CELLULOSE CHEMISTRY AND TECHNOLOGY

PHASE SEPARATION IN XANTHAN SOLUTIONS

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Paper dedicated to Professor Bogdan C. Simionescu
on the occasion of his 70th anniversary

The effect of ethanol (Et), isopropanol (IP) and NaCl addition on xanthan gum (XG) separation from aqueous solution was investigated by turbidimetry at 25 °C. The turbidity started to increase in the presence of alcohols (above 10% Et and 5% IP), independently of the XG concentration in the initial aqueous solution. In contrast, the NaCl content, which determines an increase of the turbidity, was dependent on the XG quantity according to an established relationship. Upon addition of the alcohols, phase separation into a polymer-poor phase and a polymer-rich one (with gel-like behaviour) was observed for both XG/water/Et and XG/water/NaCl/IP systems. After one week at rest, the gel-like phase of XG/water/Et system was more structured compared to that of XG/water/NaCl/IP system, in which XG precipitate was observed. NaCl addition reduced the alcohol quantity required to determine the separation of XG due to the enhancement of the polymer–polymer interactions in the presence of salt. The reduced viscosity of XG/water/Et system, higher than those of XG/water/NaCl/IP (at a constant XG/NaCl ratio), has also confirmed that the XG chains present a more compact conformation in the presence of salt.

Keywords: xanthan, turbidity, phase separation, salt, alcohol, viscosity

INTRODUCTION

In the last years, xanthan gum (XG) received great attention from both researchers and manufacturers due to its non-toxicity, biocompatibility, biodegradability and acceptable cost, which permit its use in various applications. In addition, the high viscosity at low concentrations and the rheological properties of its solutions make XG applicable as a thickener in food,[1,2] cosmetics,[3,4] pharmaceutical and biomedical products[5-7] *etc*., as a rheological control agent in aqueous systems for water-based paints or as a stabilizer for emulsions or suspensions in the food industry.[8,9] The role of XG as a thickener and stabilizing agent in food formulations has been investigated in different combinations with guar gum,[10] rice starch,[11] amidated low methoxyl pectin,[12] chitosan,[13] gelatin B,[14] soy protein hydrolysate[15] *etc*.

Various formulations based on mixtures of XG with neutral or charged polymers have been developed in order to extend the range of applications of this polysaccharide. The usual modifications of biochemical parameters and the morphological changes induced by neomycin sulphate can be excluded by its administration together with a XG/chitosan hydrogel complex, resulted from the interaction of the amino groups of chitosan with the anionic ones of XG.[16] Knowing the capacity of XG to improve the viscoelastic properties of hydrogels and its bioactivity, Sehgal *et al*.[17] developed a bioactive tissue-engineered scaffold containing xanthan, gellan and chitosan nanoparticles for bone regeneration. The eco-friendly hydrogels with superabsorbant properties were prepared by including different amounts of XG in a polymer matrix obtained by chemical crosslinking of cellulose in different allomorphic forms.[18] The literature reports many investigations on the mixtures of XG with natural (chitosan,[19] guar gum,[20] xyloglucan[21] *etc.*) or synthetic (Pluronic F127,[22] poly(ethylene oxide),[23] PVA[24] *etc.*) polymers in order to elucidate the nature of the interactions established between the polymer chains that are responsible for the synergistic properties observed for these systems.

XG is an anionic natural polysaccharide produced mainly from glucose by *Xanthomonas campestris* bacteria. The macromolecules have the main chain with the structure of cellulose (β-1,4-linked D-glucose units), to which side chains of trisaccharides (two mannose and one glucuronic acid) are attached. Due to its structure, strong polymer-polymer interactions are established between XG chains or with other polymers in multicomponent systems. In aqueous solution at 25 °C, the XG backbone is extended, but in partially ordered form (randomly broken helix), due to the two types of possible interactions between the chains: electrostatic repulsive interactions and attractive interactions by hydrogen bonds.[25] XG undergoes a transition from the ordered to the disordered conformation as a function of the external conditions (temperature, addition of salt, pH, solvent quality) and the structural factors (acetate and pyruvate substituent content).[26-28] The nature of ordered and disordered structures, as well as the conditions in which XG chains adopt one or another of these conformations, are still a matter of debate.

