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Alcohol Precipitation of Xanthan Gum from Pure Solutions and Fermentation Broths

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ABSTRACT

Xanthan gum was precipitated from pure solutions and fermentation broths using either ethanol, isopropanol, or *tert*-butanol. The compositions of the precipitate and supernatant phases were determined as a function of alcohol concentration and used to construct binodal solubility curves with tie lines. Xanthan did not precipitate at bulk-mixture alcohol concentrations below 37.5% (wt) for ethanol, 35% for isopropanol, and 31% for *tert*-butanol. As the alcohol concentration increased beyond this point, the precipitates first were heavy gels with low xanthan concentrations. At higher alcohol concentrations, the precipitates were compact and fibrous. The maximum xanthan concentration in the precipitate was 14.5% at 60% ethanol, 23.5% at 50% isopropanol, and 33.5% at 40% *tert*-butanol in the pure solution precipitation experiments. At alcohol concentrations beyond 75%, the precipitates were brittle and needle-like, which made separation from the supernatant difficult. The results for the fermentation broth experiments were very similar to those of the pure solution experiments. Thus, precipitation using ethanol required the highest alcohol usage and resulted in the lowest xanthan concentration in the precipitate. Conversely, *tert*-butanol required the least alcohol for precipitation and formed the precipitates highest in xanthan concentration.

Key Words. Xanthan gum; Precipitation; Fermentation broths; Binodal solubility curve; Ethanol; Isopropanol; *tert*-Butanol

INTRODUCTION

Xanthan gum is an anionic polysaccharide produced by the Gram-negative bacterium *Xanthomonas campestris* and has a molecular weight of approximately 3.0 to 7.5×10^6 dalton (1). Solutions of xanthan gum are shear thinning and highly viscous. In addition, the viscosity of these solutions is unusually stable with respect to ionic concentration, pH, and temperature (2).

The typically high viscosities and low polymer concentrations (1 to 4%) of xanthan fermentation broths makes the recovery of xanthan gum on a commercial scale both difficult and costly (3). Xanthan can be isolated from the fermentation broth in several ways including precipitation through salt addition, direct evaporation through spray or drum drying, or precipitation through the addition of water-miscible organic solvents (4).

The salt precipitation processes are limited due to problems of purity and cost. Typically, xanthan gum precipitated by polyvalent cations is in an insoluble form and must be further purified to convert it to the soluble form (5, 6). Quaternary ammonium salts can be used to precipitate xanthan, but must be recovered and recycled to make the process economically viable (7, 8). Because of their high toxicity and extra purification requirements, neither polyvalent cations nor quaternary ammonium salts are used on a commercial scale to recover food-grade xanthan gum.

Evaporation processes, which include drum drying and spray drying, also result in an industrial-grade product of inferior quality unless further purification steps are employed (9, 10).

Currently, the only commercially viable recovery method utilizes isopropyl alcohol (IPA) to isolate and purify the polysaccharide gums. Alcohol precipitation has the advantage of simultaneously isolating and purifying the polysaccharide gum; however, this process is inefficient and expensive due to the large amount of IPA required and the utility costs of distilling the alcohol for reuse (4).

Research on the alcohol precipitation of xanthan gum has been limited until recently. Gonzales et al. (11) examined the effects of salt type, salt addition, alcohol concentration, and temperature on the precipitation of xanthan gum by ethanol (EtOH). Subsequently, the same authors investigated the phase equilibria of xanthan gum in pure solutions of EtOH and water (12).

García-Ochoa et al. (13) studied the effect of solvent type on the precipitation of xanthan from pure solutions. EtOH, IPA, and acetone were used as precipitating agents in the pure solution precipitation experiments. In addition, the authors studied the effects of xanthan concentration and salt addition on the precipitation of xanthan from both pure solutions and fermentation broths. IPA was used as the precipitating agent in the fermentation broth experiments.

The purpose of this work is to investigate the effect of alcohol type and concentration on the precipitation of xanthan gum from both pure solutions and fermentation broths. Binodal solubility curves comparing the behavior of xanthan gum in mixtures of water and either EtOH, IPA, or *tert*-butanol (tBA) have been constructed for both pure solutions and fermentation broths.

