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EFFECT OF CONCENTRATION, pH AND TEMPERATURE ON XANTHAN CONFORMATION: A PRELIMINARY STUDY BEFORE CROSSLINKING

Anca BEJENARIU,[a,b] Marcel POPA,[a *] Luc PICTON [b] and D. LE CERF [b]

[a] "Gh.Asachi" Technical University Faculty of Industrial Chemistry and Environmental Protection, Department of Natural and Synthtic Polymers, Bd. D. Mangeron, 71 A, 700050 Iasi, Roumanie
[b] University of Rouen, FRE 3101 CNRS, F-76821 Mont Saint Aignan, France

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Before to crosslink xanthan by adipic acidic dihydrazide in acidic medium and by sodium trimetaphosphate in alkaline medium, we have studied by rheology and dichroism circular the conformation of xanthan chains in concentrated condition and different temperature. We have shown that xanthan chains are always in double helix conformation in acidic medium. Otherwise in alkaline medium xanthan chains exhibit partially conformation transition to coils which is favourable by high temperature. These parameters should influence the final properties of obtained hydrogels.

INTRODUCTION

Hydrogels are three-dimensional networks, which absorb water by swelling without dissolution. They have found widespread applications in various domains. They are obtained by chemical or physical crosslinking of polymers.[1] Amongst the polymers, polysaccharides are interesting because of their biocompatible character and their possible biodegradation.[2,3]

Many research deals on adaptative hydrogels, which can entrap and release active compounds with controlled manner in function of extrinsic parameters (temperature, pH, ionic strength…). Usually, this control is obtained by specific function on polysaccharide backbone or on crosslink agent. For example, carboxylic functions can retain cationic compounds and can release their by modification of pH or ionic strength.[4,5] An amphiphilic character is necessary to solubilise hydrophobic compound in hydrophobic clusters. This character can be bring by alkyl pendant groups on polymer backbone[6,7] or by used amphiphilic crosslinker.[8,9] For obtain temperature dependent systems, the literature is abundant on the used of lower critical solution temperature (LCST) polymer as Poly(N-isopropylacrylamide) [poly(NIPAM)][10] or poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymers (Pluronic®).[11] Such polymer systems are given as « intelligent » or « smart » materials.

An other way consists of the crosslinking of polysaccharide, which presents a conformation transition order disorder. One of them is scleroglucan. Its main chain consists of (1→3)-linked β-D-glycopyranosyl units where every third unit bears a (1→6)-linked β-D-glycopyranosyl monomer. Chains are organized in triple helix, which can dissociate to single disordered chains in function of different effects: i) in water dimethyl sulfoxide mixture (water weight fraction $W<0.13$);[12] ii) by increasing the temperature above the triple-helix melting temperature $Tm=130°C$;[13] iii) in alkaline medium ($pH>13$).[14] This transition is irreversible. Scleroglucan was able to give strong physical gels in presence of borate anions ($B(OH)^-_4$). It has been shown that these hydrogels shrink in very alkaline media in reason of conformational transition.[15]

* Corresponding author: marpopa@ch.tuiasi.ro

Xanthan is an extracellular polysaccharide produced by *Xanthomonas campestris*. The backbone consists of D-glucose linked in β1-4 (as cellulose) with a trisaccharide side chain composed with α mannose, α glucuronic acid and β mannose attached on every second unit. The terminal mannose may be substituted by a pyruvate acetal and the inner mannose is frequently acetylated.[16]

The xanthan chain presents a reversible conformation transition between helix and coil in function of ionic strength, nature of added salt and temperature. This transition, well define in dilute regime is very sensitive to the ionic concentration and the temperature due to low intrachain secondary interactions.[17] It has been monitored by various techniques including optical rotation,[18] light scattering,[19] circular dichroism,[20] N.M.R.[21]

At room temperature, low concentration xanthan solution exhibits high viscosity and a pronounced pseudoplastic flow due to helical conformation stabilized by hydrogen bonds between backbone and side chain. In higher concentration (C>C*) xanthan solutions behaves as weak gel at low shear rates and in very high concentration (C>C**) these solutions could present liquid crystalline phase.[22]

Three dimensional networks can be obtained with different mechanisms. We can described hydrogels, which result of physical interaction between chains: (i) coacervation between anionic and cationic polymers as the system chitosan – xanthan;[23] (ii) ionic interaction between di- or trivalent cation and anionic polymer ac Cr^{III} or A^{III} xanthan;[24] (iii) synergetic behaviour between two polymers as galactomannan – xanthan.[25]

An other possibility is the action of di- or trifunctional molecule (crosslinker) on polymer, which gives chemical hydrogel. These hydrogels are very abundant in reason to the high diversity of crosslinker.

