1–1% (w/w) of unused carbohydrate, and salts and other chemicals in small quantities which are added to the medium for cell growth. Successful xanthan recovery must eliminate nearly 95% of the broth. For this purpose both physical and chemical methods must be used (1), including:

Preliminary treatments for degradation and removal of the cells
Polysaccharide precipitation
Final steps, including washing, dewatering, drying, milling, and packing

Several techniques have been proposed and used to achieve these objectives, and the methods used on a commercial scale often depend on the aim of the processing and the cost. Thus, preliminary treatments used are of a thermal (4, 5), chemical (5, 6), and/or physical (5–8) nature. The final steps depend on the commercial properties and uses of xanthan gum.

Xanthan in solution can be precipitated by decreasing its solubility through the addition of alcohols or ketones of low molecular weight, such as methanol (4), ethanol, isopropanol (4, 5, 7), *t*-butanol (7), acetone (4), etc. In most research (4, 5, 7) isopropanol (IPA) is employed at a ratio between 1.8:1 to 2.5:1 (v/v, IPA/broth). An excess of from 8 to 25% of this quantity of IPA is used for washing.

Other authors (6–13) have proposed the addition of polyvalent cations to polysaccharide solutions, producing xanthan precipitation based on the polyelectrolytic character of the polysaccharide. Xanthan can be precipitated by the addition of calcium salts (6, 10) at a basic pH (between 8.5 and 12) or by the addition of aluminum salts (11) at an acid pH (between 4 and 4.5). In this way an insoluble xanthan salt is obtained which must be converted to a soluble salt (sodium or potassium) for commercial uses (11). Other authors have proposed employing quaternary ammonium salts (11–13), but the product must finally be converted to a sodium or potassium salt. The xanthan obtained in this way cannot be used in the food industry (1) due to the high toxicity of quaternary ammonium salts. Therefore, this method is not used on a commercial scale.

Smith (4) has pointed out the possibility of adding both salts (calcium, magnesium, and zinc) and IPA. In this way a smaller quantity of IPA is needed.

The aim of this work is to study xanthan precipitation from aqueous solutions, taking into account the influence of the precipitating agent, the xanthan concentration, and the effect of salt addition. Three different precipitants have been considered (ethanol, IPA, and acetone) for precipitating xanthan from solutions of different concentrations. The effect of the addition of mono- and divalent cation salts on the quantity of IPA needed for precipitation has been studied, and different sodium and calcium chloride concentrations have been studied to determine the influence of salt concentration. Finally, xanthan recovery from fermentation broth has been carried out by using both IPA and salt addition and taking into account the initial salt content of the broth.

EXPERIMENTAL

Xanthan gum of Technical Grade (ref. TF9418) was supplied by Jungbunzlauer A.G. Xanthan solutions were obtained by mechanical stirring at a controlled temperature (25°C) for 1–2 hours.

Fermentation broths were fermented in a Biostat (MD Braun) using sucrose at a concentration of 20 g/L as the substrate and a medium with the composition given in Table 1.

The precipitating agent was added to the solution and the mixture was again mechanically stirred. When salts were employed, the salt was added to the xanthan solution prior to IPA addition.

The xanthan which precipitated was removed by filtration under vacuum. It was then dried at 60°C for several hours and then weighed. Xanthan concentrations in solution, before and after precipitation, were checked by viscosity measurement in a Brookfield viscometer by using a no. 18 spindle with a thermostated microsample. The viscosity of the xanthan solutions, previously diluted to a convenient concentration range, has a constant relationship to the biopolymer concentration (14) according to a power law.

The ethanol, isopropanol (IPA), and acetone were of industrial quality, 96% (w/w), 85% (w/w), and 98% (w/w) in content, respectively. All salts used were of PRS quality, around 98–99% (w/w) in purity.