EXPERIMENTAL

Preparation of Xanthan Solutions

Ten liters of a pure solution of xanthan gum, consisting of 1.7% (w/w) xanthan (Keltrol T food-grade xanthan, Kelco Division of Merck & Co., Inc., San Diego, California), 1% (w/w) potassium chloride (analytical reagent grade, Mallinckrodt, Inc., Paris, Kentucky), and 0.02% (w/w) sodium azide (Sigma Chemical Co., St. Louis, Missouri) were prepared by mixing at 400 rpm for 24 hours to insure homogeneity and stored at ambient temperature (14).

The microorganism *Xanthomonas campestris* ATCC 13591 (American Type Culture Collection, Rockville, Maryland) was used to produce the fermentation broths used in the precipitation experiments. The fermentations were conducted in 2 L baffled shake flasks containing 200 mL of the medium given by García-Ochoa et al. (15). A 5% inoculum, grown on Y-M medium (Difco Laboratories, Detroit, Michigan), was used. The 2-L flasks were incubated for 48 hours at 30°C and 120 rpm in a reciprocating shaker.

The broth xanthan concentration at the end of the fermentation was approximately 1% (w/w). Upon completion of the fermentation, the broth was pasteurized, unstirred, at 90 to 95°C for at least 1 hour in a water bath to inactivate cells and enzymes. After the pasteurized broth was cooled to room temperature (overnight), KCl was added to the fermentation broth in the form of a 20% (w/w) solution by stirring at high speed with a propeller-type mixer for 2 hours. The total KCl concentration in the broth was approximately 1% (w/w).

Precipitation Experiments

The alcohols used in the precipitation experiments with xanthan gum were EtOH (Absolute, U.S.P. Punctilious grade, Quantum Chemical Co., Tuscola, Illinois), IPA (Certified A.C.S. grade, Fisher Scientific, Pittsburgh, Pennsylvania) and tBA (Certified grade, Fisher Scientific, Pittsburgh, Pennsylvania). The alcohols were each 99% or greater in purity.

Xanthan gum solution (either pure solution or fermentation broth) and the test alcohol (EtOH, IPA, or tBA) were mixed according to the procedure outlined in Gonzales et al. (12). The solutions were combined in the desired ratios in preweighed 250 mL centrifuge bottles to a total solution mass of approximately 100 g in each bottle. The bottles were sealed tightly, shaken vigorously by hand for 1 minute, and placed in a shaking water bath at 120 rpm and 15°C for 24 ± 2 hours to reach equilibrium.

After shaking, the bottles were centrifuged at 15,000*g* and 15°C for 30 minutes. For each bottle the supernatant was poured from the bottle immediately after removal from the centrifuge, and 50 mL of it was saved in an airtight tube for later analysis. Supernatant that remained after pouring was decanted off carefully with a Pasteur pipet. The wet precipitate was weighed in the bottle and stored at 4°C for up to 72 hours before analysis.

Analytical Methods

Cell concentration in the broth was measured by first diluting a known mass of broth 1:20 to decrease the viscosity and then centrifuging at 15,000*g* and 5°C for 30 minutes to isolate the cells. The resulting cell pellet was washed with distilled water and centrifuged again at the same conditions. Finally, the washed pellet was resuspended and dried at 70°C to constant mass in a tared aluminum pan (12 hours). Cell concentration was calculated by dividing the dry cell mass by the initial mass of the broth.

In the precipitation experiments, the water concentration in the supernatants was measured, in duplicate, by Karl Fisher titration (Model 701 KF Titrino, Metrohm Ltd., Herisau, Switzerland).

The total solids concentration in the precipitates was measured, in duplicate, by drying a known mass of the wet precipitate in a tared aluminum pan to constant mass at 70°C. Total solids concentration was calculated by dividing the dry solids mass by the wet precipitate mass.

Xanthan concentration in the precipitates was measured by dialyzing a known mass of the precipitate for 3 days in 50,000 dalton MWCO dialysis tubing (Spectra/Por 6 Molecularporous Dialysis Membrane, Spectrum, Houston, Texas). The tubes were dialyzed against 3 to 4 L of distilled water, which was changed daily. Dialyzed precipitate was dried in tared aluminum pans to constant mass at 70°C (12 to 24 hours). Xanthan concentration was calculated by first dividing the dry, dialyzed precipitate mass by the initial wet precipitate mass, then subtracting off the cell concentration.

