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Temperature-Induced Extension and Dissociation of Native Xanthan

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ABSTRACT

Light scattering and viscosity changes accompanying the temperature-induced chirooptically-recorded denaturation of high weight-average molecular weight ($\bar{M}_w$) native xanthan coming from a fermentation broth are reported. The $\bar{M}_w$ and $|\eta|$ changes as a result of heating the initially ordered native xanthan (0·1 M aq. NaCl, T = 25°C) at a temperature above the melting temperature T_m then cooling to the initial temperature for reaching a reordered form can be interpreted by a two-step extension–dissociation mechanism. The first step, during which the viscosity increases whilst $\bar{M}_w$ is unchanged, consists of an extension of the native dimeric structure that is preserved after a return to the initial condition of temperature. The second step is rather slow and requires temperatures higher than that adequate for inducing the optically-detected transition. The molecular weight is roughly halved, therefore indicating a complete dissociation of the dimeric form. The results are compared with those from literature data showing no evidence of a complete dissociation and a tentative explanation is given.

INTRODUCTION

Xanthan has a well defined primary structure consisting of a linear chain of (1 → 4)-linked β-D-glucopyranosyl residues with charged trisaccharide side-chains attached to alternate residues of the main chain to give a pentasaccharide repeat unit (Jansson *et al.*, 1975). It is also well docu-

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mented that the xanthan molecule exists in an ordered semi-rigid rod-like conformation in aqueous salt solution in a temperature range that depends on the salt content (Rees, 1972; Holzwarth, 1976; Morris *et al.*, 1977; Milas & Rinaudo, 1979). As the temperature is increased, or the salt concentration decreased, the ordered xanthan molecule undergoes a denaturation to a more flexible, disordered conformation, as evidenced by changes in optical rotation.

The conformational changes associated with such an order–disorder transition may be monitored by a variety of techniques, for example viscosity and light scattering. However, a controversy exists concerning the exact structure of the helical, ordered xanthan, and conflicting literature data show that it is a matter of discussion whether the ordered form is a single or a double strand (Holzwarth, 1976; Holzwarth & Prestridge, 1977; Morris *et al.*, 1977; Rinaudo *et al.*, 1983; Muller *et al.*, 1984; Norton *et al.*, 1984; Paradossi & Brant, 1982; Sato *et al.*, 1984*a*, *b*, *c*; Muller *et al.*, 1986; Stokke *et al.*, 1987). It should be mentioned that such a debate also exists concerning the exact secondary structure of other polysaccharides such as κ-carrageenans (Morris *et al.*, 1980; (Grasdalen & Smidsrød, 1981).

Recently the present authors reported on the molecular weight ($\bar{M}_w$), viscosity (η) and optical activity (α) changes of a native xanthan sample brought to the disordered conformation by exposure to low salt concentration and then returned to the initial salt concentration (0·1 M aq. NaCl) where the so-called 'reordered conformation' prevails. The observed phenomena associated with such a treatment were explained by putting forward the existence of three different ordered structures. A compact, double-stranded native structure in 0·1 M aq. NaCl was suggested to elongate to a less compact (and possibly stiffer) one upon exposure to low salt concentration (i.e. 10^{-3}–10^{-4} aq. NaCl) and to dissociate into single-stranded chains only at even lower salt levels. Both the elongated double-stranded structure and the single strand remain stable without further aggregation when the initial salinity was restored (Lecourtier *et al.*, 1986, 1987).

Other recent literature data concern the temperature-induced conformation changes of xanthan. Sato *et al.* (1984*a*) reported that the dissociation of ordered xanthan could be induced by addition of cadoxen or by heating in pure water at 95°C. Liu *et al.* (1987) reported that the molecular weight of xanthan remains unchanged and almost twice as large as that in cadoxen during the thermally induced transition in 0·01 M aq. NaCl below 80°C, suggesting that an intramolecular process occurs in the dimer itself. Hacche *et al.* (1987) also claimed that the temperature-driven conformation change of low $\bar{M}_w$ xanthan in

5×10^{-3} M aq. NaCl occurs without noticeable $\bar{M}_w$ change in the temperature range they examined. The same $\bar{M}_w$ constancy in 0·01 M aq. NaCl below 55°C has been reported by Milas & Rinaudo (1986); however, they concluded that the conformation change occurs in a single chain with an increase in the overall dimension.

