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On the Role of Thermal Treatments on the Properties of Xanthan Solutions

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ABSTRACT

The aim of the present study was to determine the effect on the xanthan molecule of the thermal treatment applied to the xanthan broth after the fermentation processes. The influence of xanthan and salt concentrations, pH, temperature and time were tested. After the treatments, the xanthan was characterized by measuring the broth and dilute solution viscosities, the intrinsic viscosity, the molecular weight and the substituent contents.

Three mechanisms are suggested to explain the experimental results. The viscosity increase may be due to the conversion of the native ordered xanthan conformation to a renatured more viscous one, or to aggregation. The aggregates seem to be reversible when they are formed from the native ordered xanthan conformation and irreversible from the denatured one. The loss in viscosity is due to xanthan degradation; this process increases with the temperature and time of treatment when the temperature is higher than 60°C.

INTRODUCTION

The role of thermal treatment on xanthan in dilute or semi-dilute aqueous solutions is still under discussion. Usually, on commercial samples, a thermal treatment is applied to the broth for sterilization. It is known to have a role in the increase of viscosity of the final polymer (Jarry *et al.*, 1987). On the other hand, it has been shown by some authors that thermal treatment can decrease the molecular weight of xanthan (Glass *et al.*, 1983; Parsons *et al.*, 1985). In that respect, it was demonstrated that the thermal stability of the polysaccharide is higher

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when it is in the ordered conformation, i.e. in excess of external salt (Wellington, 1980; Ash *et al.*, 1983; Lambert & Rinaudo, 1985; Seright & Henrici, 1986; Foss *et al.*, 1987).

Nevertheless, it has been demonstrated that the first effect of heating at temperatures above the temperature of the conformational change, T_m, is to induce, after cooling, a change in the stiffness of the molecule, for which intrinsic viscosity increases greatly (this conformation is called the renatured form) compared with the native state (Milas & Rinaudo, 1986).

In this work, the role of the thermal treatment, up to 130°C on xanthan solutions, is examined for various polymer concentrations and ionic contents.

EXPERIMENTAL

The thermal treatments are performed directly or after dilution on two different non-pasteurized broths (I and II) produced by Rhône-Poulenc Co. The initial concentration of the broths was around 30 g/litre in polymer. The exact concentrations were determined from the recovered weight after precipitation of a given volume of broth (Milas & Rinaudo, 1986). A knowledge of the polymer broth concentration allows them to be diluted to 1 or 0·5 g/litre for viscosity determinations.

All thermal treatments were performed on 5 ml of broth in 10 ml capped pyrex tubes of 1 cm diameter immersed in a glycerol bath at a controlled temperature; the pH was adjusted with H_2SO_4 or NaOH before heating and the role of the salt content was examined by addition of 1·5% Na_2SO_4 (by weight) to the broth. The reaction was stopped by immersion in cold water. The viscosity of the broth was obtained with a Carrimed CS50, equipped with cone and plate geometry ($\alpha = 4°$; diameter 2 cm). The different experiments are described in Fig. 1. The viscosity of the solutions after dilution to 0·5 g/litre (or 1 g/litre) with $2{\cdot}5 \times 10^{-3}$ M Na_2SO_4 were determined in the low shear-rate Newtonian region with a Low Shear 30 viscometer from Contraves at 25°C. Before heating, xanthan in these solutions is in the native treated state. After heating above T_m (the temperature of conformational transition) and cooling, xanthan will be in the renatured treated state. Whatever the initial pH, after dilution at 25°C with $2{\cdot}5 \times 10^{-3}$ M Na_2SO_4, the melting temperature, T_m, of the ordered conformation is never obtained at ambient temperature. Thus xanthan remains in the native treated state. As T_m (checked by optical rotation for all the native treated state solutions) is around 60°C, after heating these solutions to 80°C, the renatured