The concentrations of the remaining components in the supernatant and precipitate were obtained through mass balances (14).

RESULTS AND DISCUSSION

Effect of Alcohol Concentration

The effect of alcohol concentration on the mass of the precipitates in the pure solution and fermentation broth precipitation experiments is plotted in Figs. 1 and 2, respectively. To account for differences in the mass of xanthan in the initial bulk mixtures, the precipitate mass was normalized by dividing it by the initial mass of xanthan gum in the bulk mixture. Alcohol concentrations are given as the weight percent (on a salt-free basis) in the initial bulk mixtures. Error bars indicate a 95% confidence interval.

A comparison of Figs. 1 and 2 shows that the alcohol precipitation of xanthan from pure solutions with added KCl is very similar to the precipitation of xanthan from fermentation broths. For both pure solutions and fermentation broths, three types of precipitates formed depending on the alcohol concentration.

It can be seen from Figs. 1 and 2 that no precipitation occurred at alcohol concentrations below 30% for any of the three alcohols in the precipitation experiments. However, between 31 to 40% alcohol (depending on the alcohol), there was a solubility transition resulting in the forma-

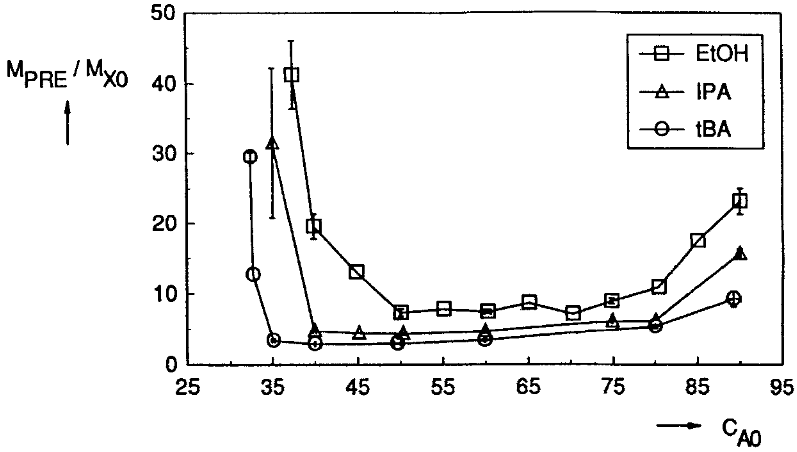

FIG. 1 The xanthan precipitate mass ratio (M_{PRE}/M_{X0}) vs the weight percent ethanol (EtOH), isopropanol (IPA), or *tert*-butanol (tBA) in the bulk mixture (C_{A0}) for pure solutions.

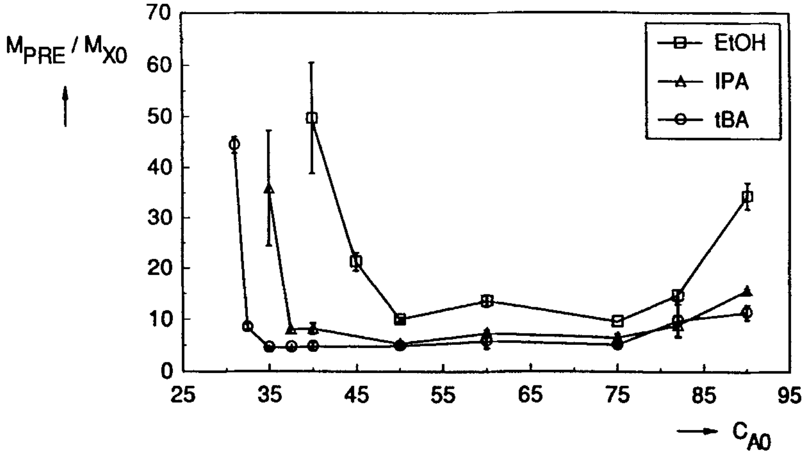

FIG. 2 The xanthan precipitate mass ratio ($M_{\mathrm{PRE}}/M_{\mathrm{X0}}$) vs the weight percent ethanol (EtOH), isopropanol (IPA), or *tert*-butanol (tBA) in the bulk mixture (C_{A0}) for fermentation broths.

tion of a gel-like precipitate. These precipitates weighed from 30 to 50 times the initial mass of xanthan. The large vertical error bars in this alcohol concentration range were due to the difficulty in separating the solid and liquid phases. In this region the two phases had nearly the same composition.

