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Behaviour of Xanthan in Cadoxen

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ABSTRACT

The stability of xanthan versus time in cadoxen at 20°C has been studied using optical rotation, viscosimetry and light scattering. For xanthan samples with a high initial molecular weight, the relative and the intrinsic viscosities, the weight average molecular weight and the radius of gyration of the samples continuously decrease with time. These values were found to be compatible with those given by Sato et al. *for partially depolymerised xanthan fractions, even if the interpretation is quite different. The decrease in molecular weight in cadoxen can be explained by a random process of depolymerisation when cadoxen is used as solvent. For lower molecular weight xanthans, no degradation is obtained in cadoxen, but the ratio* $\bar{M}_{\mathrm{w\ NaCl}}/\bar{M}_{\mathrm{w\ cadoxen}}$ *is found to be less than 2. Because of the depolymerisation observed for high molecular weight xanthan it is necessary to take great care when measuring and interpreting the properties of xanthan when cadoxen is used as a solvent.*

INTRODUCTION

Xanthan is a water-soluble, extracellular polysaccharide produced by the bacterium *Xanthomonas Campestris.* The nature of its ordered conformation is still under discussion (Sato *et al.*, 1984*a*, *b*; Kitagawa *et al.*, 1985; Lecourtier *et al.*, 1986; Milas *et al.* 1986*a*; Stokke *et al.*, 1986; Jones *et al.*, 1987). One of the pieces of evidence used to support the existence of a xanthan double helix in solution is the work of Sato *et al.* (1984*a*, *b*) using cadoxen as solvent; they found in this solvent a molecular weight which is half the value of that in 0·1 M NaCl.

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In this work we studied the stability of the xanthan in cadoxen under the conditions used by Sato *et al.* (1984*a*, *b*).

MATERIALS AND METHODS

Materials

Three different xanthans were studied: the previously investigated Na salt xanthans from Shell and Rhône-Poulenc (Milas *et al.*, 1986*b*) and a commercial xanthan from Kelco used without purification (Keltrol). The solvent cadoxen was prepared, all at one time, following strictly the procedure given by Sato *et al.* (1984*a*). The cadoxen solutions were prepared either by mixing weighed amounts of the Na salt xanthan with pure cadoxen or a cadoxen–water mixture with a cadoxen volume fraction $v_{\text{cad}} = 0{\cdot}8$; the solutions were stirred until the xanthan completely dissolved. This took from 4 to 9 h. In another preparation, the xanthan was first dissolved in pure water, and cadoxen was added later to reach $v_{\text{cad}} = 0{\cdot}95$. This procedure dispenses with the stirring of xanthan in cadoxen and allows measurements to be made immediately after the addition of the cadoxen. After xanthan dissolution the solutions were kept, usually at 20°C, without stirring.

Methods

Viscosity measurements were carried out in the low shear limit using a Contraves Low-Shear viscometer thermostatted at 25°C. Intensities of light scattered from cadoxen solutions of Na salt xanthan at 25°C were measured on a Fica 50 light scattering photometer in the angular range from 30° to 150° at 546 nm and on a Chromatix KMX6 LALLS at 6–7° and 633 nm. Optical clarification of cadoxen solutions was done either by filtration through 0·5 μm Millipore filters (Type FM) or by centrifuging for 3 h at 20 000 *g*.

According to the results of Sato *et al.* (1984*a*) the value of $\mathrm{d}n/\mathrm{d}c$ used was $0{\cdot}164\ \mathrm{cm}^3\ \mathrm{g}^{-1}$. The effect on $\mathrm{d}n/\mathrm{d}c$ of wavelength and water content in cadoxen which is found by Sato *et al.* (1984*a*) to be lower than 2% were not taken into account in this work. The xanthan solutions were not dialysed as no semi-permeable membrane resistant to the solvent was available. In any event, due to the high ionic strength of the solvent and its composition (28 wt% aqueous solution of ethylene diamine) dialysis is not necessary. Optical rotation was determined at $\lambda = 300$ nm on a Spectropol 1b from Fica. We used a 5 cm long quartz cell, thermostatted at 25°C. The optical rotation measurements were made within 24 h after solution preparation.