For applications in the food industry, the knowledge of the effect of different additives (for example, salt or alcohol) on the viscoelastic properties and on the stability of the systems containing XG is very important. The decrease of xanthan solubility in water can be realized by the addition of salts,[27,29,30] water miscible non-solvents[28,31-33] (for example, methanol, ethanol, isopropanol, acetone) or salt/non-solvent mixtures,[34,35] and by increasing the solution concentration.

By addition of a non-solvent (ethanol, isopropanol or tert-butanol), the direct precipitation of XG from its aqueous solution does not occur. The following stages were observed as a function of the composition of the solvent/non-solvent mixture: 1) up to about 30% alcohol in the alcohol/water mixture XG does not precipitate; 2) between 30% and 40% alcohol (as a function of the nature of the alcohol), the solubility decreases leading to the formation of two phases: a XG-poor phase and a XG-rich one with gel-like properties; 3) for 50%-75% alcohol, a clear separation of the liquid and gel phases was obtained; 4) for an alcohol content higher than 75%, a brittle precipitate, difficult to separate by centrifugation, is formed.[31]

The presence of salt in the XG aqueous solutions decreases the amount of alcohol required to determine the precipitation of the polymer. The conformational changes and polymer-polymer or polymer-solvent interactions from the XG solutions are influenced by a large number of factors, such as alcohol structure, pH, temperature, addition of salts and concentration of components in the mixture.

The study of stability of XG aqueous solutions in the presence of salt or alcohols represents an important topic for research, as well as for practical applications, because many food formulations or personal care products include salt and/or alcohol. In this context, the purpose of this paper is to investigate the phase separation occurrence in XG in water/ethanol and water/NaCl/isopropanol mixtures by turbidimetry and to analyse the viscometric behaviour in the presence of non-solvent or salt. In addition, visual observations will be made to support the conclusions obtained by turbidimetry and viscometry concerning the phase separation in the studied XG solutions.

EXPERIMENTAL

Materials

A xanthan gum (XG) sample was provided by Sigma-Aldrich and it was used as received. The viscometric molecular weight of XG, determined previously,[27] was 1.165 $\times 10^6$ g/mol. NaCl, ethanol (Et) and isopropanol (IP) were also purchased from Sigma-Aldrich and were used without further purification. Homogeneous XG aqueous solutions, with concentrations between 0.003 g/dL and 0.4 g/dL, were prepared by the dissolution of the polymer in Millipore water (obtained from a Milli-Q PF apparatus) at room temperature under gentle stirring.

Viscosity measurements

The viscometry measurements were carried out at 25 °C, using an Ubbelohde viscometer of type I with a capillary diameter of 0.63 mm, and by sequential dilution. The viscometer was connected to an automatic system Lauda LMV 830 Instrument, Germany. Two XG solutions, with the concentration of about 0.02 g/dL in water/Et and water/NaCl/IP mixtures, were prepared. One solution was diluted with water/Et mixture (11% Et) and the second one was diluted with water/IP mixture (6% IP), keeping the XG/NaCl ratio constant during the viscometric measurements. The quantity of NaCl was calculated by using the relationship between XG and NaCl, as determined from the experimental data reported in the present paper.

Turbidimetry investigations

The turbidity measurements were performed on a HACH 2100AN turbidimeter in the range of 400-600 nm wavelengths. For turbidimetry measurements, a small quantity of NaCl solution (with set concentration) or alcohol (Et or IP) was added into XG aqueous solutions and, after each addition, the turbidity in terms of NTU (Nephelometric Turbidity Unit) was determined.

RESULTS AND DISCUSSION

The behaviour of XG in aqueous solution and the effect of different non-solvents on the phase separation represent subjects still discussed in the literature. The establishment of the conditions for XG phase separation requires good knowledge of the solution behaviour and of the mechanism involving polymer separation. The present paper is focused on the effect of non-solvent (ethanol, isopropanol) or salt (NaCl) addition on the separation of a native XG from aqueous solution at 25 °C.