The present paper deals with the temperature-induced conformation changes accompanying the optically-recorded denaturation of a high molecular weight native highly-pyruvated xanthan as deduced from viscosity and low angle laser light scattering.

EXPERIMENTAL

Polymer and solutions

The xanthan samples studied came from pasteurized (xanthan A) and unpasteurized (xanthan B) fermentation broths manufactured by Rhône-Poulenc, France. The broths were diluted to a concentration of about 0·4 g litre^{-1} with 5 g litre^{-1} NaCl solution as solvent. The solutions were clarified by successive filtrations through Millipore filters (3 μm, 1·2 μm and 0·65 μm) to remove cells and macroimpurities according to a procedure described elsewhere (Chauveteau & Kohler, 1984). All measurements were performed on solutions of xanthan obtained by dilution of the stock solution (0·4 g litre^{-1}) to the desired concentration with prefiltered, deionized water containing sodium azide and salt. As the method of preparing solutions was found to be a critical parameter which strongly affects the light scattering data (Muller *et al.*, 1986), all precipitation and drying steps were avoided. Both initial xanthan samples contained approximately one pyruvate group and one acetyl group per side chain. The polymer concentration was determined with a Dohrmann Carbon Analyser DC80.

The characteristics of the different conformations of unpasteurized xanthan B as a result of the previously reported salt-induced order–disorder–reorder cycle are given in Table 1.

Procedure for testing the effect of temperature

The 'native ordered xanthan' (State 1, 0·1 M aq. NaCl) was brought to the disordered conformation (State 2) at constant salinity by heating at a given temperature and then returned to the initial temperature to obtain a 'reordered conformation' (State 3). The influence of temperature was studied by comparing the viscosity and light scattering data of States 1

TABLE 1

Effect of the Salt-Induced Order–Disorder–Reorder Cycle on the Conformational Properties of Xanthan B Determined in Ordered Conformation (0·1 M aq. NaCl, pH = 7, $T = 30°C$)

$\bar{M}_w \times 10^{-6}$	$\lvert\eta\rvert$ (ml g^{-1})	q (nm)[a]	*Secondary ordered structure*	*Transition process*
4·8	3400	—	Compact double strand	Native
4·0[b]	8500	150	Extended double strand	Intramolecular
2·1[c]	6100	60	Single strand	Intermolecular

[a] q is the persistence length of the worm-like xanthan molecule.
[b] After treatment in 10^{-3}–10^{-4} M aq. NaCl.
[c] After treatment in pure water.

and 3, i.e. under the same salt and temperature conditions, therefore avoiding problems due to changes of the specific refractive index increment dn/dc with temperature.

Viscosity and molecular weight

Intrinsic viscosities were determined in the low shear limit using a Contraves Low Shear 30 viscometer.

Intensities of light scattered from aqueous NaCl solutions of native and reordered xanthan were measured at 25°C with a low-angle laser light scattering photometer Chromatix KMX6. Optical clarification of solutions was effected by filtration through Millipore filters (0·22 μm) as they flowed into the measuring cell.

RESULTS

Optical activity measurements indicate that an ordered conformation exists at room temperature for salinity above 5×10^{-3} M aq. NaCl. The thermal treatment was carried out in 0·01 or 0·1 M aq. NaCl as a solvent and in the presence of oxygen. Comparison with experiments performed in the absence of O_2 ($< 5 \times 10^{-9}$ g ml^{-1}) has shown that no oxidative degradation of xanthan occurs for the conditions of temperature and salinity used in the experimentally examined time-scale (Lund *et al.*, 1988).

Viscosity data in 0·1 M aq. NaCl

Figure 1 shows the effect of preheating ($T = 110°C$) the native xanthan B in 0·1 M aq. NaCl on the low shear reduced viscosity measured after return to the initial condition of temperature ($T = 25°C$). The viscosity is observed to increase in the early stage of treatment then reach a limiting value which is strongly dependent on the preheating temperature. A close examination of the data reveals that viscosity changes associated with the heat denaturation correspond to an extension process similar to that reported for salt-induced denaturation (Lecourtier *et al.*, 1986). The kinetics of this extension are temperature dependent. The upper limiting viscosity value after 5 min at 110°C is the same as, for example, that obtained after 30 min at 95°C. Upon heating at 110°C, the viscosity is observed to decrease, reaching a lower constant value ($|\eta| \simeq 6000$ ml g^{-1}) after more extended heating times ($t = 15$ and 20 min).