TABLE 1
Fermentation Broth Composition

Component	Concentration (g/L)
Boric acid	0.006
Citric acid	2.10
Hydrochloric acid	0.0002
Calcium carbonate	0.02
Ferric chloride	0.0024
Zinc oxide	0.006
Monobasic potassium phosphate	2.866
Magnesium sulfate	0.507
Sodium sulfate	0.089
Urea	1.083
Sucrose	20.00

RESULTS AND DISCUSSION

Influence of Precipitating Agent

Figure 1 shows the different volumes of precipitating agent needed to precipitate xanthan from solution. As can be seen, a much greater quantity of ethanol than of IPA or acetone is needed. The quantities of IPA and acetone are similar, but it is evident that a somewhat smaller quantity of IPA is needed than of acetone. Thus, for total xanthan precipitation, a V_{agent}/V_0 ratio of 6 is needed with ethanol, while values of 3.6 and 3.2 for this ratio are enough when acetone or IPA is used. Methanol was also used, but the xanthan precipitate was not easily formed; a gel which was very difficult to recover was obtained.

Influence of Xanthan Concentration

Xanthan was precipitated from solutions having different initial concentrations of polysaccharide, as shown in Fig. 2, using IPA as the precipitating agent in all cases. The initial xanthan concentrations used were 3, 4, 5, 6, 7, and 8 g/L. It is evident that the dependence of xanthan concentration in solution on the amount of IPA added varies with the initial xanthan concentration, but the final quantity of IPA needed for the total precipitation of xanthan is the same: a V_{IPA}/V_0 ratio equal to 3.2.

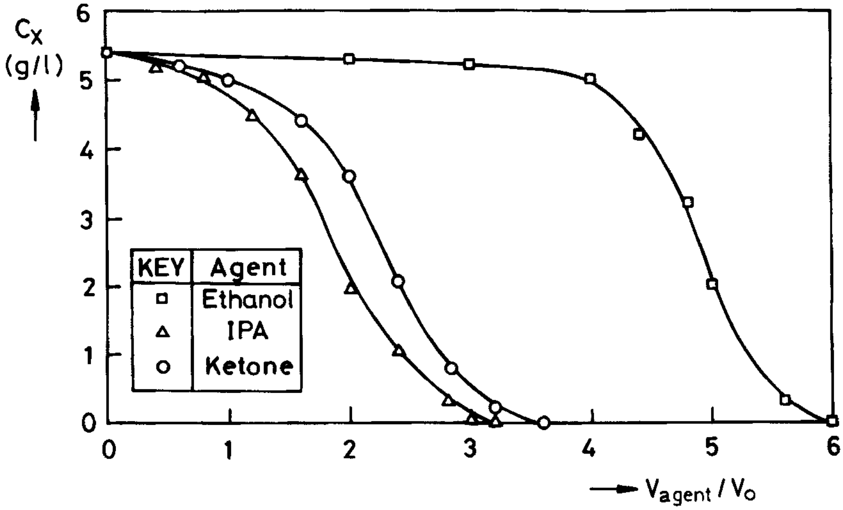

FIG. 1 Xanthan concentration in solution vs precipitating agent volumes added using ethanol, ketone, and IPA.

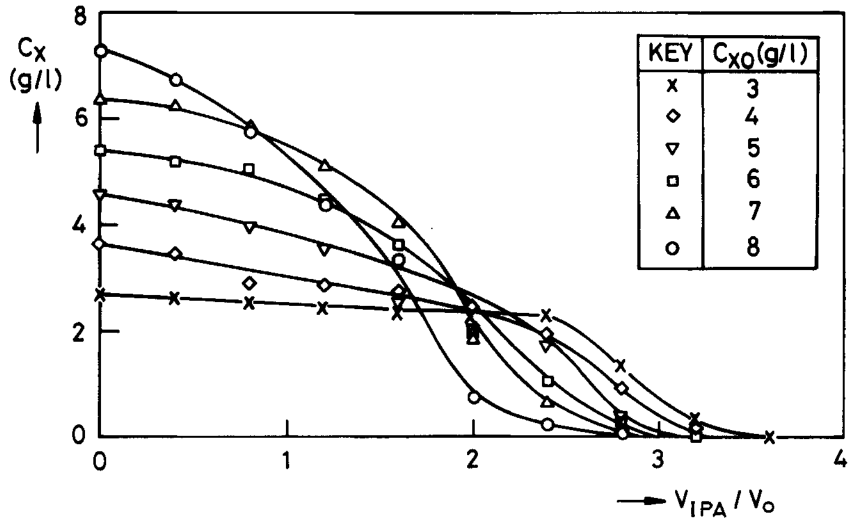

FIG. 2 Xanthan concentration in solution vs IPA volumes added for different initial xanthan concentrations.