RESULTS

In Fig. 1 the specific optical rotation of xanthan solutions is given versus cadoxen–0·01 M NaCl v/v fractions. The curve is similar to that obtained by Kitagawa *et al.* (1985) although we found an initial decrease in $[\alpha]$ when a very small amount of cadoxen is added. This change in $[\alpha]$ is due to the deacetylation of the xanthan in the basic medium (Callet *et al.*, 1987). The pH of these solutions except when $v_{\text{cad}} = 0$ is always higher than 13.

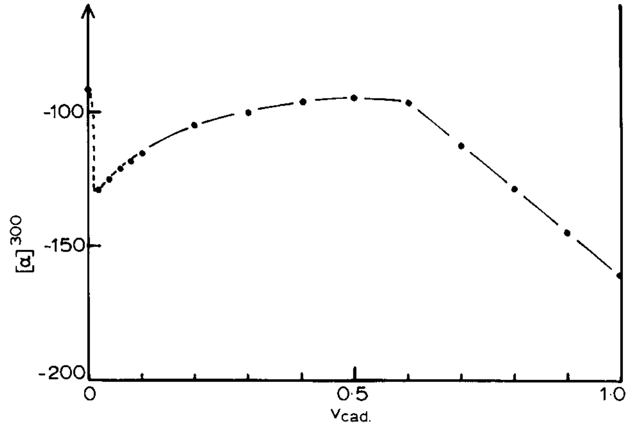

Fig. 1. Dependence of $[\alpha]^{300}$ on v_{cad} in cadoxen–0·01 M NaCl solutions for Shell xanthan at $C_p = 1$ g/litre and 25°C.

No variation in optical rotation with time was observed for any of the solutions studied for at least one week. In Fig. 2 we report the specific viscosities for the three xanthans used in cadoxen–water mixture with $v_{\text{cad}} = 0{\cdot}8$ versus storage time. The viscosities decrease with time. For the Kelco sample, we show in Fig. 3 the effect of the solution preparation conditions and cadoxen–water ratios. When the xanthan is first dissolved in water (see Materials, $v_{\text{cad}} = 0{\cdot}95$) we find a high initial viscosity followed by a large viscosity decrease. The lower viscosities obtained from the two other solutions, prepared directly by dissolving xanthan in cadoxen, are perhaps due to an additional initial shear-induced degradation of xanthan during stirring in cadoxen as suggested by Sato *et al.*

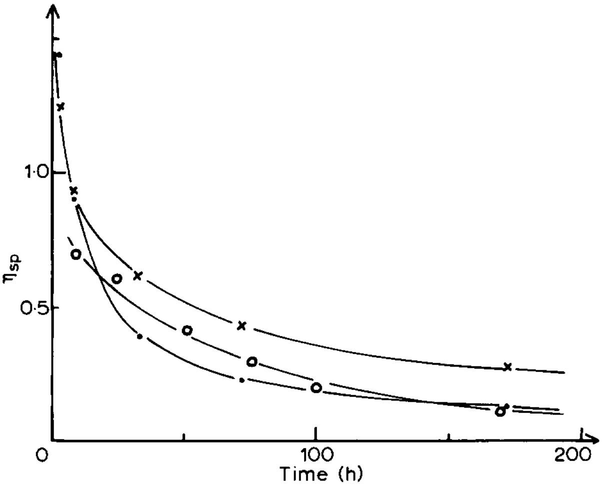

Fig. 2. Specific viscosities at 25°C for the three xanthans used in $v_{cad} = 0{\cdot}8$, versus storage time at 20°C. ●, Shell; ○, Kelco samples: $C_p = 0{\cdot}5$ g/litre, ×, RP sample $C_p = 1$ g/litre.