As the alcohol concentration was increased to nearly 45%, the precipitate mass quickly dropped to less than 10 times the initial xanthan mass. Between 50 to 75% alcohol the precipitates were very compact and fibrous, resulting in a good separation of solid and liquid phases.

Above 75% alcohol the precipitates were characterized by brittle, needle-like particles that were difficult to separate completely from the supernatant by centrifugation. Precipitate mass increased to in this region presumably due to entrained solvent in the precipitate phase.

The behavior of xanthan gum precipitation in the various alcohol concentration regimes may be attributed to the change in affinity between the polymer and the solvent with increasing alcohol concentration. At low alcohol concentrations (below 30%) there is a high degree of polymer–solvent affinity, and xanthan is soluble. In other words, the highly charged polymer is stabilized through hydrogen bonding interactions with water. However, above 30% alcohol, the solvent becomes sufficiently nonpolar that polymer–polymer affinity becomes comparable with polymer–solvent affinity (16). The result is the formation of weak bonds between polymer molecules which are stabilized through binding with the various cations present (in this case K^+). This effect can be seen macroscopically as the precipitation of a solid phase in the form of a gel. Gonzales et al. (12) reported similar behavior in the low-alcohol range for xanthan gum precipitation by EtOH. They saw a sharp transition from soluble to insoluble over a narrow range, giving rise to a "gel-like and swollen" precipitate. The formation of a gel is undesirable industrially because it contains large amounts of entrained solvent and is difficult to separate from solution.

At higher alcohol concentrations (above 45%) enough water is displaced such that interactions between xanthan molecules are predominant over other interactions, and the polymer becomes virtually insoluble. The increasingly nonpolar nature of the bulk solution forces the charged xanthan molecules to aggregate. Smith and Pace (16) discussed the aggregation of xanthan coils due to high intermolecular attractive forces which squeeze out most of the entrapped solvent. Physically, the precipitate appears compact and fibrous and is much lower in moisture content.

At the highest alcohol concentrations, the repulsion between the alcohol and xanthan is most likely considerable. Thus, the shrinking of the xanthan coils and expulsion of entrapped solvent becomes nearly complete, producing a precipitate that is brittle in texture.

The precipitates from the fermentation broths contained an undesirable yellow pigment not present in the precipitates from the pure solutions (14). Since the supernants in the recovery experiments were also yellow, these pigments were probably soluble in alcohol–water mixtures. The precipitates (and supernatants) lightest in color were formed at the highest alcohol concentrations, where the pigments were probably leached from the cells and diluted by the large amount of alcohol used for precipitation.

Binodal Solubility Curves for Pure Solutions

Figures 3, 4, and 5 contain the results of the pure solution precipitation experiments using EtOH, IPA, and tBA, respectively. The weight percent of xanthan in the precipitate, bulk-solution, and supernatant phases is plotted against the weight percent alcohol in the corresponding phases. Error bars, where used, indicate a 95% confidence interval. Open circles without error bars are for single data points. The dotted lines in Figs. 3, 4, and 5, which encompass the two-phase region for each alcohol, are the binodal solubility curves. Theoretically, any bulk-mixture composition which falls underneath one of these curves will separate into a precipitate and a supernatant phase. The composition of these phases is defined by the solid tie line which passes through the bulk-mixture composition point. Furthermore, the position of the bulk-mixture composition point on the tie

