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# Physical Properties of Xanthan, Galactomannan and Their Mixtures in Aqueous Solutions

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SUMMARY: Some new information on the conformation of xanthan in aqueous solutions is given. A single helical chain conformation characterizes xanthan in the native state. When heated over the critical temperature for conformational change ( helix→ coil), the xanthan is denatured and renatured when it is cooled down in a localy double helical structure. A galactomannan was also characterized and its persistence length was obtained (Lp ≈ 90A°). Then mixtures of the galactomannan and xanthan were investigated to propose a mechanism for the specific gelation. From the results of microcalorimetry and circular dichroism, it is concluded that a complex is formed between one disordered xanthan chain and one galactomannan chain and that an ordered conformation is stabilized at temperatures lower than 25°C when the galactomannan has a M/G ratio of ≈ 3. This temperature corresponds to the sol-gel transition. This is the first time that a structure of the crosslink points is demonstrated.

## Introduction

There are many papers in the literature investigating the conformation of xanthan. Xanthan is a bacterial ionic polysaccharide produced by the bacterium *Xanthomonas campestris* ; it was the first bacterial polysaccharide produced on large industrial scale all over the world. It was recognized as a good thickening polymer in aqueous solution giving an original stability of the viscosity in the presence of external salt and having high intrinsic viscosity. This stability is related to the existence of a helical conformation with a relatively high persistence length1). In addition, it was early recognized that thermal treatments increase the viscosity of solution. The objective was then to relate this change in hydrodynamic behavior with change in the conformation2). A helix-coil conformational change is observed when temperature increases to a temperature $T_m$ which depends on the ionic strength as is usual with other charged

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biopolymers. But the helical conformation is still under discussion : some people claim that it is a double helix, others that it is a single helical chain[3,4]. This point is discussed in this paper.

Galactomannan has a simpler structure as no ordered conformation exists in aqueous solution ; it is a neutral polymer extracted from different leguminous seeds. Variability resides in the M/G ratio and distribution of galactose substitution along the mannan chain [5,6].

These two polymers are water soluble and are just thickening agents but when they are mixed, an original gelation occurs. The mechanism is still under discussion, but stronger gels are formed when the xanthan is in its disordered conformation; then the β1→4 glucose chain of xanthan should be able to form cooperative interaction (H bonds) with the galactomannan chain. Nevertheless, this mechanism is not generally admitted and interaction with ordered xanthan chains is also described in the literature[7,8]. This point is analyzed in this paper to better understand the mechanism of gelation.

## Experimental

**Materials**. Different samples of xanthan were used : first, a sample given by Rhône-Poulenc (Melle, France) isolated from unpasteurized fermentation broth. The native conformation (NX) was preserved during purification by using a salt content (NaCl) equal to at least 0.01M during the process[2]. The polymer was isolated in the sodium salt form.

An industrial sample ( powder form) " Keltrol RD " , was obtained from Kelco (San Diego, CA, USA). This sample was purified as previously described [9].

These two samples were also degraded by sonication to a lower molecular weight (~ $10^6$ ) to allow convenient steric exclusion chromatography (SEC). The temperature of conformational change was determined by optical rotation ; the ordered conformation was demonstrated by optical rotation or circular dichroïsm [10].

The galactomannan was extracted from the endosperm of *Schizolobium parahybae* (Vell.) Blake (M/G= 3)[11] . The extraction was carried out in water at 25°C. The supernatant was precipitated with one volume of ethanol and dried.

**Instrumentation.** SEC experiments were performed using multidetection giving directly the molecular weight distribution. The solvent was 0.1 M $NaNO_3$ in the presence of 0.2g/l $NaN_3$

as preservative at 25°C$^{12)}$. Viscosities of dilute solutions in the Newtonian regime were measured using a Low Shear 30 rheometer from Contraves ; for higher polymer concentrations, viscosity was measured with a Carrimed CS50 rheometer. Microcalorimetric experiments were performed using a MicroDSC III from Setaram. Circular dichroïsm spectra were obtained using the DC V instrument from Jobin-Yvon.