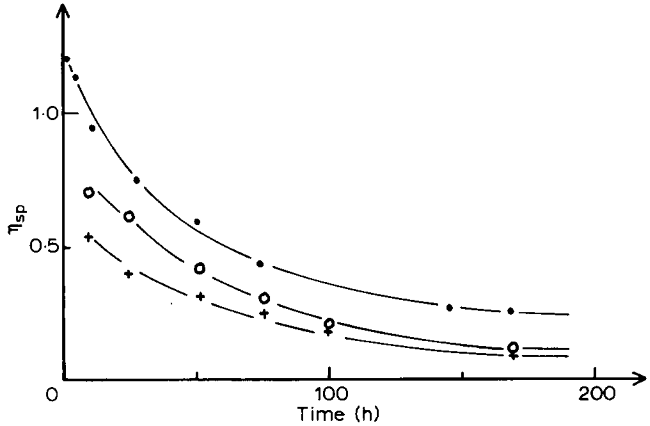

Fig. 3. Specific viscosities at 25°C for Kelco sample ($C_p = 0{\cdot}5$ g/litre) versus storage time at 20°C for three different solution preparations: ●, $v_{cad} = 0{\cdot}95$ (xanthan was first dissolved in water); +, $v_{cad} = 1$; ○, $v_{cad} = 0{\cdot}8$.

(1984*b*). Similar results (not given here) are found for the purified Shell sample. To understand this behaviour we measured the intrinsic viscosities $[\eta]$, the weight average molecular weights $\bar{M}_w$ and the radius of gyration $\langle S^2\rangle^{1/2}$ of xanthan, versus time for different cadoxen solutions. Table 1 summarises the values of $\bar{M}_w$, $[\eta]$ and $\langle S^2\rangle^{1/2}$ for the Shell sample in a cadoxen–water mixture with $v = 0{\cdot}95$. Figure 4 shows light scattering envelopes after 3 and 99 h in this mixture and in 0·1 M NaCl. After 50 h,

TABLE 1

$\bar{M}_w$, $[\eta]$ and $\langle S^2 \rangle^{1/2}$ at 25°C of the Xanthan from Shell Versus the Storage Time at 20° and $C_p = 0{\cdot}2$ g/litre in Cadoxen–Water Mixture, for a Cadoxen Volume Fraction 0·95 (the Xanthan Was First Dissolved in Water, Fica L.S. Measurements)

Time (h)	*3*	*8*	*26*	*55*	*99*[a]
$\bar{M}_w \times 10^{-6}$	5·5	4·1	3·2	2·12	(0·9)
$[\eta]$ (ml/g)	1 600	1 320	960	550	(400)
$\langle S^2 \rangle^{1/2}$ (Å)	1 750	1 400	1 220	980	(500)

[a] This solution contains aggregates.

aggregates appear in solution which perturb light scattering at low scattering angles (see Fig. 4(c)). These points are not taken into account for the M_w determination. A continuous decrease of these three values is observed versus time. A double-logarithmic plot of $[\eta]$ against $\bar{M}_w$ is shown in Fig. 5 for different solution preparations and storage times. Figure 6 shows the molecular weight dependence of $\langle S^2 \rangle^{1/2}$. In these two last figures we report also the values of Sato *et al.* (1984*a*, *b*) obtained from samples with different initial molecular weights.

For lower molecular weights (below 2×10^5 in 0·1 M NaCl) no degradation appears with storage time in agreement with the results of Sato *et al.* (1984*a*). But the ratio $\bar{M}_{w\,\mathrm{NaCl}}/\bar{M}_{w\,\mathrm{cadoxen}}$ is found to be lower than 2 (Table 2).

DISCUSSION

In agreement with Kitagawa *et al.* (1985), the change in optical rotation of xanthan in cadoxen–water mixtures (Fig. 1) can be attributed to an order–disorder transition when the volume fraction of cadoxen in the mixed solvent increases from 0·6 to 0·8. For cadoxen fractions higher than 0·8, Sato *et al.* (1984*a*, *b*) and Kitagawa *et al.* (1985) attribute the loss in viscosity and molecular weight of xanthan to a dissociation of double helices to single chains. Figures 2 and 3 show that the viscosities of the cadoxen solutions decrease continuously with time. In Figs 5 and 6 a striking agreement between our values obtained at different storage times for the intrinsic viscosity and radius of gyration and the values of Sato *et al.* (1984*a*, *b*, 1985) and Kitagawa *et al.* (1985) from samples with different initial molecular weights is found. As our xanthan and that used by Sato *et al.* (1984*a*) give the same $[\eta]$: $\bar{M}_w$ curve in 0·1 M NaCl (Milas *et al.*, 1986*b*) for $\bar{M}_w$ lower than 3×10^6, the change in xanthan molecular weight and viscosity in cadoxen are expected to be mainly due