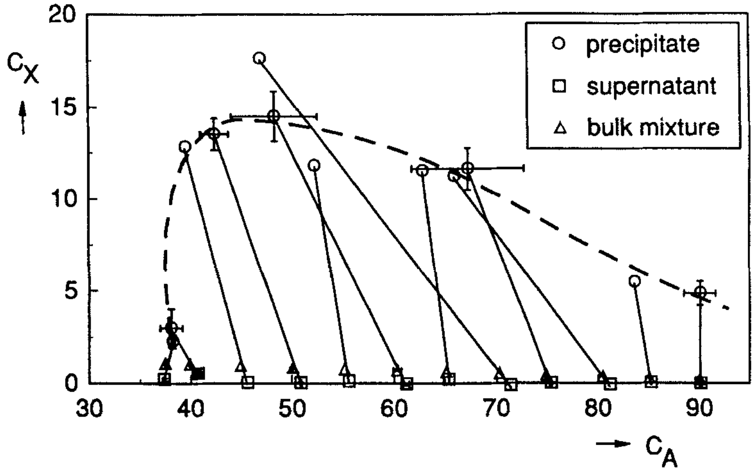

FIG. 3 The weight percent xanthan in the precipitate (C_X) vs the weight percent ethanol (C_A) for pure solutions.

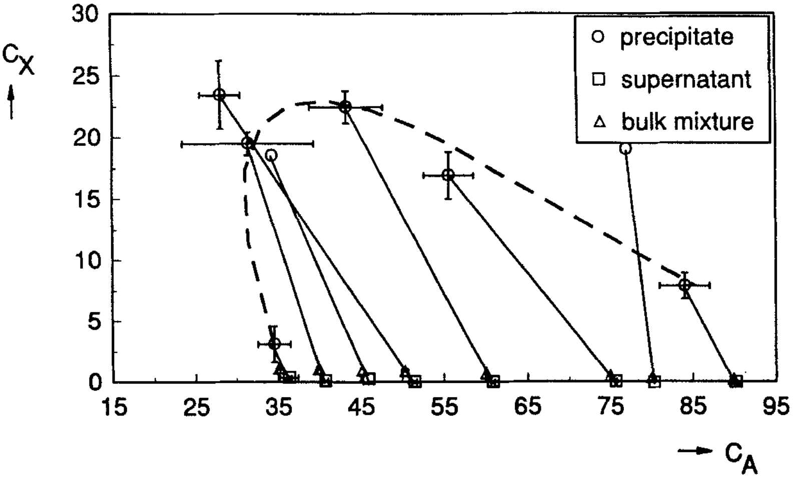

FIG. 4 The weight percent xanthan in the precipitate (C_X) vs the weight percent isopropanol (C_A) for pure solutions.

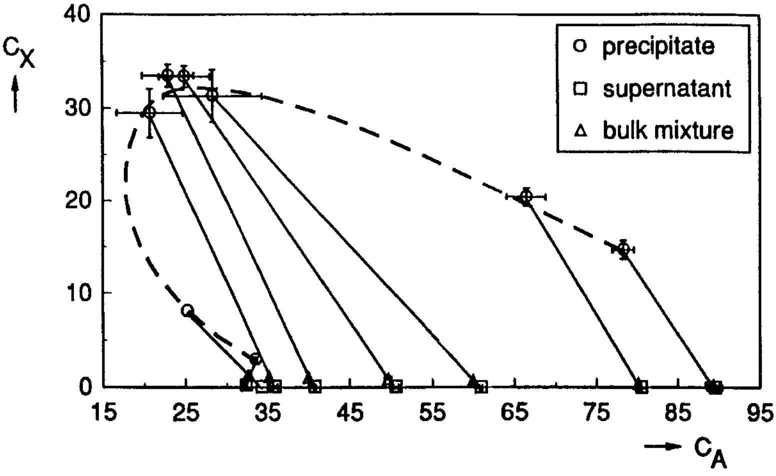

FIG. 5 The weight percent xanthan in the precipitate (C_X) vs the weight percent *tert*-butanol (C_A) for pure solutions.

line will determine the relative amounts of the supernatant and precipitate phases according to the lever rule (17).

In Figs. 3, 4, and 5 the generally backwards slope of the tie lines in the two-phase region has an important practical consequence. The alcohol concentration in the precipitate will be lower than the alcohol concentration in the supernatant. In other words, the alcohol migrates preferentially to the supernatant where it is more easily recovered. Gonzales et al. (12) also reported backward-sloping tie lines.