Effect of Et addition on the turbidity of XG aqueous solutions

By addition of Et, the XG aqueous solutions exhibit an increase of the turbidity, which is more pronounced for the solutions with higher polymer concentration (Fig. 1).

The increase of turbidity starts around 10% Et (critical c_{Et}) and a plateau is reached after adding approximately 20%-30% Et in the system. The chemical structure of XG favours the formation of polymer–polymer interactions, which are intensified upon addition of Et. Above 10% Et, the number of intermolecular associations formed between the XG chains becomes so important that they determine the increase of turbidity. For the solutions with low XG concentration (below 0.01 g/dL), a slight shift to higher values of critical c_{Et} was observed. The XG precipitate obtained at higher XG concentrations swells in the liquid medium and it appears as a gel-like phase. In the literature, it was reported that the formation of weak gel structures can occur upon adding up to 30% Et into solutions with high XG concentration.[36] For the total precipitation of XG from solutions with concentrations higher than 0.3 g/dL, an ethanol volume to solution volume ratio of 6 was necessary. This ratio was found to be independent of the polymer concentration in the initial solution.[34]

Effect of IP addition on the turbidity of XG/NaCl/water mixtures

The effect of salt addition on the solubility of XG in water depends on the valence of the cations. Thereby, the salts with monovalent cations (for example, sodium) do not affect the XG solubility, in contrast to the salts containing polyvalent cations, such as calcium, aluminum, quaternary ammonium, which cause XG precipitation.[28,37] The use of quaternary ammonium salt in food applications is not indicated because of its toxicity.

In the present study, a slight increase of the turbidity was evidenced upon addition of NaCl into XG solutions with concentrations between 0.01 g/dL and 0.10 g/dL (Fig. 2).

In water at 25 °C, the XG chains are disordered or partially ordered and more extended because of the repulsion electrostatic forces between them.[25] The addition of NaCl screens the ionic interactions between COO^{-} groups on the side chains, the molecular dimensions of XG chains decrease due to the reduction of the electrostatic interactions when a more compact conformation is formed.[27]

For a highly pyruvated single-stranded XG sample, Muller *et al.*[38] observed that there are no molecular weight changes during the conformational transition induced by the salt addition, showing that the intramolecular interactions are responsible for this process.

From the plot of the XG amount (Q_{XG}, g) as a function of the NaCl quantity (Q_{NaCl}, g) corresponding to the point from which the turbidity starts to increase, the following dependence was obtained (inset of Fig. 2):

$$Q_{XG} = 0.0214 \cdot Q_{NaCl} - 0.0035 \qquad (1)$$

Although the addition of monovalent cations does not cause XG precipitation, the presence of these salts favours the polymer separation when the alcohol is added. Thus, a more efficient separation occurs by combining the effect of salt with that of alcohol on the chain conformation in aqueous solution and on the degree of association of XG chains. In addition, the quantity of the alcohol used for XG separation is decreased, when a certain amount of salt is added. In this context, the effect of adding IP into the XG solution, in the presence of NaCl, was investigated by turbidimetry.

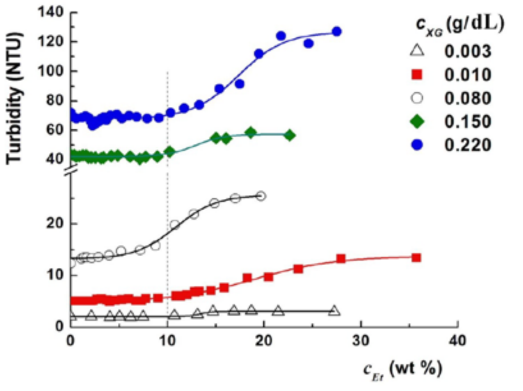

Figure 1: Effect of ethanol addition on the turbidity of xanthan aqueous solution