Effect of Salt Addition

Due to the polyelectrolytic character of xanthan molecules, several authors (6–13) have proposed precipitating xanthan from solutions by adding salts having cations with different valences. But because of commercial standards, particularly those for food additives, it seems more convenient to use IPA and salts to reduce the quantity if IPA required, as pointed out by Smith (4). In the present work, different salts (with mono- and divalent cations) were added to the xanthan solution and then xanthan was precipitated by IPA addition. Chloride was the anion in all salts, while the cations were calcium, magnesium, ammonium, sodium, and potassium. A salt concentration of 1 g/L was used in all cases. Figure 3 is an example of the results obtained for an initial xanthan concentration of 6 g/L. The results with other initial xanthan concentrations (3 and 8 g/L) were very similar. The addition of divalent salts produces a much greater reduction in the IPA volume needed for xanthan precipitation than was produced by the addition of monovalent salts. Thus, total precipitation of xanthan is reached with a V_{IPA}/V_0 ratio smaller than 0.9 with divalent salt addition, while this ratio is around 1.8 with monovalent salts. With respect to the cation of the salt, calcium gives a slightly greater reduction in required IPA than magnesium, but the results are very close; sodium, ammonium, and potassium salts also yield very similar results. Despite the advantage of di-

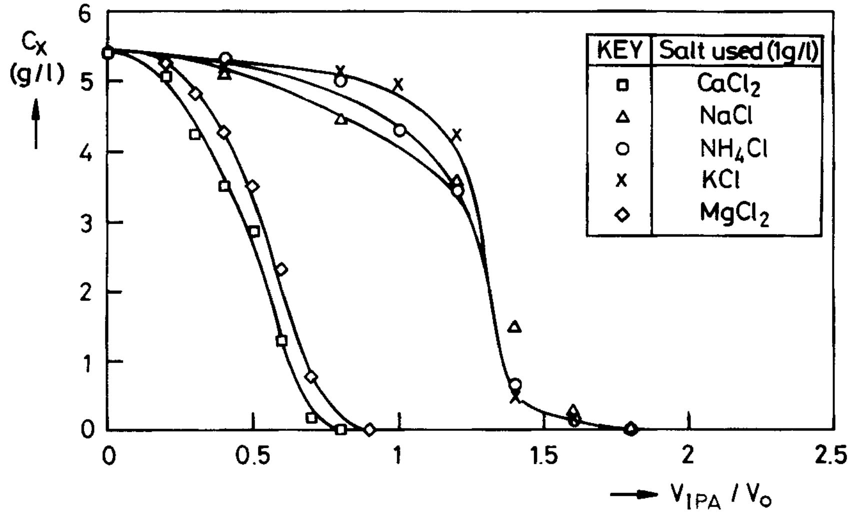

FIG. 3 Xanthan concentration in solution vs IPA volumes added with the addition of 1 g/L different salts.

valent salts in producing a greater reduction of IPA volume required for xanthan precipitation, these salts have the disadvantage of producing an insoluble xanthan gum.