Figure 2 illustrates the dependence of the reduced viscosity at zero shear rate on polymer concentration measured in 0·1 M aq. NaCl before and after heating at 110°C. The values of intrinsic viscosities are

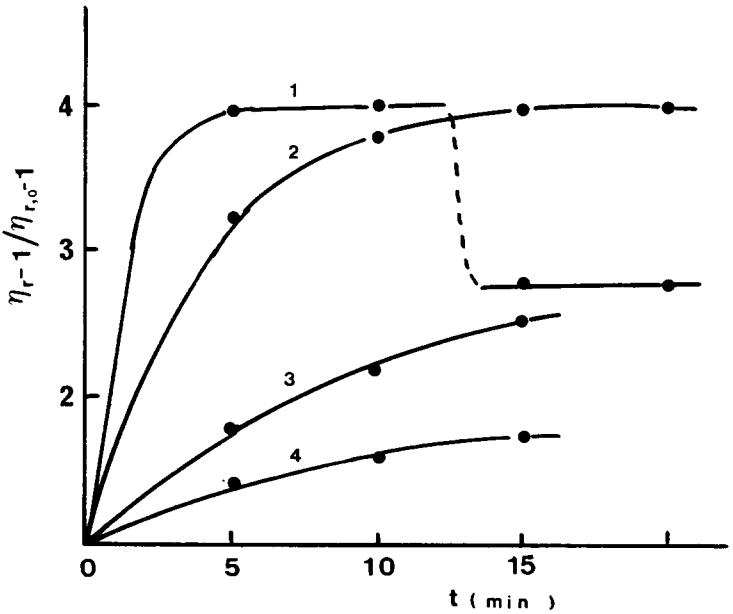

Fig. 1. Effect of temperature (T, °C) and heating time on the specific viscosity (measured at 25°C in 0·1 M aq. NaCl) of xanthan B ($C = 2{\cdot}7 \times 10^{-4}$ g ml^{-1}). $\eta_r - 1$ and $\eta_{r,0} - 1$ respectively refer to specific viscosity measured at 25°C after heat treatment at 1, 110°C; 2, 95°C; 3, 90°C; and 4, 85°C and before treatment.

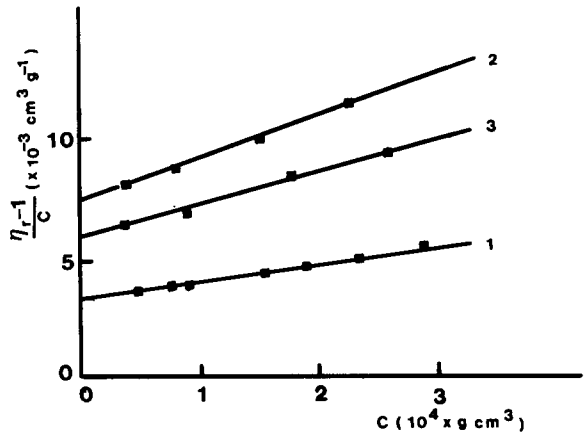

Fig. 2. Concentration dependence of the reduced viscosity of xanthan B in 0·1 M aq. NaCl (25°C). 1, Native compact form (before heating); 2, after 10 min at $T = 110$°C (extended double strand); 3, after $t > 15$ min at 110°C (single strand).

TABLE 2
Molecular Parameters of Xanthan B as a Function of Heating Time t at 110°C (0·1 M aq. NaCl)

	t (min)				
	0	*5*	*10*	*15*	*20*
$\bar{M}_w \times 10^{-6}$	4·8	4·2	4·2	2·5	2·5
$\lvert\eta\rvert$ ml g^{-1}	3400	7600	7600	6000	6000
k'	0·4	—	0·4	0·4	—

reported in Table 2 together with the molecular weight $\bar{M}_w$ and the Huggins constant value ($k' = 0{\cdot}4$). The value of k' is that expected for non-interacting molecules, supporting the view that under these conditions xanthan does not aggregate (Noik *et al.*, 1987).