In literature only xanthan crosslink with epichlorhydrine is described.[26] This hydrogel is obtained in alkaline medium at high temperature.

We want to synthesize new adaptative hydrogels capable to entrap cationic or hydrophobic compounds. Consequently we have used two crosslinker : trisodium trimetaphosphate which bring phosphate groups[27] and adipic acidic dihydrazide[28] which bring hydrophobic clusters formation. Crosslinking reaction realizes in acid medium with adipic acidic dihydrazide and alkaline medium with sodium trimetaphosphate.

The objective of this paper was to know the conformation of xanthan macromolecules in high concentration, which is necessary to obtain effective crosslinking.[29] By rheology and circular dichroism (CD) spectroscopy, we have studied the effect of pH and temperature. At the view of these results, syntheses of hydrogels with different crosslinkers will realize with xanthan in different conformation to obtain xanthan hydrogel with different internal structure.

RESULTS AND DISCUSSION

1. Choice of experimental conditions

Preliminary synthesis have shown that effective crosslinking reaction requires high concentration up to 15 $g.L^{-1}$ and the addition of salt up to 0.001 $mol.L^{-1}$ to screening phosphate groups grafted on polymer. It is important to note that the ionic strength is not constant in the different xanthan solutions. First of all the scale of concentration is large and ionic strength increases with the xanthan concentration due to counterions to the glucuronate and pyruvate residues (around 0.005 and 0.025 $mol.L^{-1}$ for 5 and 25 $g.L^{-1}$ respectively). Secondly the acid or alkali used for adjustment of pH also contribute to ionic strength (0.001 $mol.L^{-1}$ fro pH 3 and 0.1 $mol.L^{-1}$ for pH 13).

Concentrated xanthan's solutions were studied in two extreme conditions of pH (pH=3 and pH=13) and at temperatures between 25°C and 90°C by rheological and circular dichroism measurements, in order to established the correct hydrogel's synthesis parameters.

2. Rheological measurements

It is well know that in dilute solution the conformation of xanthan chains is dependent of ionic strength, pH and temperature. In concentrated regime, with the aim to synthesis hydrogels, oscillation measurements are greater adaptive.

Figures 1 and 2 show the curves of elastic modulus (G') and loss modulus (G'') vs frequency for xanthan solutions at 15, 20 and 25 $g.L^{-1}$ in function of temperature (25 and 90°C) and pH (3 and 13) in $LiNO_3$ 0.001 $mol.L^{-1}$.

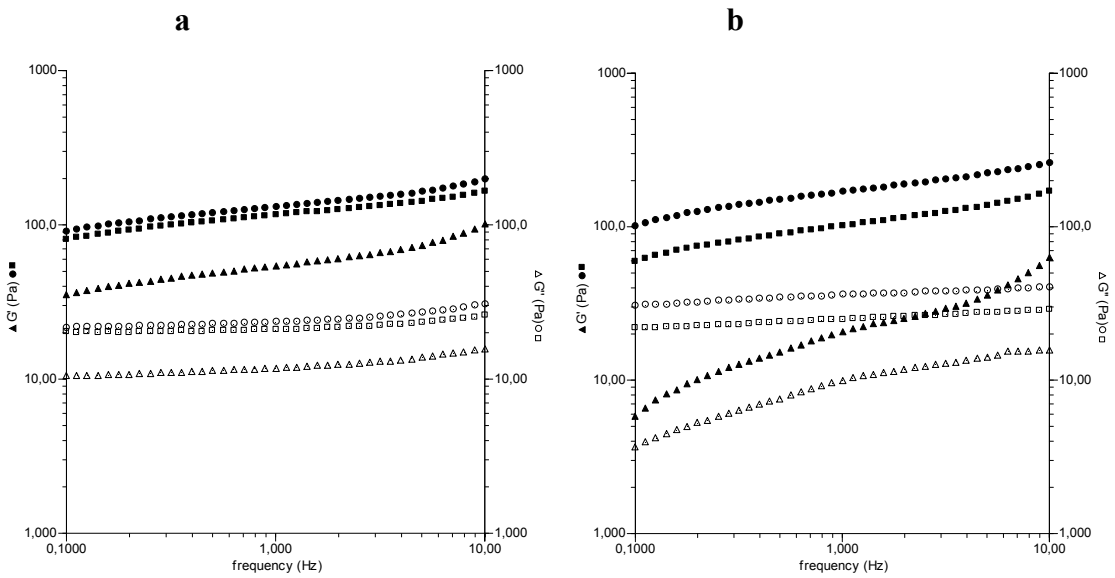

Fig. 1 – Dependence of the storage modulus G' (filled symbols) and the loss modulus G'' (open symbols) on frequency for xanthan solutions at different concentration (circle: 25 $g.L^{-1}$ - square: 20 $g.L^{-1}$ - triangle: 15 $g.L^{-1}$) Condition pH 3, and 0.001 mol L^{-1} $LiNO_3$ **a : 25°C b : 90°C.**