## Results and Discussion

The characteristic behavior of xanthan and galactomannan will be summarized using our last experimental data. Then, the mechanism of interaction of these two polymers on mixing will be discussed.

**Xanthan behavior.** The majority of our experimental data were obtained on the sample of xanthan directly isolated from the broth in such conditions that the native conformation is preserved. In this case, the single chain helical conformation was demonstrated at 25° C in salt excess greater than $10^{-3}$M, in dilute conditions$^{4,13)}$. Xanthan is well-known to present a reversible conformational transition (helix - coil) when an aqueous solution is heated, which is characterized by a characteristic temperature Tm .The values of Tm depend on the polymer concentration and on the external salt concentration reflected by the total ionic concentration ($C_T$ equiv.$L^{-1}$) of the solution, including the uncondensed counterions and the salt excess. In the presence of monovalent counterions, one found $^{10)}$:

$$Tm^{-1}(K^{-1}) = 2.16 \times 10^{-3} - 0.5 \times 10^{-3} \log C_T$$

Nevertheless, experimental evidence does not support the hypothesis of a single chain helical conformation $^{3)}$. More recently, from SEC experiments using a multidetectors, we have proposed the following steps $^{4)}$:

$$\text{Native} \xrightarrow{T\nearrow\ \mu\searrow} \text{Denaturated} \underset{T\nearrow\ \mu\searrow}{\overset{T\searrow\ \mu\nearrow}{\rightleftarrows}} \text{Renatured}$$

The renaturated conformation (RX) has the same molecular weight as the native conformation (NX) but a $M_L$ ($M_L$=mass per unit length) corresponding to a double helix; the conformational change, at least for sufficiently high molecular weight, is an intramolecular process[4,13] (Fig. 1). Then, when the native molecule is heated at a temperature higher than the temperature of conformational change (Tm), after cooling, it appears to be a double helix.

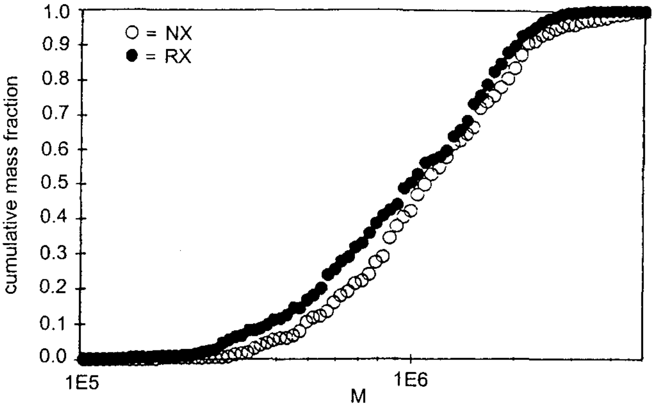

**Figure 1.** Cumulative molecular weight distribution for native and renaturated xanthan ( ref. 13 reproduced with kind permission from Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands)

The persistence lengths were determined to be about 300 and 1250 A°, respectively, for the native and renatured conformation from data obtained using the multidetection SEC equipment and the proposed analysis[4] (Fig. 2) . It must be mentioned that the temperature for conformationnal change is the same for the two conformations. This transition is relatively large, which indicates a low cooperativity.

**Galactomannan behavior.** This molecule presents no ordered conformation in solution and is neutral. The rheological behavior of its aqueous solutions is characteristic of a polymeric solution with a non Newtonian character at high concentrations and/or shear rates[11,14]. The specific viscosity at zero shear rate is represented by

$$\eta_{sp} = C[\eta] + k'(C[\eta])^2 + B(C[\eta])^n$$

as is usually found for single- chain polymers.