Comparing the data presented in Tables 1 and 2 it is worth noting that the viscosity value obtained for heating times above 15 min at 110°C is very close to that measured after treatment at very low salinity ($\simeq 10^{-5}$ M aq. NaCl) which has been shown to correspond to that of a single strand. On the other hand, the upper viscosity limit after shorter heating times ($\lvert\eta\rvert \simeq 7600$ ml g^{-1}) is lower than that found after treatment in a salinity

range where denaturation was shown to occur without dissociation of the native double structure ($|\eta| \simeq 8500$ ml g^{-1}). Such a difference could be ascribed to the contribution of electrostatic repulsions between charged groups which induce an extension of the xanthan molecule at very low salinity. Here the thermal treatment was performed at a salt concentration (0·1 M aq. NaCl) where the effect of electrostatic repulsion is screened. Figure 3 shows that decreasing the salinity of the heated xanthan from 0·1 to 10^{-4} M aq. NaCl and then restoring it to 0·1 M aq. NaCl results in an increase in the viscosity up to a value which corresponds to that of the fully-extended double strand.

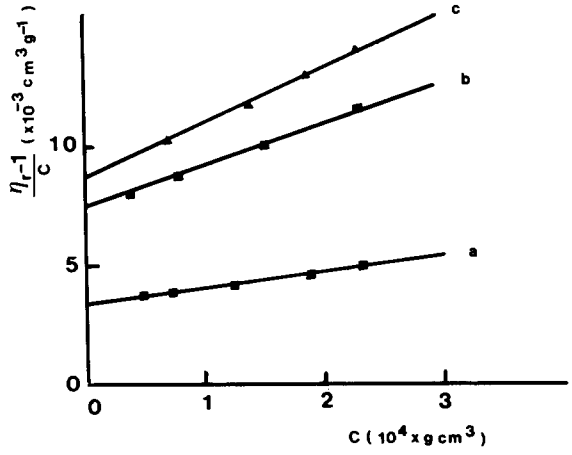

Fig. 3. Concentration dependence of the reduced viscosity of xanthan B in 0·1 M aq. NaCl (25°C). a, Native form; b, heated for 10 min at 110°C in 0·1 M aq. NaCl; c, after decreasing salinity of heated xanthan to 10^{-4} M aq. NaCl then restoring it to 0·1 M aq. NaCl.

Static low-angle laser light scattering

Light-scattering measurements were performed in order to elucidate the origin of the above-reported viscosity changes with temperature and to gain more precise information on the exact nature of the thermal transition.

Figure 4 shows the changes in weight-average molecular weight $\bar{M}_w = \bar{R}_\theta / KC$ ($\bar{R}_\theta$ is the measured excess Rayleigh ratio, K is the usual