The alcohol concentration range corresponding to the solubility transition for xanthan gum depended on the type of alcohol used. In the precipitation experiments using EtOH (Fig. 3), the transition from completely soluble to completely insoluble for xanthan gum occurs over an alcohol concentration range of 37.5 to 50%. In the IPA precipitation experiments (Fig. 4), xanthan became completely insoluble over a narrower range of alcohol concentrations from 36 to 40%. Finally, in the tBA precipitation experiments (Fig. 5), the alcohol concentration range for xanthan insolubility was between 32.5 to 35%.

García-Ochoa et al. (13) found that solubility transition occurred at a lower alcohol concentration for IPA and acetone than for EtOH. In their work, the xanthan solubility transition occurred as the IPA concentration in the bulk mixture was increased from 40 to 50% (wt), which is slightly different from that of this work, where the xanthan solubility transition occurred between 36 to 40% alcohol (Fig. 4). One possible explanation for this difference is the lower KCl concentration used in the precipitation experiments of García-Ochoa et al. (1 g/L) as compared to this work (10 g/L). Lower salt concentrations require a higher alcohol concentration to achieve complete precipitation of the xanthan gum (11).

Gonzales et al. (12) also reported that the xanthan solubility transition occurred over a narrow alcohol concentration range (20 to 30%) for the precipitation of xanthan gum from pure solutions. The lower solubility transition for EtOH compared to this work may be due to differences in pyruvate substitution on the side chain of the xanthan gum molecule. Sandford et al. (18) used a gradient in EtOH concentration to precipitate xanthan gum into fractions which had different extents of substitution of pyruvate groups. Fractions with a higher degree of pyruvylation required more EtOH to precipitate.

Gonzales et al. (12) speculated that the solubility of xanthan gum in alcohol–water solutions may be a function of the dielectric constant of the bulk solution. They proposed a critical bulk-mixture dielectric constant of 65 and suggested that the sharp solubility transition for xanthan gum occurs when enough alcohol is added to the bulk-mixture to lower the solvent dielectric constant below this number.

In the pure solution precipitation experiments in this work, it was found that xanthan became completely insoluble at a dielectric constant of approximately 50 for all three alcohols. Bulk-mixture dielectric constants were estimated using equations given by Åkerlöf (19), which relate dielectric constant with alcohol concentration and temperature. In general, tBA is the most efficient of the three alcohols at lowering the bulk-mixture dielectric constant since it is the most nonpolar; EtOH is the least efficient.

Thus, the differences between the alcohols with regard to the solubility transition of xanthan gum may be directly related to their efficiency in lowering the bulk-mixture dielectric constant. tBA lowers the dielectric constant quickest and is required in the least amount to completely precipitate xanthan gum. Conversely, EtOH is the least efficient at lowering the bulk-mixture dielectric constant and is required in the highest concentration to precipitate xanthan.

At the solubility transition in Figs. 3, 4, and 5, the concentration of xanthan in the supernatant was approximately 0.2 to 0.3% and decreased to nearly zero for all higher alcohol concentrations. Gonzales et al. (12) reported similar supernatant xanthan concentration behavior when EtOH was used as the precipitating agent. García-Ochoa et al. (13) compared the precipitation of xanthan gum with EtOH, acetone, and IPA. For each precipitating agent the concentration of xanthan in the supernatant decreased to zero with increasing concentration of alcohol or ketone. This result is desirable because it means that nearly all of the xanthan is recovered in the precipitate.

A major difference between Figs. 3, 4, and 5 is the concentration of xanthan in the precipitates from the three alcohols. tBA precipitates had the highest concentration of xanthan at every alcohol concentration, while EtOH precipitates had the lowest. The maximum xanthan concentration in the precipitate was 14.5% at 60% EtOH, 23.5% at 50% IPA, and 33.5% at 40% tBA in the pure solution precipitation experiments.

The concentration of xanthan in the precipitate is related not only to the type of alcohol, but also to the method used in isolating and separating the wet precipitate from the supernatant. Gonzales et al. (12) determined the maximum xanthan concentration in the precipitate to be less than 5% in their pure solution precipitation experiments using EtOH. This result is much smaller than the 14.5% precipitate xanthan concentration for EtOH in this work. The difference may be due to differences in technique in separating the precipitate phase.