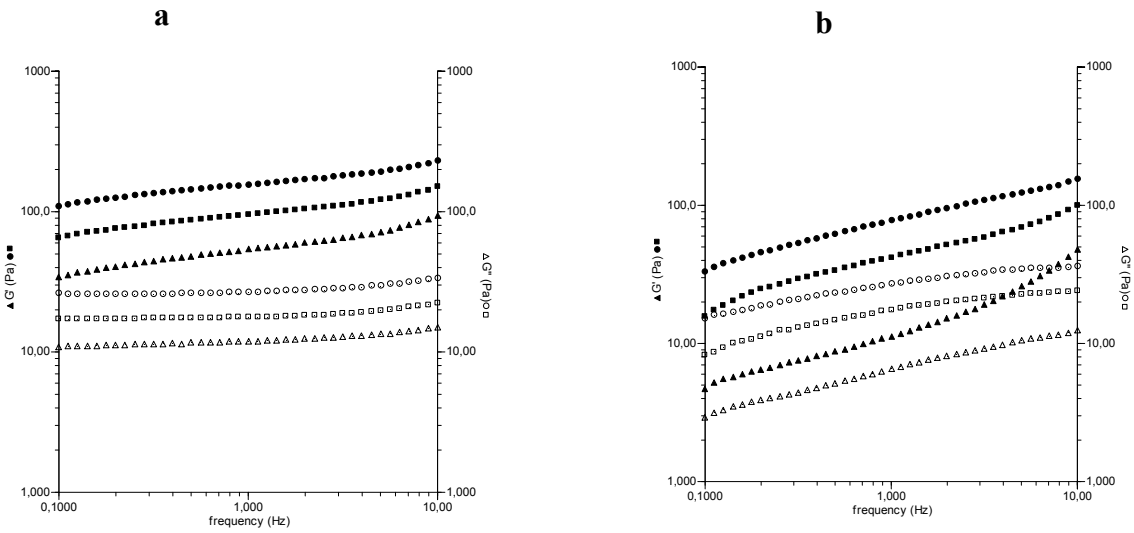

Fig. 2 – Dependance of the storage modulus G' (filled symbols) and the loss modulus G'' (open symbols) on frequency for xanthan solutions at different concentration (circle: 25 $g.L^{-1}$ - square : 20 $g.L^{-1}$ - triangle: 15 $g.L^{-1}$) Condition pH 13, and 0.001 mol L^{-1} $LiNO_3$ **a : 25°C b : 90°C.**

At 25°C, wathever pH, G' and G'' are constant in a large scale of frequency (figures 1a and 2a). At high concentration, all xanthan solutions exhibit pseudo-gel behaviour. When the measures are realized at 90°C, we observe a decrease of G' and G''. Thermal agitation facilities the chains mobility. At this temperature, the pH has an effect on the behaviour. In acid medium, at 20 and 25 $g.L^{-1}$ the pseudo-gel behaviour is conserved with only a very slight decrease of G' and G''. At 15 $g.L^{-1}$ a great dependence from frequency is observed and shows viscoelastic behaviour. In this condition, conformation transition is possible. In very alkaline media (pH=13), hydrogen bonds between backbone and side chain are destroyed. Wathever concentration, we observe viscoelastic behaviour. Coil conformation is preferential but high G' suggests coexistence of double helixes with coils and/or partially denaturation.

We have realized temperature rate between 30 to 90°C and measured G' and G'' (figure 3).