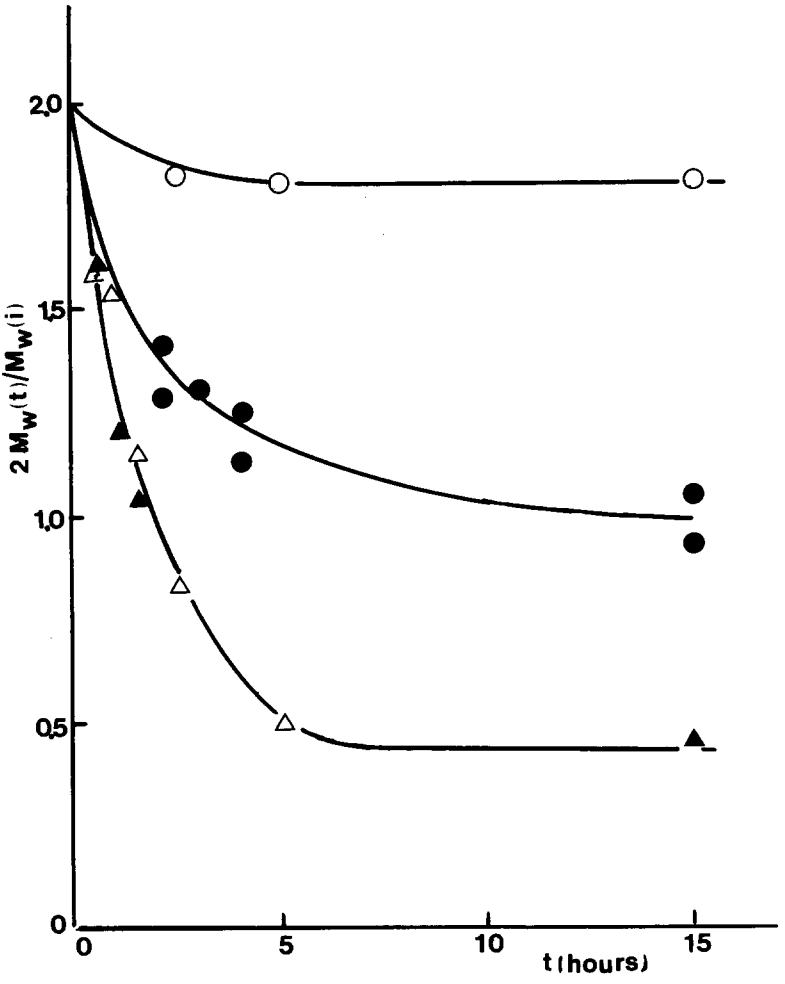

Fig. 4. Change in weight-average molecular weight of xanthan A (○, △) and B (●, ▲) as a result of heat treatment in different salt conditions (○, ●: 0·1 M aq. NaCl; △, ▲: 10^{-4} M aq. NaCl). The $\bar{M}_w$ are determined from the values of $\bar{R}_\theta$ measured in 0·1 M aq. NaCl and $T = 25°C$ before heating ($\bar{R}_{\theta,i}/KC = \bar{M}_{w,i}$) and after heating ($\bar{R}_{\theta,t}/KC = \bar{M}_{w,t}$) ($K$ is the usual optical constant with $dn/dc = 0{\cdot}155$ ml g^{-1}).

optical constant and C is the concentration in g ml^{-1}) as a result of heating xanthans A and B under different salt conditions. Data are expressed as $2\bar{M}_w(t)/\bar{M}_w(i)$ where $\bar{M}_w(t)$ and $\bar{M}_w(i)$ refer to the $\bar{M}_w$ measured in 0·1 M aq. NaCl after and before heating under different conditions of salinity respectively. With such a representation, the above ratio should vary from 2 to 1 if the transition results in a halving of the molecular weight

(Yukioka *et al.*, 1985). Figure 4 clearly shows that heating at low salt levels leads to chain degradation for both xanthan samples, confirming the much lower chemical stability of the disordered conformation (Lambert & Rinaudo, 1985). On the other hand, the two samples behave quite differently at higher salinity i.e. when they are predominantly in the ordered form during the thermal treatment. The molecular weight of xanthan A changed only slightly, showing that the chirooptically-detected disruption of the ordered, low-temperature form upon heating only implies a simple reorganization of the secondary structure through the side chains. Obviously this is not true for xanthan B, as $\bar{M}_w$ is approximately halved. The results presented thereafter refer to thermal treatments performed at salt concentrations high enough for the same native ordered conformation to exist before heating and for no degradation of the main chain to occur on heating. These conditions are fulfilled in both 0·01 and 0·1 M aq. NaCl. Figure 5 illustrates the light scattering data for sample B in 0·1 M aq. NaCl before and after heating at 110°C. From the measured changes in $\bar{R}_\theta$ (Fig. 5A) it is found that $\bar{M}_w(i)/\bar{M}_w(t) \simeq 2$, as deduced from the concentration dependence of $KC/\bar{R}_\theta$ for