Calcium and sodium chloride were chosen as being representative of di- and monovalent salts, with the advantages and disadvantages mentioned above, and then different salt concentrations were studied (0.1, 0.2, 0.3, 0.4, 0.5, and 1 g/L) to optimize the salt concentration in both cases. Figures 4 and 5 show the results obtained, the first for NaCl and the second for $CaCl_2$. As can be seen, when a greater salt concentration is used, the effect of calcium chloride is more marked than that of sodium chloride. In both cases the reduction rate in the volume of IPA required for xanthan precipitation decreased when the salt concentration was increased. Therefore, a salt concentration addition of 1 g/L can be considered as the optimum value because the addition of a greater quantity would produce a very small improvement because the reduction in the volume of IPA required would be very small. Total precipitation of xanthan is achieved for a salt concentration of 1 g/L for the V_{IPA}/V_0 ratio of 1.8 with NaCl and a ratio of 0.8 with $CaCl_2$ when using an initial concentration of 6 g/L xanthan.

Finally, the effects of both initial xanthan concentration and added salt concentration were studied. Figure 6 shows the results obtained when xanthan is precipitated from solutions containing 4, 6, and 8 g/L by adding

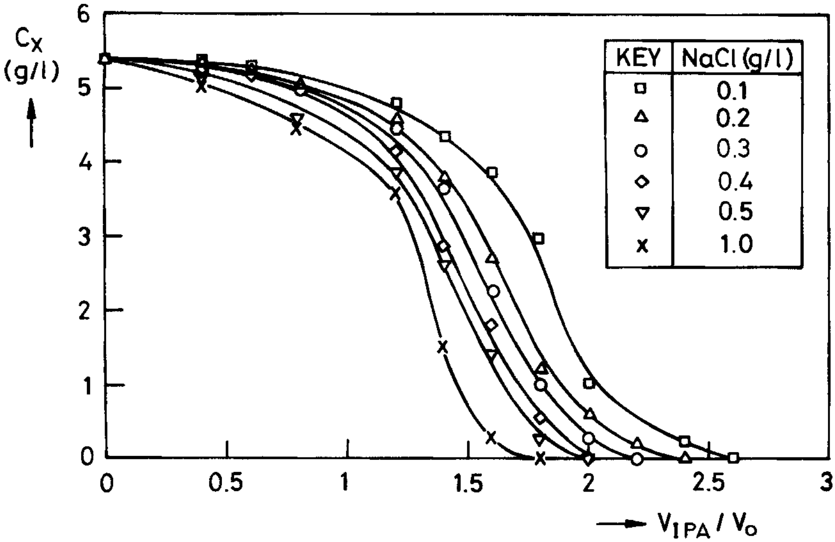

FIG. 4 Xanthan concentration in solution vs IPA volumes added with the addition of sodium chloride in different concentrations.

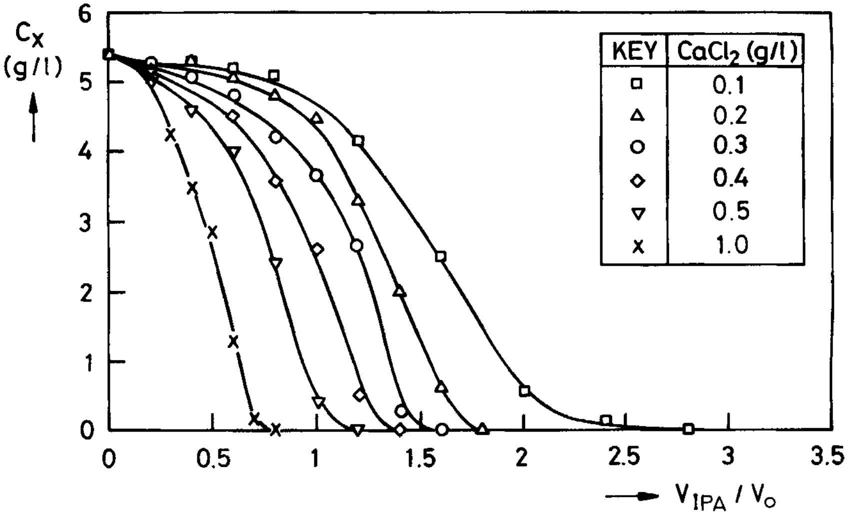

FIG. 5 Xanthan concentration in solution vs IPA volumes added with the addition of calcium chloride in different concentrations.