Binodal Solubility Curves for Fermentation Broths

Figures 6, 7, and 8 contain the results of the precipitation experiments on fermentation broths. Error bars, where used, indicate a 95% confidence interval. Open circles without error bars are for single data points. The ties lines and binodal solubility curves in these figures are exactly analogous to those in Figs. 3, 4, and 5.

From Fig. 6, the EtOH concentration range (in the initial bulk mixture) for the xanthan solubility transition was 40 to 50%. In Fig. 7 the concentration range for IPA was 35 to 40%. In the tBA experiments (Fig. 8), xanthan solubility decreased to zero over the alcohol concentration range of 30 to 35%. These results are very similar to the alcohol concentration ranges reported earlier for the xanthan solubility transition in the pure solution recovery experiments.

García-Ochoa et al. (13) also found that the precipitation of xanthan gum from fermentation broths closely resembled the precipitation from pure solutions containing salt. The authors reported the solubility transition for xanthan gum to occur between 33.5 to 45% (wt) IPA in their precipitation experiments on fermentation broths containing 1% gum.

As in the precipitation experiments from pure solutions, the precipitates from the three alcohols have noticeably different concentrations of xanthan in the precipitate. For all three alcohols the precipitates had nearly the same xanthan composition (2 to 5%) near the solubility transition, where the gel-like precipitates formed. However, the concentration of

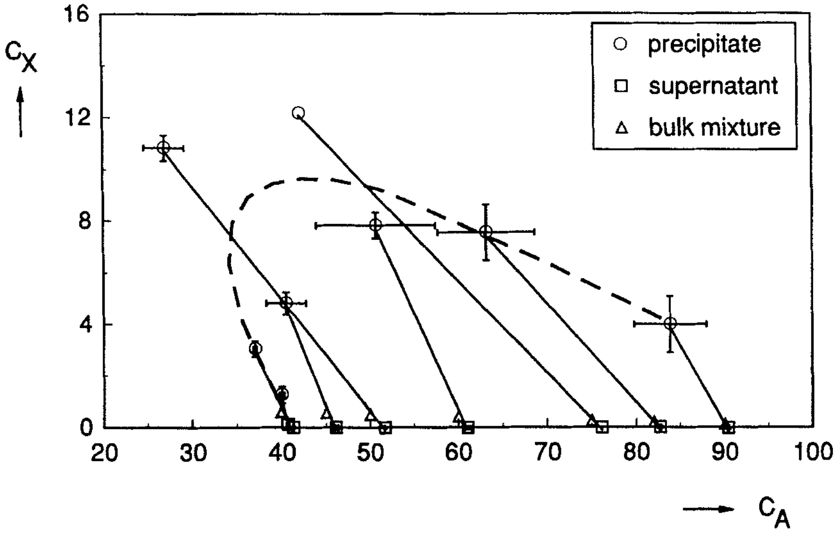

FIG. 6 The weight percent xanthan in the precipitate (C_X) vs the weight percent ethanol (C_A) for fermentation broths.

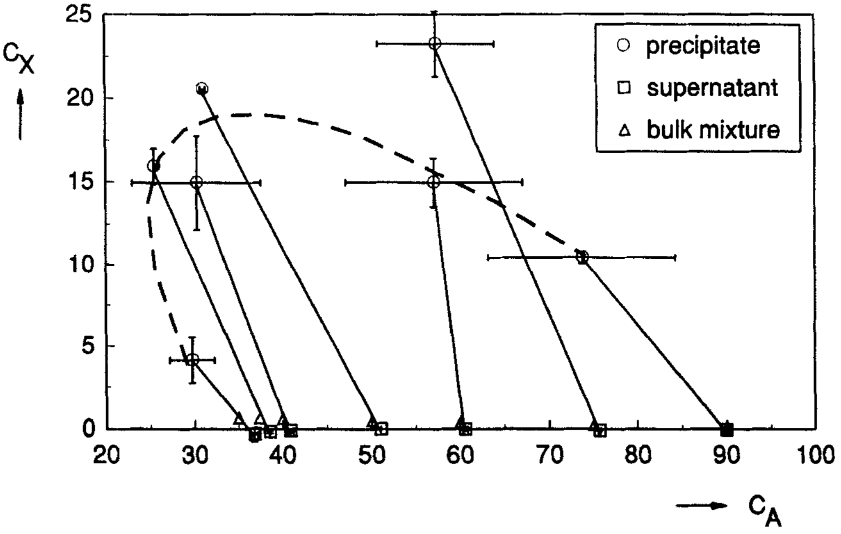

FIG. 7 The weight percent xanthan in the precipitate (C_X) vs the weight percent isopropanol (C_A) for fermentation broths.