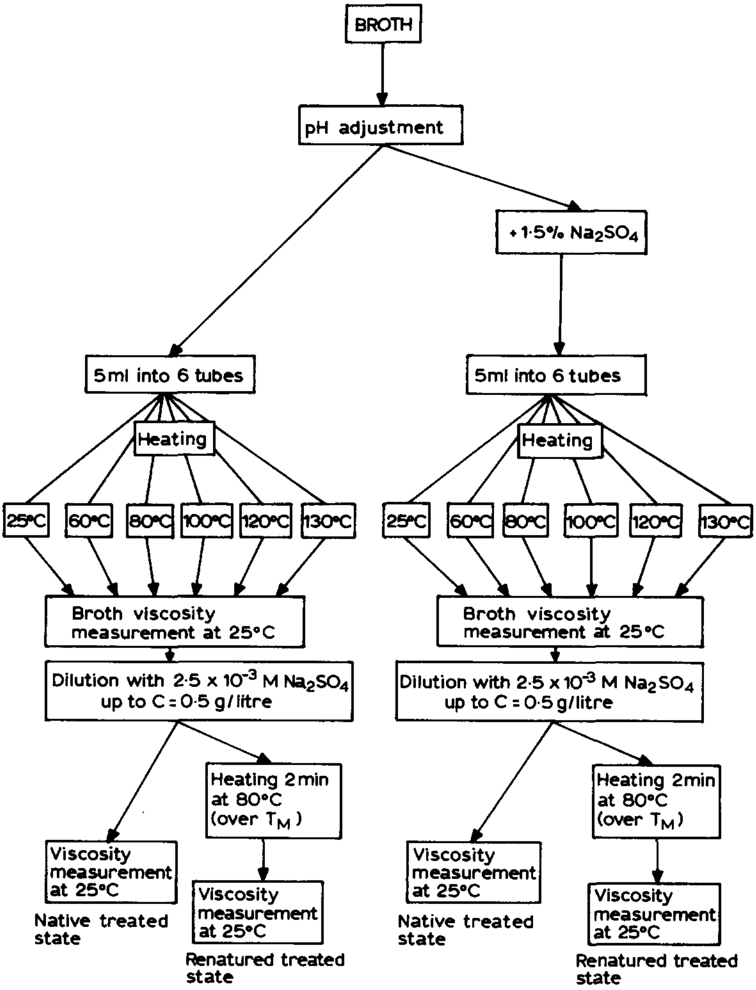

Fig. 1. Experimental design.

treated state is always obtained after subsequent cooling to ambient temperature.

For intrinsic viscosity and molecular weight determinations, solutions of purified xanthan in the sodium salt form were prepared in 0·1 M NaCl. The average molecular weight, $\bar{M}_w$, of the initial and treated samples were obtained by light-scattering measurements, with a Chromatix KMX 6 (Milas & Rinaudo, 1986). Relative viscosities in the low shear-rate Newtonian region have been measured with a Low Shear 30 viscometer from Contraves. The corresponding, reduced specific viscosities have been extrapolated to zero concentration to yield the intrinsic viscosity $[\eta]$. The xanthan concentrations used for $\bar{M}_w$, and $[\eta]$ determinations were in the range 0·150 to 0·0125 g/litre. Before measurement, all the solutions were filtered through a 0·2 μm Millipore filter. These filtrations were used as a test of filtrability: the viscosity of the initial solution (0·150 g/litre in xanthan) was compared with that of the filtered one; if there was some decrease in viscosity, or in flow rate, the sample was considered to contain aggregates which were causing the plugging of the filter membrane.

RESULTS AND DISCUSSION

Taking into account that thermal degradation is directly related to treatment time (Callet, 1987), a 10 min heat treatment was adopted, except for the measure of the residual substituents when the treatment was increased to 20 min in order to increase the accuracy of the determination.

The experimental data were obtained on two different broths (referred to as I and II). The characteristics of the corresponding xanthans are given in Table 1.

Role of the temperature and pH of the treatment

The separation of the effect of conformational change from that of molecular weight on the viscosity, during thermal treatment, was attempted as follows. The broths were heated, then cooled to 25°C and/or directly diluted to constant polymer concentration (0·5 g/litre or 1 g/litre) with Na_2SO_4, and the viscosity was measured to determine the degree of degradation. These diluted solutions were heated again above

TABLE 1
Characteristics of the Xanthan Used

Broth	$\bar{M}_w \times 10^{-6}$	$[\eta]_{native}$ *(ml/g)*	$[\eta]_{renatured}$ *(ml/g)*	$\eta_{rel}{}^{a}$ *(0·5 g/litre)*	*Pyruvate (%)*	*Acetate (%)*
I	8·9	13 600	17 000	155	38	70
II	7	9 000	13 000	35	20	50

[a] The relative viscosity in the renatured conformation is given at 25°C.

T_m and cooled to 25°C to investigate whether the conformational transition occurred, or not, during the initial heating. The scheme for these tests is given in Fig. 1. The main results are given in Figs 2 and 3.

The initial value, taken as the reference for the untreated broth, corresponds to the points at 25°C. The temperatures given on the figures are those of the thermal treatments. All the viscosity measurements were performed at 25°C. The role of the ionic content and nature of the electrolyte added is of secondary importance. (Callet, 1987).