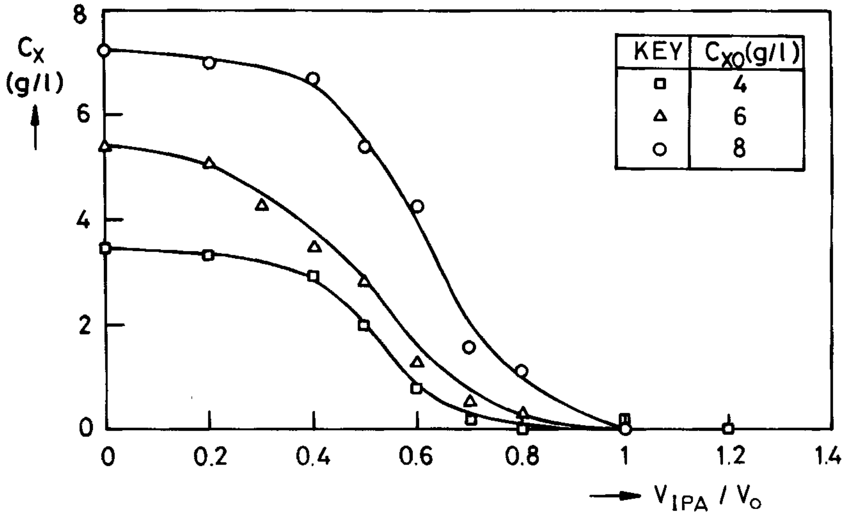

FIG. 6 Xanthan concentration in solution vs IPA volumes added for different initial xanthan concentrations with the addition of 1 g/L calcium chloride.

calcium chloride at a concentration of 1 g/L. The dependence of xanthan concentration in solution on V_{IPA} is different (see Fig. 6) from that found when salt was not added, as shown in Fig. 2. This is because, apart from the reduction in IPA volume (from a value of the V_{IPA}/V_0 ratio of around 3 to a value of 0.8 to 1 when 1 g/L $CaCl_2$ is added), the curves now obtained for different initial xanthan concentrations have a parallel dependence; that is, the effect of the xanthan concentration on the dependence is smaller. The reason for this behavior is probably that when salts are not present, associative interaction among the xanthan molecules in solution is favored at greater xanthan concentrations, and the higher apparent molecular weight produces the precipitation of some of these molecules, as shown in Fig. 2 by the crossing of the curves, but such interactions are blocked when salts are added, so the curves do not cross.

Precipitation of Xanthan from Fermentation Broths

Figure 7 shows plots of the results obtained in the precipitation of xanthan from fermentation broths using IPA as the precipitating agent and with different initial xanthan concentrations. As can be appreciated, the behavior is very similar to that observed with xanthan solutions when salts are used together with IPA, surely due to the salt content of the fermentation broth. This effect is clearly pointed out in Fig. 8 where the results

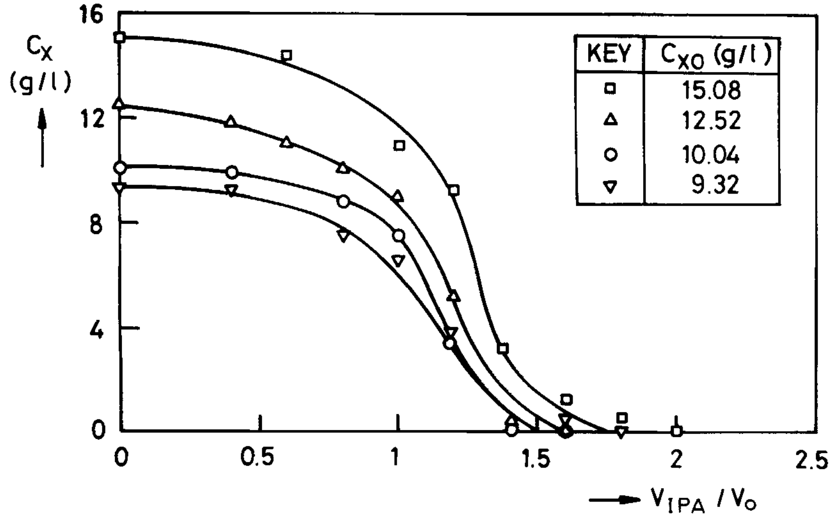

FIG. 7 Xanthan concentration in solution vs IPA volumes added for different fermentation broths with different initial xanthan concentrations.