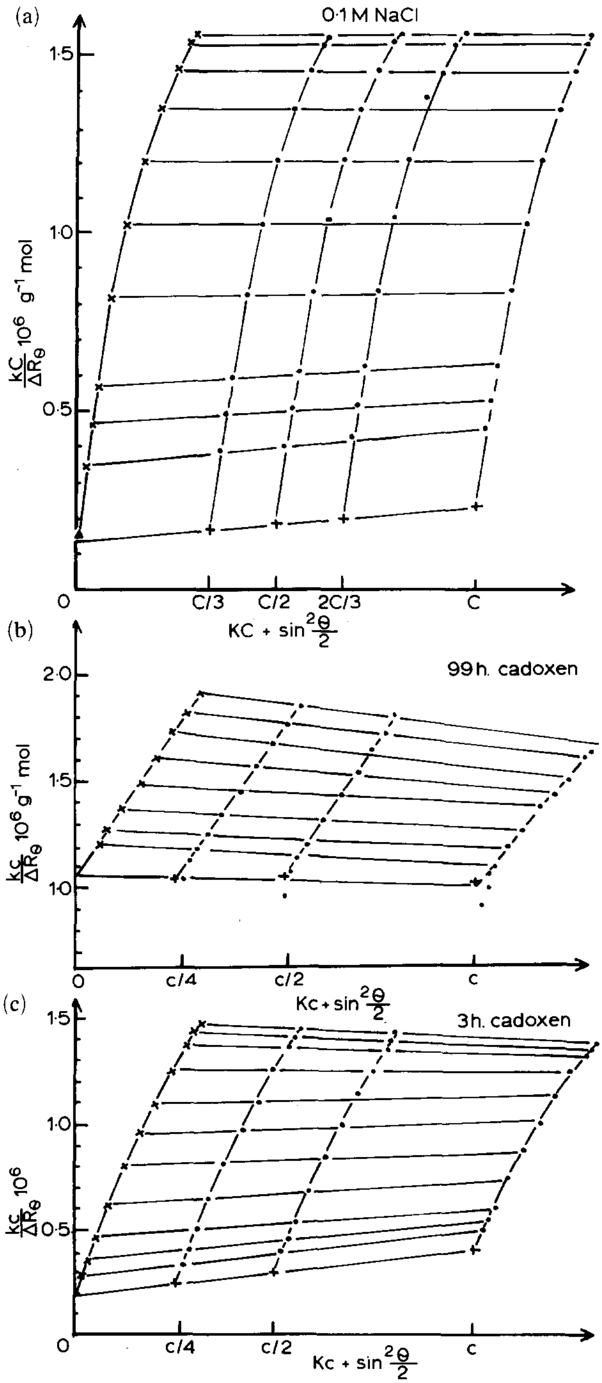

Fig. 4. Light scattering Zimm plots for Shell xanthan: (a) in 0·1 M NaCl (▲, KMX 6 value); (b), (c) in cadoxen solution ($v_{\mathrm{cad}} = 0{\cdot}95$) 3 h and 99 h after solution preparation respectively.