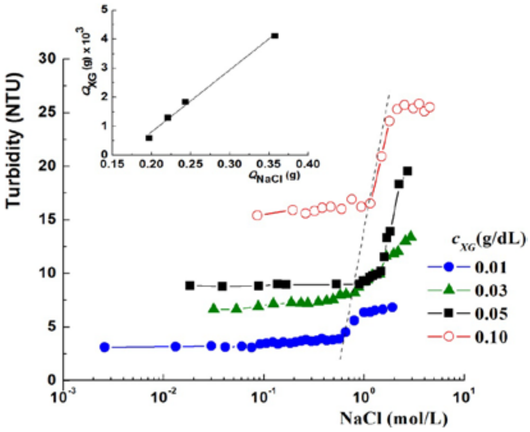

Figure 2: Turbidity variation by addition of NaCl for XG solutions with different concentrations; the inset figure shows the XG and NaCl amounts corresponding to the points where the turbidity starts to increase

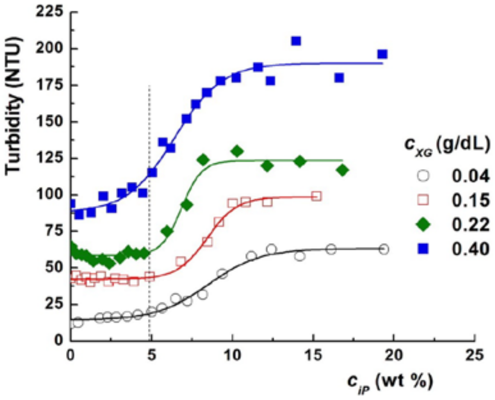

Figure 3: Variation of turbidity of XG/NaCl/water mixture by addition of IP

The amount of NaCl for each sample was calculated according to Equation 1 and different quantities of IP were added to each XG solution, which was further investigated with regard to turbidity.

Figure 3 shows the variation of the turbidity of salted XG solution upon addition of IP. An increase of the turbidity was observed at a critical concentration of IP, c_{IP}, which is independent of the XG concentration in the initial solution and it is located around 5% IP. Generally, the turbidity reaches a plateau above 11% IP.

García-Ochoa *et al.*[34] reported that the XG total precipitation from aqueous solution occurs at a non-solvent volume/initial XG solution volume ratio of 6 for Et, 3.2 for IP and 3.6 for acetone. The addition of divalent salts causes a higher reduction of the IP amount necessary to precipitate XG than the monovalent salt addition.

Phase separation in XG aqueous solution by addition of alcohol

The addition of alcohol to XG aqueous solution determines a decrease of polymer solubility and, above a certain content of alcohol, phase separation occurs.[33] In the presence of alcohol, the XG chains form a weak gel-like phase, due to the high polymer concentration, which favours the formation of entanglement networks.[39,40] The stability of the XG/water/Et systems up to about 60% Et in the water/Et mixture allows the use of XG as thickener for the products based on water/alcohol.[41]

Figure 4 illustrates the phase separation in XG aqueous solution by addition of 63% Et (in the absence of NaCl) and 42.5% IP (in the presence of a NaCl amount calculated by Equation 1). The mechanism that controls the XG separation from aqueous solution is still unclear. The addition of alcohol into XG aqueous solution reduces the water-XG interactions and, at the same time, it enhances the polymer–polymer interactions through the cations present in the medium, leading to aggregate structures that form microgels.

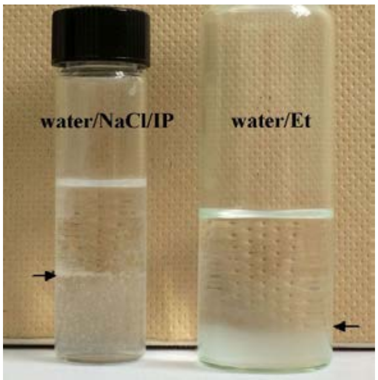

Figure 4: Phase separation in XG aqueous solution by addition of NaCl/IP (42.5% IP in water/IP mixture, left) and Et (63% Et, right); the NaCl amount for XG in water/NaCl/IP was calculated according to Equation 1