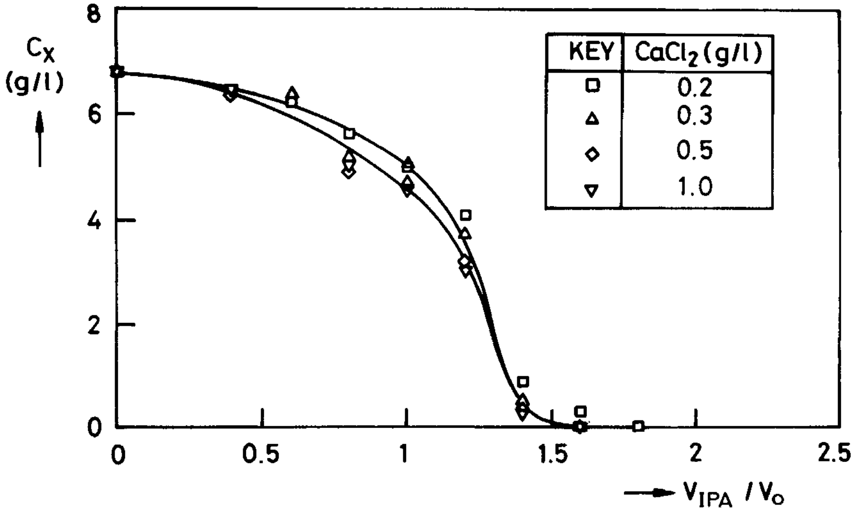

FIG. 8 Xanthan concentration in solution vs IPA volumes added for a fermentation broth with the addition of calcium chloride in different concentrations.

obtained with a diluted broth, with around 7 g/L xanthan, are presented. It is evident that salt addition—from 0.2 to 1 g/L calcium chloride—has hardly any effect on the quantity of IPA required for xanthan precipitation, with V_{IPA}/V_0 being around 1.5 for every initial xanthan concentration and added salt concentration.

CONCLUSIONS

The V_{agent}/V_0 ratio for total xanthan precipitation changes with the precipitant employed. Thus, that ratio is 6 for ethanol, 3.6 for acetone, and 3.2 for IPA. With methanol, the xanthan precipitate was not obtained, only a gel which was very difficult to recover was formed.

The addition of divalent salts produces a much greater reduction in the IPA volume needed for xanthan precipitation than that produced by the addition of monovalent salts. For a salt concentration of 1 g/L, total precipitation of xanthan is achieved for a V_{IPA}/V_0 ratio smaller than 0.9 with divalent salt addition and around 1.8 with monovalent salt. The cation of the salt has a slight influence on the reduction of IPA required. The dependence of xanthan concentration in solution on V_{IPA} is different when salt is added, as is shown in Fig. 6, from that found when salt was not added, as shown in Fig. 2. Not only is a reduction in IPA volume achieved (from a value of the V_{IPA}/V_0 ratio of around 3 to a value of 0.8 to 1 when 1 g/L $CaCl_2$ is added), but the curves now obtained for different initial xanthan concentrations when salt is added have a parallel dependence because the effect of xanthan concentration on the dependence is smaller than when salts are not present.

For a fermentation broth, salt addition—from 0.2 to 1 g/L calcium chloride—has hardly any effect on the quantity of IPA required for xanthan precipitation, with V_{IPA}/V_0 being around 1.5 for every initial xanthan concentration and added salt concentration. This must be due to the salt previously contained in the broth.

NOMENCLATURE

C_X	xanthan concentration in solution (g/L)
C_{X0}	initial xanthan concentration in solution (g/L)
V_{agent}	volume of precipitating agent added
V_{IPA}	volume of IPA added
V_0	volume of xanthan solution

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