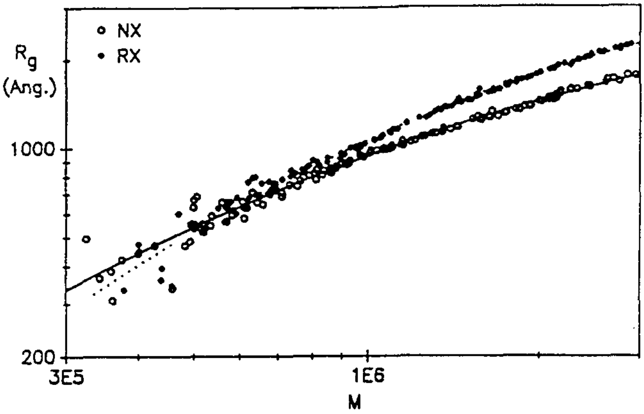

**Figure 2.** Radius of gyration as a function of the molecular weight for native and renaturated xanthan. —— and --------- are the theoretical prediction corresponding to $M_L$= 90 and 200 g/A° and Lp= 300 and 1250 A°, respectively, for the native and the renaturated xanthan ( ref.13 reproduced with kind permission from Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands).

Nevertheless, even if the molecular weight and the intrinsic viscosity ([η]) are moderated, high viscosity is observed at finite concentration in relation with a large n exponent. This exponent is often much larger than 3.4 due to interchain interactions (Fig. 3). The value of n increases when the M/G ratio increases (G= galactose unit; M=mannose unit) 11, 15). From SEC experiments, one obtains the values of $R_G$ as a function of the molecular weight for a galactomannan with M/G = 1. From this curve, using a wormlike chain model, one deduces a persistence length (Lp) of 93A°, in good agreement with the value calculated from molecular modeling 15).

**Xanthan-Galactomannan mixtures**. Neither polymer is a gel forming polymer, but when they are mixed, they form a loose gel. From viscosity measurements, it was proved in dilute solution that a large increase of viscosity, followed by gelation, is obtained for a composition of the mixture around 1/1(w/w). The strongest interaction was obtained when xanthan was in the disordered conformation. The better stability was obtained with a deacetylated xanthan 16). Then the role of the chemical structure and of the conformation of xanthan was demonstrated 17). The interaction of the mannan chain with the cellulosic backbone of xanthan was predicted by Millane 7) and Chandrasekaran 18).

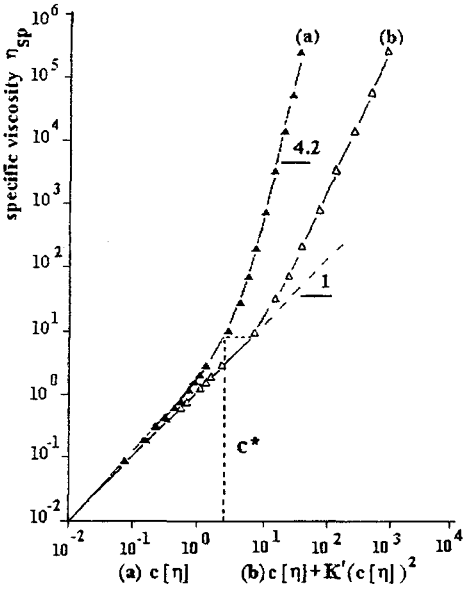

**Figure 3.** Variation of the specific viscosity in the Newtonian plateau as a function of the overlap parameter (a) C [η] and (b) C [η] + k' ( C[η] ) $^2$ ( ref.11 reproduced with kind permission from Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands).

In experiments performed on a galactomannan with a M/G ratio of 3 so as to get stronger interactions, rheological and microcalorimetric measurements were combined 19). When the two polymers are mixed at high temperature ( above Tm), the viscosity increases on cooling ; in the same conditions, two separated peaks occur in microcalorimetry. The higher temperature peak corresponds to the conformational transition of xanthan; the second one is located at 25°C and corresponds to gelation ( Tgel). This result indicates that a specific interaction exists between the two polymers. The position of the Tgel peak is independent of the ionic concentration. Its amplitude depends on the degree of xanthan helicity. A larger interaction occurs for a lower degree of helical conformation (Fig. 4 ). On the other hand, the peak corresponding to the conformational transition of xanthan moves in relation with the ionic content.