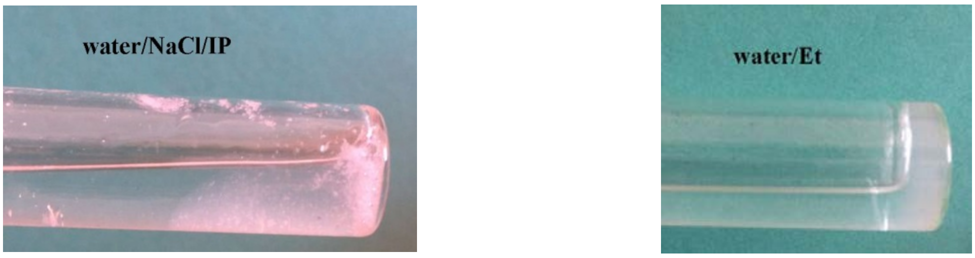

Figure 5: Images of the gel-like phases in XG aqueous solutions to which NaCl/IP was added (42.5% IP in water/IP mixture, left) and Et (63% Et, right) after one week at rest; the NaCl amount for XG in water/NaCl/IP was calculated according to Equation 1

By increasing the quantity of added alcohol, the aggregation of microgels occurs, leading to a network of microgels and a XG-rich phase (gel-like) appears. The change of the concentration of cations present in the medium can influence the XG phase separation from aqueous solution. Thereby, the addition of salt determines an increase of the number of cations in the system and the quantity of alcohol necessary for phase separation decreases. As can be seen in Figure 4, the XG separation was observed at about 42.5% IP in the presence of NaCl and at 63% Et when the salt is absent from the system. The degree of structuring of the gel-like phase after keeping the samples at rest for a week was observed visually (Fig. 5).

The presence of NaCl favours the appearance of XG aggregates visible in the weak gel-like phase formed by IP addition (Fig. 5, left). The gel-like phase obtained by adding Et shows a more compact structure as a result of the strong interactions established between free XG chains and the microgels formed in the system (Fig. 5, right).

Viscosity of XG solutions in water/alcohol mixture

The viscosity of the long XG chains in various media can be discussed taking into account the values of reduced viscosity (η_{sp}/c) defined as the specific viscosity η_{sp} (= η_{rel} – 1) related to the polymer concentration, c. η_{rel} is the relative viscosity defined as the viscosity of the polymer solution related to that of pure solvent and it is calculated as the ratio between the flow time of the solution and that of the solvent.

The viscometric measurements were performed in the dilute regime of concentration, characterized by relative viscosity values between 1.2 and 1.9, ensuring the existence of isolated macromolecules surrounded by solvent molecules.[42]

Figure 6 exhibits the dependence between η_{sp}/c and c at 25 °C for XG in water and water/alcohol mixtures in the absence/presence of NaCl. The NaCl amount was calculated with Equation 1, considering that the XG content in the system and the XG/NaCl ratio were kept constant during the dilution in the viscometric measurements.

It can be observed that the reduced viscosities for XG in water/NaCl/IP are twice lower than those obtained in water/Et, suggesting that the polymer–polymer interactions are more favourable in the presence of salt due to the conformational change.

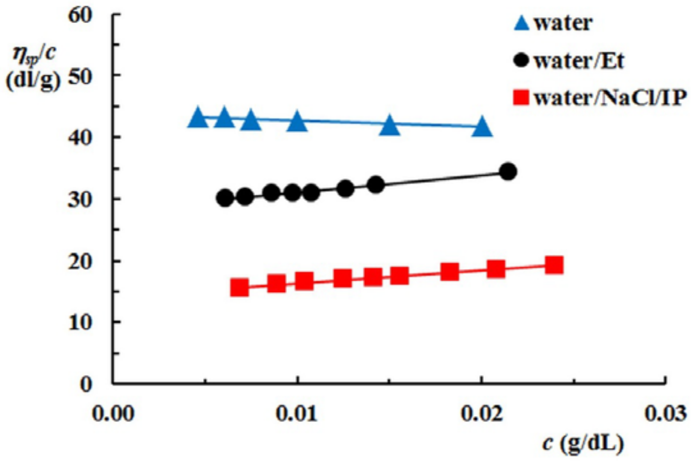

Figure 6: Dependences of η_{sp}/c *versus* c for XG in water[27] and water/Et, water/NaCl/IP (present data) at 25 °C

In Figure 6, the variation of η_{sp}/c for XG in water at 25 °C (previous data[27]) is also given in the concentration range where $\eta_{rel} > 1.2$. When the solvent is pure water, the highest values of η_{sp}/c are obtained as a result of the extended chains due to the electrostatic repulsion interactions between COO^{-} groups located in the side chains distributed along the XG macromolecule. These interactions cause the alignment of the macromolecules and their association by hydrogen bonding, leading to an ordered structure.