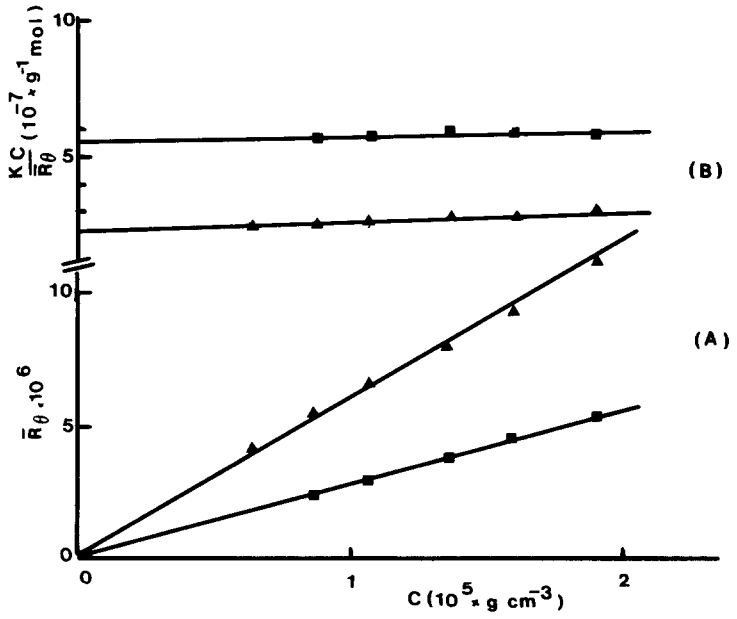

Fig. 5. Light scattering of xanthan in 0·1 M aq. NaCl. Concentration dependence of the excess Rayleigh ratio: A, $\bar{R}_{\theta=5°}$ and of B, $KC/\bar{R}_\theta = 1/\bar{M}_w + 2A_2C$; for xanthan B before heating (■) and after heating at 110°C (▲).

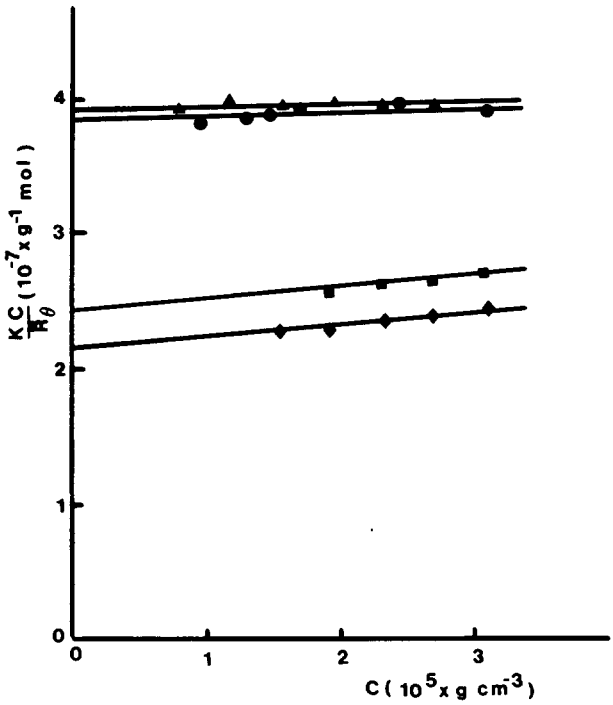

Fig. 6. Light scattering of xanthan B in 0·01 M aq. NaCl ($T = 25°C$): Native untreated (◆); after 15 (■), 50 (●) and 120 (▲) min at $T = 110°C$.

$\theta \simeq 5°$ (Fig. 5B). A similar halving of $\bar{M}_w$ was observed on heating sample B at the same temperature but in 0·01 M aq. NaCl. Figure 6 displays the plots of $KC/\bar{R}_\theta$ versus concentration for Sample B heated at $T = 100°C$ in 0·01 M aq. NaCl. For shorter heating times the molecular weight $\bar{M}_w$ is only slightly decreased, whereas the viscosity was found to strongly increase (Fig. 1); this supports an extension of the initial double strand without separation into single chains. This is confirmed by a recent careful analysis of the changes in the angular dependence of the scattered light as a result of heat treatment of native xanthan. It is observed that the radius of gyration of heated xanthan is much larger than that of the native one as a consequence of both the destruction of compact aggregated particles and the extension of the molecule upon heating (Muller *et al.* (1988*a*, *b*).

In contrast, longer heating times result in dissociation of the native structure as evidenced by the infinite-dilution value of $KC/\bar{R}_\theta$ after heating for 35 and 100 min. This gives a value of $\bar{M}_w$ close to $2{\cdot}5 \times 10^6$, whereas the initial $\bar{M}_w$ was near $4{\cdot}8 \times 10^6$. This $\bar{M}_w$ decrease agrees with the viscosity decrease reported in Fig. 1.