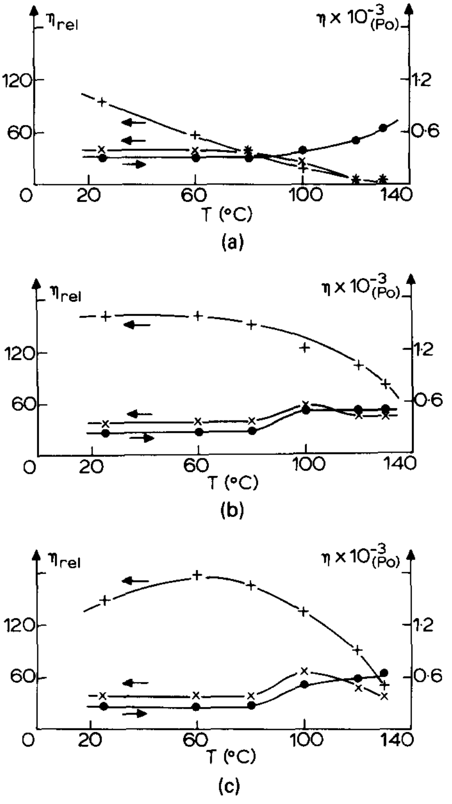

Fig. 2. Viscosity of Broth I (●) (expressed in poises) and relative viscosity of the native (×) and renatured (+) treated states at $C_p = 0{\cdot}5$ g/litre and 25°C (see Fig. 1) after heating the broth at different temperatures (T) and pH. (a) pH 4; (b) pH 7; (c) pH 8; time, 10 min, without salt.

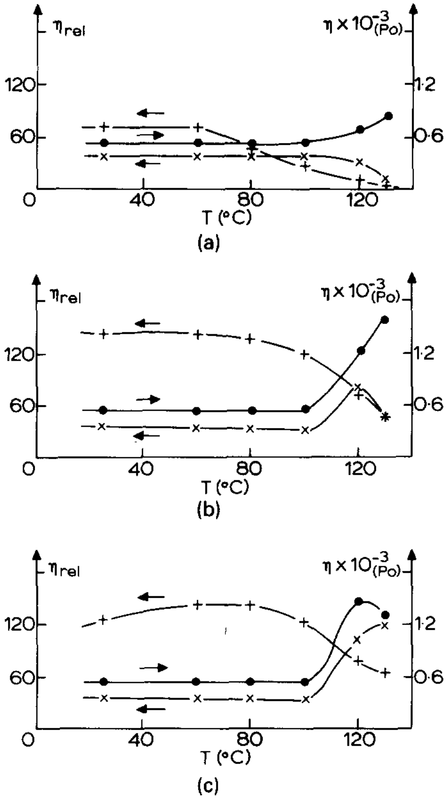

Fig. 3. Viscosity of Broth I (●) and relative viscosity of the native (×) and renatured (+) treated states at $C_p = 0{\cdot}5$ g/litre and 25°C (see Fig. 1) after heating the broth at different temperatures (T) and pH. (a) pH 4; (b) pH 7; (c) pH 8; time, 10 min, in the presence of 1·5% w/w Na_2SO_4.

From Figs 2 and 3 it is clear that the pH has a large effect on the viscosity. Better stability is obtained at pH 7 or 8. Usually an increase of viscosity of the broth and of the native heated form is obtained for the higher temperature treatments as mentioned by Jarry *et al.* (1987). However, after dilution and a conformational transition, the depolymerization is demonstrated by the decrease in viscosity obtained in the renatured treated state. In this way, it is demonstrated that thermal treatments of the broth, even just over 60°C for 10 min at pH 7, induce a decrease in viscosity due to polymer degradation. Then, over 100°C, at least two processes occur. The first is a degradation of the polymer causing a decrease in viscosity of the renatured form. The second is an increase in viscosity, due to a possible aggregation discussed in the following which explains the increases in the viscosity of the undiluted or diluted broth (native treated state) usually observed under these conditions. In some cases, depending on the origin of the broth, some gelation was observed in presence of excess of salt after thermal treatment (Callet, 1987). If a partial melting of the native conformation, in the experimental conditions adopted for the thermal treatment, occurs, it is masked by the two processes mentioned previously.

The presence of excess external salt increases the zone of viscosity stability by up to 20 degrees. It also produces gel formation in higher temperature treatments. In addition, when pH 8 is fixed initially, a decrease in pH is observed after thermal treatment. This is due to hydrolysis of the acetyl substituents (Milas *et al.*, 1988).