For salt-free polyelectrolyte solutions, a deviation from the linearity of the dependence of η_{sp}/c on c appears in the region with low polymer concentration (where η_{rel} values are very low), due to the electrostatic interactions established between the charged macromolecules.[27,43-46] Such deviations make impossible to determine the intrinsic viscosity, $[\eta]$, by extrapolation of the experimental data to zero polymer concentration by the traditional Huggins method. The $[\eta]$ value for XG in water, determined by a recently developed method by Wolf,[47] was 49.20 dL/g.

Phase transition in the XG/water/H_3PO_4 ternary system was investigated and three phases were distinguished: a completely separated phase, a liquid crystalline miscible phase and an isotropic miscible phase.[48] The optical and viscoelastic properties are very sensitive to phase transition phenomena.[48,49] The liquid crystalline phase presents a specific elastic behaviour during deformation,[49] the stored energy is much higher as compared to those of isotropic polymer fluids. The interactions responsible for the supramolecular XG structure are shrunk by reducing the ionic strength or by increasing temperature when an order-disorder conformational transition occurs. This transition is associated with a change of the solution viscosity due to a substantial decrease in hydrodynamic volume.[50]

Because of the complexity of the XG structure, the phase separation depends mainly on the macromolecular size and structure of the side chains (the pyruvate or acetate content), as well as on the adopted conformation that influences the supramolecular structure (ordered or disordered conformation). The conformational characteristics of the XG chains are strongly affected by the temperature, presence of salts, as well as solvent quality (which is varied by using solvent/non-solvent mixtures). All these parameters confer to XG special and attractive properties and they strongly affect the phase separation and purification of the polymer. Sanford *et al.*[51] evidenced the influence of the XG structure (pyruvate content) on the phase diagram in water/Et at 15 °C, in the presence of 1% w/v KCl, and achieved the precipitate with 20%-30% Et. Higher Et content improves the purity of XG, but does not increase the polymer yield. The effectiveness of precipitation methods is limited by the conformation of XG, which also restricts the composition range where each method is applicable.

Thus, the recovery process from the fermentation broths by precipitation in the presence of alcohols has the advantage of simultaneously isolating and purifying XG, but requires a high amount of alcohols.[31,36] The precipitation through NaCl addition improves this process, reducing the amount of alcohol required for polymer precipitation.

The high potential of XG for various applications[33,52-54] in food and food packaging, agriculture, oils, paints, cosmetics, controlled drug delivery or tissue engineering, justifies the efforts made to achieve a better understanding of the behaviour of this versatile polymer.

CONCLUSION

The effect of adding alcohol (Et or IP), in the absence/presence of NaCl, on the phase separation in XG aqueous solution at 25 °C was investigated and discussed. The turbidity of XG aqueous solution started to increase at about 10% Et in the water/Et mixture, due to the intensification of polymer–polymer interactions, which lead to the formation of aggregates and microgels. In the presence of NaCl, the cation content in the medium is modified, the polymer–polymer interactions are favoured and the quantity of alcohol that determines the phase separation became lower. The turbidity of XG/water/NaCl started to increase at about 5% IP in the water/IP mixture. The consistency of the gel-like phases formed upon addition of alcohol depends on the presence of salt. Thereby, after one week, the gel phase from the XG/water/Et system was more structured due to the strong interactions established between the formed aggregates. NaCl addition caused the formation of a weak gel-like phase, in which the XG precipitate was observed. The viscometry data confirmed the conclusions concerning the effect of NaCl and alcohol on the XG separation from aqueous solution obtained from turbidimetry and visual analysis. The XG/water/Et system showed reduced viscosity values higher than those of XG/water/NaCl/IP (at a constant XG/NaCl ratio) due to the electrostatic repulsion interactions between COO^{-} groups along the XG chains, which determine the extension of the macromolecules and their association by means of hydrogen bonds.

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