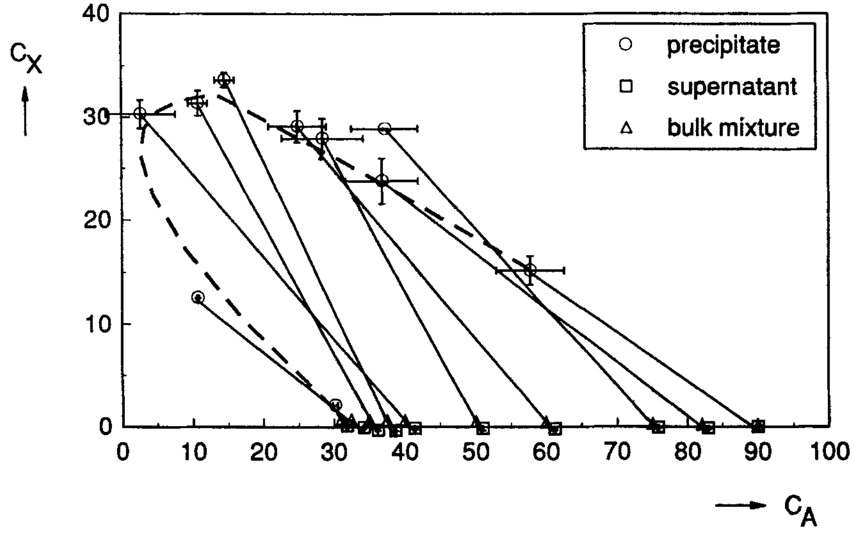

FIG. 8 The weight percent xanthan in the precipitate (C_X) vs the weight percent *tert*-butanol (C_A) for fermentation broths.

xanthan in the precipitate was highly dependent on the type of alcohol after the point where xanthan became completely insoluble.

In the EtOH precipitation experiments (Fig. 6), the maximum xanthan gum concentration in the precipitate was 12.1%, which is lower than the precipitate xanthan concentration (14.5%) in the pure solution experiments with EtOH (Fig. 3). In the precipitation experiments using IPA (Fig. 7), the highest xanthan concentration in the precipitate was 23.2%, which is nearly the same (23.5%) as in the IPA recovery of pure solutions (Fig. 4). The maximum precipitate xanthan concentration for the tBA experiments was 33.6%. This result also closely matches the maximum (33.5%) observed for pure solutions precipitated with tBA (Fig. 5).

The fermentation broths had lower xanthan concentrations than the pure solutions (1 vs 1.7%). This difference did not seem to affect the solubility transition. However, the lower precipitate xanthan concentration in the fermentation broth precipitation experiments using EtOH may be due to the lower initial xanthan concentration. Nevertheless, the trend of higher xanthan concentrations in the tBA precipitates than in the IPA or EtOH precipitates can still be seen.

In their precipitation experiments on fermentation broths using IPA, García-Ochoa et al. (13) did not report the concentration of xanthan in the precipitate phase.

CONCLUSIONS

Binodal solubility curves comparing the behavior of xanthan gum in mixtures of water and either EtOH, IPA, or tBA were presented for both pure solutions and fermentation broths. It was established that the mass and texture of the xanthan precipitates was dependent on the initial alcohol concentration. It was also determined that precipitation using EtOH required the highest alcohol usage and resulted in the lowest xanthan concentration in the precipitate. Conversely, tBA required the least alcohol for precipitation and formed the precipitates highest in xanthan concentration. Finally, it was found that precipitation from fermentation broths closely resembled precipitation from pure solutions which contain salt.

NOMENCLATURE

C_A	weight percent of alcohol
C_{A0}	weight percent of alcohol in bulk-mixture
C_X	weight percent of xanthan
M_{PRE}	mass of wet xanthan precipitate (g)
M_{X0}	initial mass of xanthan in bulk-mixture (g)

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