For some samples, the chemical modifications resulting from the thermal treatments were tested (Table 2). These results confirm previous conclusions (Milas *et al.*, 1988). During heating in acidic pH, the pyruvates are hydrolysed but, under basic conditions, the acetates are liberated and the pH decreases. In the absence of pH adjustment (broth pH = 5·7), hydrolysis of pyruvate is also observed to a lower extent.

TABLE 2

Chemical Modifications of the Xanthan after Thermal Treatment at Different pH (time, 20 min; temperature, 100°C; polymer concentration, 30 g/litre) (Broth II)

Substituent	*pH*			*Initial sample*
	4	*5·7*	*8*	
Acetate[a]	0·40	0·50	0·26	0·50
Pyruvate[a]	0·10	0·15	0·20	0·20

[a] Average number per xanthan side chain.

Role of the polymer concentration

Thermal treatments for 10 min at pH 7 and different temperatures were applied to polymer solutions with different polymer contents and Na_2SO_4 concentrations lower than $2{\cdot}5 \times 10^{-3}$ M. The results are given in Fig. 4. The data compare the native and renatured states at constant polymer concentration and ionic content. It is clear that the lower the polymer concentration during the thermal treatment, the lower the degree of degradation.

The same viscosities, observed in Fig. 4 for the both states at 1 g/litre, means that T_m was exceeded during the thermal treatment. When the polymer concentration increases, differences exist between both states, especially after heating at 100°C. This behaviour indicates that the conformation of the heated polymer is still at least partially in the native conformation. In relation to T_m, the difference in viscosities of the two solutions decreases when the polymer concentration decreases and the temperature increases. In contrast, after heating the solutions at 130°C, the small differences between the native and the renatured treated states are an indication that T_m is reached during the heating of the solutions at

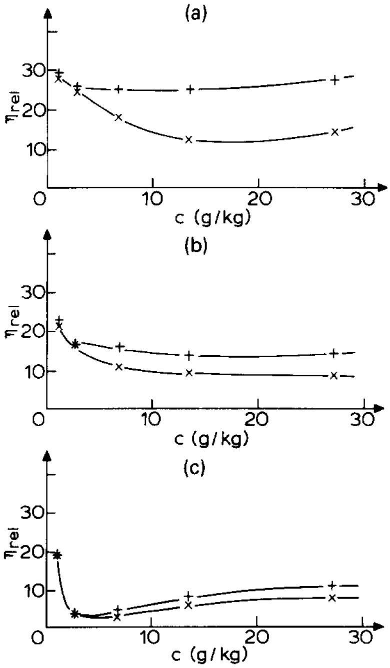

Fig. 4. Relative viscosity of the native (×) and renatured (+) treated states (see Fig. 1) (Broth II, $C_p = 0{\cdot}5$ g/litre, $T = 25$°C) after heating the broth at different xanthan concentrations, c (dilution with $2{\cdot}5 \times 10^{-3}$ M Na_2SO_4) and different temperatures. (a) $T = 100$°C; (b) $T = 115$°C; (c) $T = 130$°C; time, 10 min, pH 7.

this temperature. The xanthan molecules are at least partially in the disordered conformation at 130°C, even at the higher xanthan concentrations used. No optical rotation measurement was possible at this temperature in order to check this assumption. The level of the viscosity of the renatured polymer is directly related to the residual molecular weight. Comparing the initial relative viscosities of the polymer in the native ($\eta_{rel} = 14$) and renaturated ($\eta_{rel} = 35$) conformations, it is clear that degradation occurs more severely at concentrations above 2 g/litre, increasing considerably with the temperature adopted for the thermal treatment. The role of temperature is noticeable both on the degree of conformational change and on the degree of degradation. The heated polymers were isolated and characterized for comparison with the initial polymer (see Table 1).

The results are given in the Fig. 5. After heating at 100°C, for solutions with polymer concentration over 7 g/litre it must be considered