Using circular dichroïsm , it is demonstrated that, when xanthan , under disordered conformation, is mixed with galactomannan at a temperature lower than Tgel , a

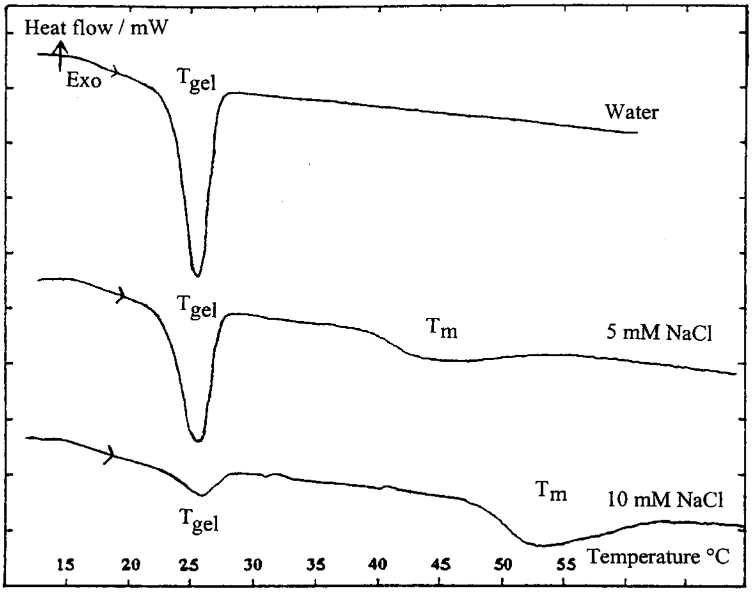

**Figure 4.** DSC thermograms obtained on heating (0.2°C/mn) of a xanthan (4g/L) + galactomannan (2g/L) mixture. (a) in water (b) in $5\times10^{-3}$ M NaCl (c) in $10^{-2}$ M NaCl . Tgel is the melting temperature of the gel, and Tm is the temperature of conformationnal transition for xanthan

conformational change is induced 19) ; it may be interpreted using the model recently given by Chandrasekaran and Radha18). A segment of double helical structure is formed involving one chain of xanthan and one chain of galactomannan.

The role of pH was also examined in absence of external salt. At low pH , xanthan is in an ordered conformation at 15 and 30°C ( fig. 5a) due to its low charge density; in these conditions, negligible interaction with galactomannan exists. During neutralization of the carboxylic groups, for xanthan alone, the coil conformation is induced. In the same conditions, it is shown from rheology that a gel is formed ( figure 6) and from CD experiments that a conformational change is induced that corresponds to the formation of an ordered complex between xanthan and galactomannan which collapses at Tgel ≈25°C (Fig. 5 b). The amplitude of the positive CD peak for the xanthan-galactomannan mixture (fig.5b) is much larger than that obtained for xanthan alone, meaning a difference in the ordered conformation formed in these two conditions.