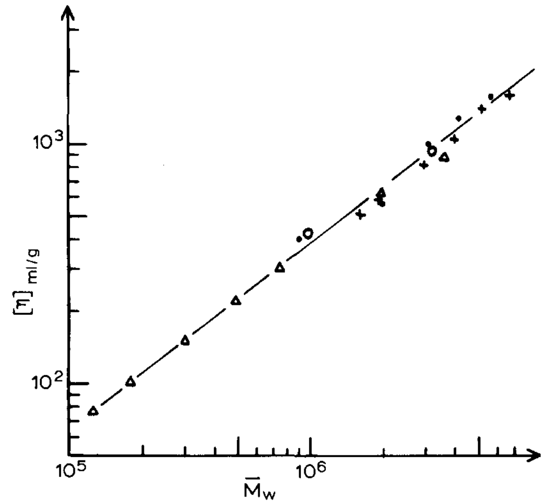

Fig. 5. Double logarithmic plot of $[\eta]$ versus $\bar{M}_w$ at 25°C for Shell sample at different storage times at 20°C, and different solution preparations: ●, $v_{cad} = 0{\cdot}95$ (xanthan was first dissolved in water, Fica L.S. measurements); +, $v_{cad} = 1$ (KMX6, LALLS measurements); ○, $v_{cad} = 0{\cdot}8$ (Fica L.S. measurements); △, data of Sato *et al.* (1984*b*) for different $\bar{M}_w$ samples.

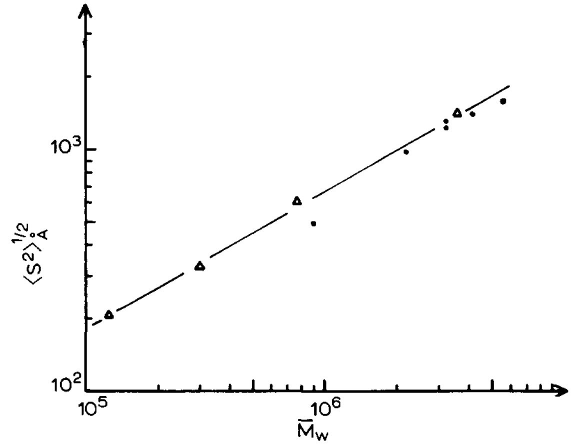

Fig. 6. Double-logarithmic plot of $\langle S^2 \rangle^{1/2}$ versus $\bar{M}_w$ at 25°C for Shell sample at different storage times at 20°C; ●, $v_{cad} = 0{\cdot}95$ (the xanthan was first dissolved in water, FICA L.S. measurements); △, data of Sato *et al.* (1984*a*) for different $\bar{M}_w$ samples.

to backbone depolymerisation; as no change in optical rotation was observed with time, this excludes a conformational or structural modification. The data are plotted in Fig. 7 according to a random degradation mechanism (Tanford, 1967; Rinaudo & Milas, 1980), i.e. a first-order reaction where the number of bonds broken is proportional

TABLE 2

$\overline{M}_w$ in NaCl and Cadoxen ($v = 0{\cdot}8$) for Enzymic Depolymerised Xanthan Samples (Milas *et al.*, 1986*b*) (KMX 6 Measurements)

$\overline{M}_{w\ \mathrm{NaCl}}$	$\overline{M}_{w\ \mathrm{cadoxen}}$	$\overline{M}_{w\ \mathrm{NaCl}}/\overline{M}_{w\ \mathrm{cadoxen}}$
175 000	110 000	1·6
85 000	63 000	1·35
42 000	37 000	1·13

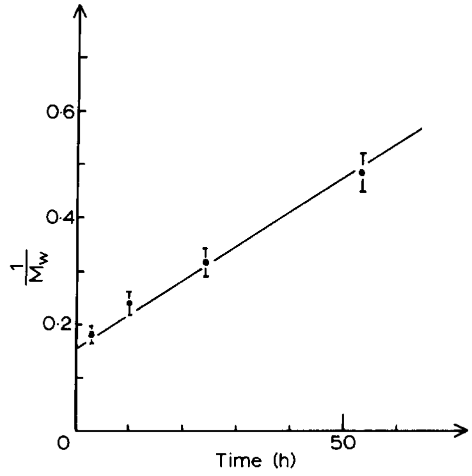

Fig. 7. $1/\overline{M}_w$ dependence at 25°C versus storage time at 20°C for Shell xanthan, $v_{\mathrm{cad}} = 0{\cdot}95$ (see Table 1).