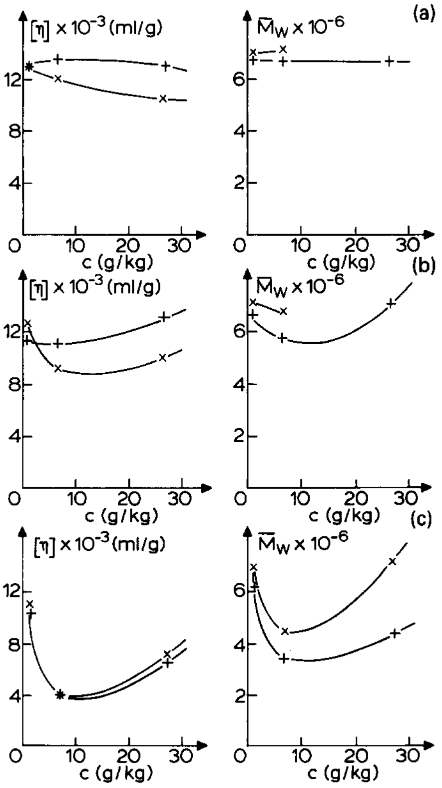

Fig. 5. Intrinsic viscosity and weight-average molecular weight of xanthan broth heating treatments under the conditions given in Fig. 4. (a) $T = 100°C$; (b) $T = 115°C$; (c) $T = 130°C$.

that aggregates are formed, leading to a loss of filtrability and preventing a meaningful molecular weight determination. In contrast, after heating the native treated states at 80°C (over T_m) the filtrability is restored, allowing accurate $\bar{M}_w$ and $[\eta]$ determinations. Then it is shown that $\bar{M}_w$ and $[\eta]$ of the samples remain unchanged compared with the initial values, in agreement with the results found in Fig. 4(a). The aggregates which perturb molecular weight determination but not viscosity measurement are dissociated during heating above T_m. From Fig. 4(a) it was concluded that T_m is not reached at 100°C for xanthan solutions with a polymer concentration over 7 g/litre, and the aggregates formed at this temperature are mainly from the native conformation. Aggregates of this type are then dissociable.

At 115°C and to a greater extent at 130°C, the apparent molecular weight of the polymer at first decreases and then increases simultaneously with $[\eta]$ for increasing polymer concentrations. These results demonstrate first a degradation of the polymer followed by aggregation when the polymer concentration is higher. It may also be assumed that the thermal stability of the polymer in this range of polymer concentration is increased. However, the increase of viscosity of broths in the presence of external salt (having a role in the stabilization of the conformation) (Fig. 3) is larger than that observed in the absence of external salt (Fig. 2) when the temperature is equal or greater than 120°C, signifying that aggregation is the main contribution to this behaviour. This hypothesis is confirmed by the large decrease in the solution viscosities of the renatured treated states (Figs 2 and 3). Then, the aggregates formed at 130°C and at high xanthan concentrations remain filtrable and only partially dissociated after heating over T_m. $\bar{M}_w$ and $[\eta]$, from the renatured treated states, still increase with the xanthan concentration used during the thermal treatment. At 130°C, in the absence of added salt or for Na_2SO_4 concentrations lower than $2{\cdot}5 \times 10^{-3}$ M, the solutions are heated over the temperature of the conformational change of the xanthan molecules, as previously discussed (Fig. 4(c)). It is then concluded that stable aggregates are formed from the disordered conformation of the polymer for xanthan concentration higher than 7 g/litre. This result contrasts with the dissociable aggregates generated from the native ordered conformation.

CONCLUSION

The experimental data discussed above, permit the understanding, at least partially, of the role of thermal treatments on the viscosity of a broth or diluted broth and, separately, on the characteristics of the xanthan molecule isolated from these broths. The observed behaviour seems to be general and depends only slightly on the nature of the broth (Callet, 1987).

In addition, the role of the conformational change and that of the degradative process are discussed. The main conclusions are:

pH has a large effect on the degree of degradation. pH 7 seems to give the best thermal stability.

The degree of degradation increases with the temperature and time of treatment of the broth as soon as the temperature is higher than 60°C.

The polymer concentration C_p, adopted for thermal treatment plays a role on the conformational change. When C_p is larger than 20 g/litre and Na_2SO_4 concentrations lower than $2{\cdot}5 \times 10^{-3}$ M, a conformational change is observed only after heat treatment to 130°C. The gain in viscosity due to the conformational change is counterbalanced by depolymerization.

Aggregation occurs for the higher polymer concentrations used ($C_p > 7$ g/litre). This seems to be reversible when it is formed from the native conformation (for example at $T = 100$°C) but produces a loss of filtrability of the heated broth. An irreversible stable aggregation, which forms filtrable large particules, is obtained for treatments at 130°C when the denatured conformation is reached during the thermal treatment.

The presence of external salt has no important role on all the processes observed in this work at qualitative level. The only difference observed was that some gels were obtained in neutral or alkaline conditions on the concentrated broth and/or in excess of external salt (Na_2SO_4 was tested).

The last conclusion is that thermal treatment always has a degradative effect on the polymer (causing a decrease of the molecular weight) even if, in some conditions, increase of the broth viscosity is observed.

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