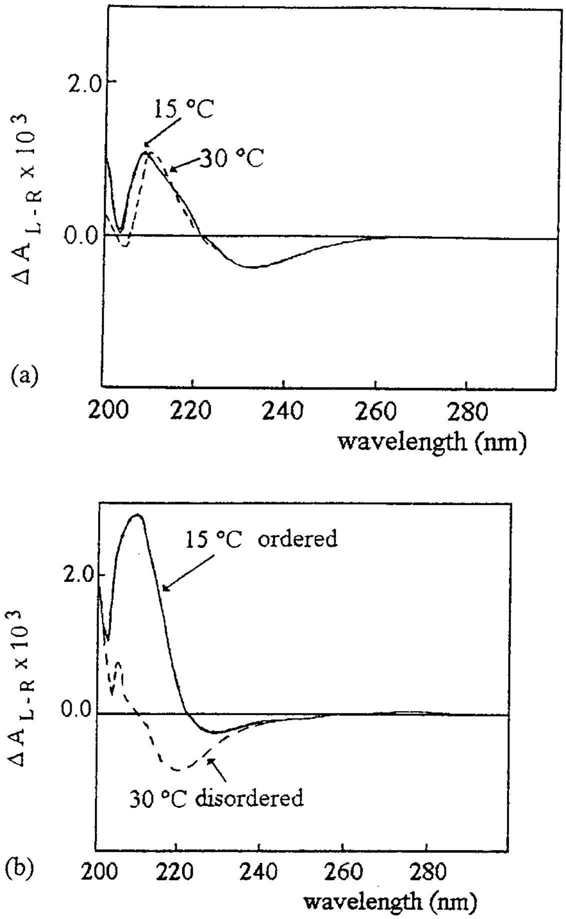

**Figure 5.** Circular dichroïsm spectra for xanthan-galactomannan mixture ( 1/1 g/L) in water at 15° and 30°C for the acid form of xanthan (a) and the neutralized form (b). The spectra (a) are similar to the spectrum obtained for acid xanthan alone at the same concentration.

## Conclusion

The mixture of xanthan and galactomannan with a low M/G ratio forms a loose gel, which formation depends on the thermodynamic conditions and on the structure of the polymers. Stronger interactions occur when xanthan is in the disordered conformation and when the galactomannan has a higher M/G ratio; but even in absence of free zones of mannose, a loose interaction occurs. The rheological properties combined with microcalorimetric data and circular dichroïsm in different conditions were analyzed. A new mechanism is proposed. It is based on mixed double helical blocks formed between the two polymers at a temperature around 25°C when M/G=3 that is independent of the polymer and the ionic concentrations. This mechanism agrees with the models recently proposed in the literature for the same system[18) and with data obtained on a very similar system [20). A stronger gel forms when the two polymers are mixed above the melting temperature of xanthan ; nevertheless, a looser gel is formed with ordered xanthan involving interaction with disordered fragments of xanthan or by a second mechanism in which side chains of ordered xanthan interact with galactomannan. These mechanisms are predicted in the paper by Chandrasekaran and Radha [18).

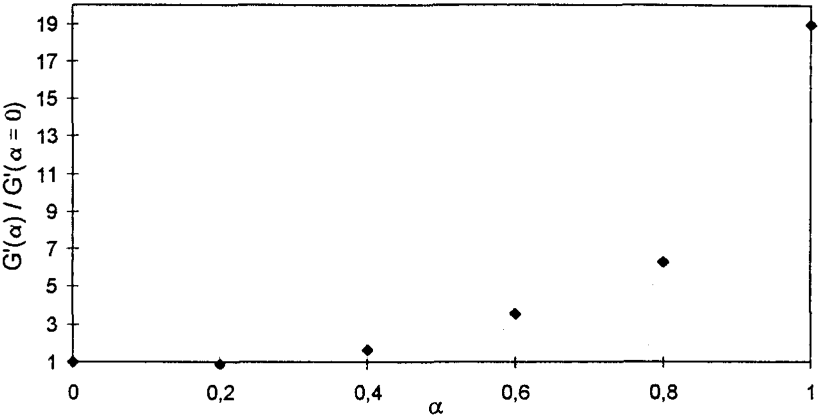

**Figure 6.** Change in the elastic modulus G'of the mixture xanthan/galactomannan (1/1 g/L) in water at 15°C and 1Hz as a function of the degree of neutralization (α) for xanthan; the reference point is obtained when the xanthan is in the acidic form ( α= 0).

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