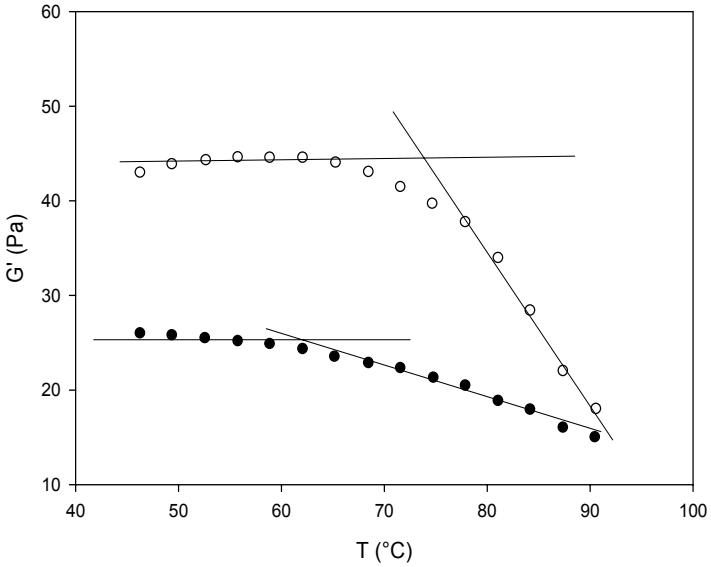

Fig. 3 – Temperature dependence of the storage modulus G' for 15g.L^{-1} xanthan solution at pH 3 (open square) and pH 13 (filled square) Condition 0.001 mol L^{-1} $LiNO_3$.

Before a temperature called Tm, G' is stable. We observed only a slight decrease of G', which is logical with the increase of temperature. At Tm, the decrease of G' is greater and is due to the transition of double helix in coil. In figure 3, we can compare the rheological response in function of pH. In alkaline media, G' is lower than in acidic pH according to a less stiff conformation. An other difference is Tm, which is lower in alkaline media. It's easy to break the hydrogen bonds. Presence of electrostatic repulsions is in favour of helix destructuration.

With the aim of compare Tm in function of external conditions, we have plotted in figure 4 Tm vs C for different pH (3, 7 and 13).

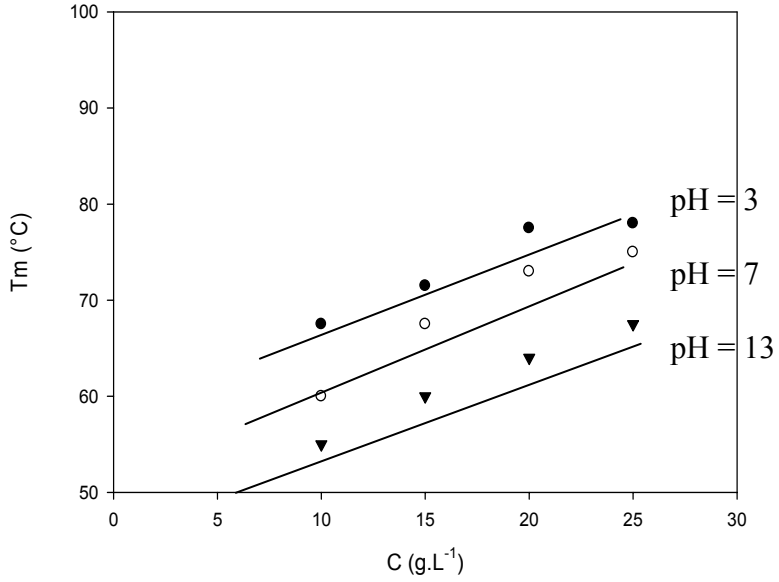

Fig. 4 – Tm vs xanthan concentration in different pH condition (filled circle: pH 3 ; open circle pH 7 ; filled triangle pH 13) Condition 0.001 mol L^{-1} $LiNO_3$.

We show that both concentration and pH have a great influence on Tm. High concentration reduces the mobility of chains that are entangled and increase the ionic strength that stabilized the helicoidal conformation. Consequently it is necessary to heat higher for destroyed hydrogen

bonds and lead to transition double helix to coil. For the pH, it is clear that alkaline media makes easier the conformational transition in spite of the high ionic strength.

3. Circular Dichroism

The CD spectrum can be used to monitor the conformational transition of xanthan.[17] For xanthan in ordered conformation, it is well know that CD spectrum exhibits a positive Cotton effect at 202 nm corresponding to the n $\rightarrow\Pi^*$ transition of the carboxylic function and a negative Cotton effect at 220 nm characteristics of the acetate function.

On figure 5, we can observe the effect of concentration and pH on the xanthan conformation. Whatever concentration, an alkaline pH decreases the ΔA_{202} intensity and shows that the formation of coils is favourable. At 5g.L^{-1}and pH = 13, $\Delta A_{202} = 0$.