DISCUSSION AND CONCLUSION

The above data show that for salt concentrations and heating times where no oxidative degradation occurs both xanthan samples, the pasteurized and the native one, behave quite distinctly with regard to the $\bar{M}_w$ changes associated with the temperature-induced transition. This confirms the earlier-reported behaviour of xanthan passing through the salt induced order–disorder conformational transition at ambient temperature (Lecourtier *et al.*, 1987). The same extension double–single strand dissociation mechanism can account for the observed changes in the molecular parameters of the native xanthan B.

For both salt- and temperature-induced denaturation, a two-step process takes place. The first step consists of an extension of the native dimeric structure, which is preserved after a return to the initial conditions of temperature and salinity (extended reordered double strand). The second step involves the complete dissociation into separate strands as indicated by the approximate halving of the molecular weight. This dissociative step may be slow and requires higher temperature (Fig. 1) and/or lower salt concentrations than required for inducing the chirooptically-detected transition.

It may be considered that the observed changes in the optical rotation more probably reflect changes in the local order than changes in the extent of double strand character of the overall molecule. The present results differ somewhat from recent literature data, showing no evidence of a complete dissociation of the xanthan molecule. A tentative explanation can be sought to account for the differences in experimental data arising from various authors.

From light scattering and kinetics measurements, Norton *et al.* (1984) concluded that conformational ordering of xanthan was an intramolecular event as no $\bar{M}_w$ change was detected on adoption of the ordered form. This corresponds to the behaviour observed in the present study for xanthan A over the entire range of salt concentration and temperature. However, an approximate doubling of $\bar{M}_w$ was observed at temperatures lower than that corresponding to the transition which the authors attributed to a side by side association of ordered single chain sequences. Milas & Rinaudo (1986), Hacche *et al.* (1987) and Liu *et al.* (1987) also reported that the molecular weight is practically unchanged upon exposure of xanthan to disordering temperature conditions as would be anticipated if the transition was associated with the partial or total disruption of a double strand structure without dissociation of the two chains. However, their results differ with regard to the dimension changes, i.e. the radius of gyration R_g and the intrinsic

viscosity η, measured during the transition. Milas & Rinaudo observed that the reordered form resulting from heating to $T > T_m$ (melting temperature) then cooling to $T < T_m$ has larger dimensions than the unheated form.

Liu *et al.* (1987) found that the dimensions remain constant or decrease depending on the $\bar{M}_w$ range of the xanthan dimer and concluded that temperature induces a partial dissociation of the xanthan molecule. Hacche *et al.* (1987) observed that temperature induces a slight increase in both $\bar{M}_w$ and R_θ. Moreover they reported that the second virial coefficient A_2 displays a clear dependence on both the salinity (a fact previously reported (Muller *et al.* (1986)) and the temperature. This agrees with the optical activity change but the change in A_2 appears at a somewhat higher temperature. They interpret their results as an aggregation process of chains, but the model proposed predicts a dissociation of xanthan into single chains at a sufficiently high temperature.

From the present experiments it is obvious that the proposed two-step denaturation process is at once salinity, temperature and time dependent. Moreover, the kinetics of the process probably depend on the degree of pyruvyl substitution of xanthan. Whereas the first intramolecular step is relatively fast and coincides with the chirooptically-detected conformation change, the strand dissociation is a slow process which requires more drastic conditions than those adequate for initiating the disordered conformation.

Between the two extreme completely-ordered and disordered forms, intermediate conformations consisting of disordered regions held together by small ordered regions can exist. Some of the literature data are in basic agreement with what was observed during the intramolecular first step, although some differences can originate in the experimental conditions used for studying the denaturation.

In this regard it should be kept in mind that both Liu *et al.* (1987) and Hacche *et al.* (1987) measured the molecular parameters in the ambient denaturing conditions i.e. while the temperature was successively elevated between 25 and 80°C in 0·01 and 5×10^{-3} M aq. NaCl. Unfortunately no information is given on the time for reaching the requisite temperature or on the time during which the molecule was maintained under the disordered conformation. These conditions greatly differ from the present experimental procedure and therefore the observed differences can be assumed to arise from kinetic effects. Moreover it should be noticed that any aggregation or molecular weight increase has never been observed with the xanthan samples in the salinity and/or temperature ranges investigated. This can be related to fermentation conditions

used for biosynthesis, giving products free of aggregating factors as suggested by the values of the Huggins constant ($k' \simeq 0{\cdot}4$).

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