to the degradation time t and with the hypothesis that the xanthan polydispersity index $\simeq 1{\cdot}4$ is not modified in the first step of the degradation (Milas *et al.*, 1986*b*). For storage times up to 50 h, a linear relationship is evident from Fig. 7 suggesting a random degradation mechanism. Moreover, the extrapolated $\overline{M}_w^{-1}$ value for $t = 0$ gives $\overline{M}_w = 6{\cdot}7 \times 10^6$, a value very close to 7×10^6, the initial value of $\overline{M}_w$ for this xanthan in 0·1 M NaCl (Milas *et al.*, 1986*b*). Thus, there is a discrepancy between this behaviour and that expected for a double helix dissociation, if this dissociation occurred within 10 h after solution preparation. So, the difference in $\overline{M}_w$ between pure 0·1 M NaCl solutions and cadoxen solutions for v_{cad} higher than 0·8 appeared to be mainly due to a depolymerisation process. This depolymerisation is favoured as a result of the xanthan being in the disordered conformation at high volume fractions of cadoxen (Kitagawa *et al.*, 1985). It is well known that the xanthan stability is much lower in this conformation (Chen & Sweppard, 1979; Ash *et al.*, 1983; Lambert & Rinaudo, 1985). In the transition region the depolymerisation rate depends on the proportion of the molecules in the disordered conformation (Rinaudo & Milas, 1980) and can explain the apparent relation between the change in molecular weight and the conformational transition found by Kitagawa *et al.* (1985). From our results, the ratio $\bar{M}_w$ (in 0·1 M NaCl)/$\bar{M}_w$ (in cadoxen) is equal to 2 ± 0·2 only for times between 9 and 25 h after addition of cadoxen to a xanthan water solution (Table 1). But these times will be longer when the cadoxen solution is kept at 5°C and shorter when the xanthan is directly dissolved in cadoxen if vigorous stirring is applied. However, in all cases, for high xanthan molecular weight a continuous decrease in viscosity with time is still observed. Consequently we think that the ratio $\bar{M}_w$ (in 0·1 M NaCl)/$\bar{M}_w$ (in cadoxen) for $\bar{M}_w$ higher than 10^6 depends on the experimental conditions selected and can be explained mainly by a depolymerisation process at least for high xanthan molecular weight.

These conclusions are different to those of Sato *et al.* (1984*a*, *b*). This disagreement cannot be explained. However, it seems quite difficult or even virtually impossible to carry out any experiment in exactly the same way as that employed by Sato *et al.* In this respect, two points seem to be particularly important: (a) the xanthan sample (the present authors think that many disagreements on xanthan behaviours observed in the literature can be related to this) and (b) the xanthan preparation procedure. This point is difficult to discuss because Sato *et al.* did not think that stirring conditions were critical, so, they do not have exact information on this point (Norisuye, T., 1988, pers. comm.). However a gradual shear-induced degradation of xanthan in cadoxen is suggested during the viscosity measurements (Sato *et al.*, 1984*a*). But higher shears are expected during the dissolution of dry xanthan in cadoxen or during filtration through membranes before light scattering measurements. From the result given in Fig. 3 it seems to be an important factor.

In Table 2 we give results of molecular weight determination in cadoxen and 0·1 M NaCl for low xanthan molecular weight obtained by enzymic depolymerisation (Milas *et al.*, 1986*b*). These samples are apparently stable in cadoxen. We found a ratio $\bar{M}_{w\ \mathrm{NaCl}}/\bar{M}_{w\ \mathrm{cadoxen}}$ lower than 2 and decreasing with molecular weight. The apparent stability of these samples can be understood as follows. Random depolymerisation shows first-order kinetics, so the decrease in molecular weight will be very much slower for the low molecular weight samples. When shear degradations occur, this will depend on the shear stresses, and hence the solution viscosity or xanthan molecular weight. On this basis the ratio of the molecular weight in NaCl to that in cadoxen should decrease with decreasing molecular weight as is indeed observed since shear degradation will not occur in NaCl.

We may conclude by saying that xanthan depolymerisation appears in cadoxen solutions at least for xanthan molecular weight higher than 10^6. Additional degradation is expected during the dissolution of xanthan in cadoxen but it is difficult to quantify this depolymerisation. In these conditions the ratio $\bar{M}_w$ in 0·1 M NaCl/$\bar{M}_w$ in cadoxen found depends on the experimental conditions and xanthan molecular weight used. The study of solutions of xanthan in cadoxen requires great care. We believe that it is difficult to use cadoxen to clarify the xanthan ordered conformation at least for the xanthan samples tested here.

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