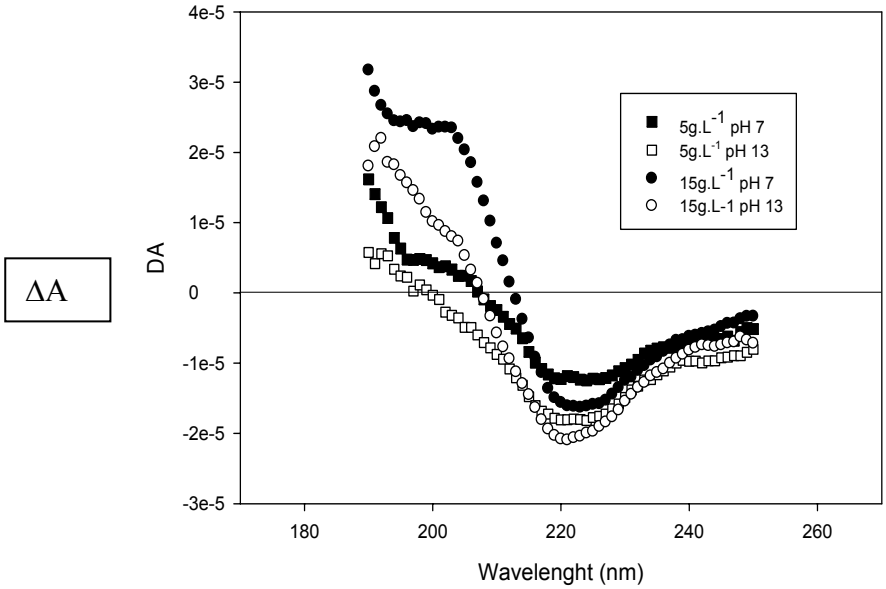

Fig. 5 – CD spectra of Xanthan solution in 0.001 mol.L^{-1} at 25°C in function of concentration (5 and 15 g.L^{-1}) an pH (7 and 13).

Electrostatic interactions happened in the macromolecule and among the macromolecules, which induced the conformation transition from order to disorder. In these conditions we can considered that all helixes are destroyed and have formed coils. With higher concentration, $\Delta A_{202} \neq 0$. Some order is persistent. Helixes are present with coils and/or helixes are partially denatured.

EXPERIMENTAL

1. Materials

Xanthan was graciously purchased from Rhodia (RHODICARE S). Its weight average molecular weight, Mw, determined by size exclusion chromatography with on line multi angle laser light scattering, was found to be about 2.10^6 g/mol. $LiNO_3$, NaOH, and HCl obtained from Acros Organics were of analytical grade.

2. Sample preparation

The xanthan is added gently in water and stirred 12 hours. Then the salt ($LiNO_3$ for 0.001 mol.L^{-1}) is added and the solution is stirred again 12 hours. If necessary, NaOH or HCl (1 mol.L^{-1}) is added to adjust pH. Reological and Circular dichroism measurements were made just after the pH adjustment. No hydrolysis of pyruvate and acetate occurs.

3. Rheological measurements

Rheological measurements were performed with a controlled stress rheometer (AR2000, TA instruments) in cone plate geometry (2° and 2 cm) with a solvent trap. Samples were loaded onto the lower plate with a spatula. Oscillatory dynamic experiments were done in the frequency range 0.1 to 10 Hz. A stress sweep was run prior to ensure that the stress chosen for the frequency sweep was in the linear viscoelastic regime. For all measurements, the stress was fixed between 0.2 and 0.5 Pa. The results are expressed in terms of the storage modulus (G') and the loss modulus (G'') which describe respectively the deformation energy stored (elastic response) and loss (viscous response) in the material.

To study the temperature dependence and the transition double helix – coil, a constant oscillation (0.1Hz) was applied and the moduli were measured as a function of temperature.

The temperature rate was choose to 0.56°C.min^{-1} and the Tm is obtained at the cross of the two straight line of G' (quite horizontal line before the transition and steep slope during the transition (see figure 3).

4. Circular dichroism

CD spectra were measured using a DC III Jobin Yvon spectrometer (France) over a range 195-250 nm with a 0.1 cm path length of rectangular quartz cell at 20°C, controlled by a thermostatic bath. Three scans were averaged per spectrum.

CONCLUSION

Rheological and circular dichroism results demonstrated that in acid media the xanthan's macromolecular chains are in double helical conformation in concentrate solutions. The synthesis reaction in acid media may crosslink the helixes between them. If the alkaline pH and high temperature indicate the presence of coils in dilute xanthan's solutions, under the same conditions in the case of concentrate xanthan's solutions there is a coexistence helix-coils. The synthesis reaction in alkaline media may crosslink two helixes or two coils or a helix with a coil. Different synthesis media's conditions will lead to different comportment networks.

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