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Comparative Depolymerization of Xanthan Gum by Ultrasonic and Enzymic Treatments. Rheological and Structural Properties

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SUMMARY

A sample of xanthan was submitted to progressive depolymerization to investigate the role of the molecular weight on the hydrodynamic behaviour of the molecule.

Two degradation methods were used: enzymic and ultrasonic. The molecular structures of the samples obtained by both techniques were compared and discussed. Sonication is the best way to prepare xanthan with a controlled molecular weight as the chemical structure is not changed, the polydispersity is low, and the dependence of the specific viscosity on the overlap parameter $C[\eta]_0$ was the same for all samples.

INTRODUCTION

Xanthan is an extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. The backbone is a $\beta(1\text{-}4)$ linked D-glucopyranose chain, as in cellulose, but with a trisaccharide side chain attached at C3 to alternate glucose residues (Fig. 1). These side chains consist of an acetylated mannose residue, a glucuronic acid residue and a pyruvate ketal linked to a terminal mannose residue. The acetate and pyruvate yield are variable depending on the fermentation and other experimental conditions used.

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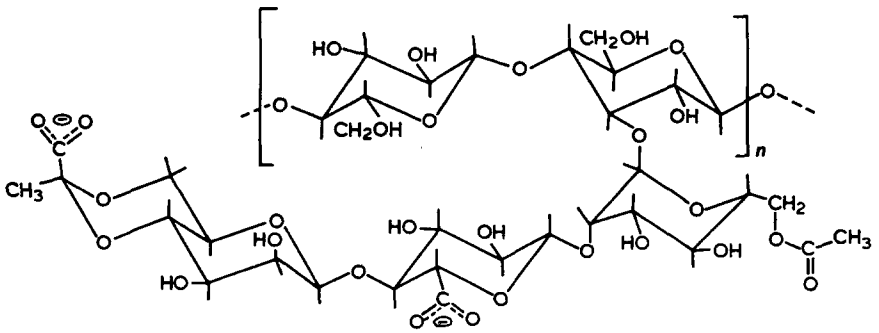

Fig. 1. Chemical structure of the repeating unit (Jansson *et al.*, 1975).

This polysaccharide has unusual rheological properties due to the stiffness of the main chain, which leads to high viscosity solutions even in excess of salt despite its polyelectrolyte character. These properties are important in industrial applications like enhanced oil recovery, drilling fluids and food thickening. The main way to establish the conformation of the xanthan in solution is to look at the molecular weight dependence of the hydrodynamic parameters. Holzwarth (1978), Paradossi & Brant (1982) and Sato *et al.* (1984) have prepared different molecular weight samples by sonication of native xanthan followed by fractionation. Rinaudo & Milas (1980) have used enzymic degradation by a cellulase. Muller *et al.* (1984) have employed fractionated samples of native xanthan prepared by surface exclusion chromatography by Lecourtier and Chauveteau (1984). This last method allows the fractionation of high molecular weight samples without modification of the xanthan molecules which may result from sonication or enzymic degradation. The aim of this work is to compare rheological, optical properties and substituent contents of xanthan samples after progressive degradation by sonication or enzymic attack.

EXPERIMENTAL

Techniques

Viscosity measurements for solutions prepared from samples with molecular weights higher than $6{\cdot}2 \times 10^5$ were carried out with a

Contraves Low Shear 30 viscometer, shear rate range $10^{-2}\ s^{-1} \leqslant \gamma \leqslant 128\ s^{-1}$. For the lowest molecular weight samples, we have used an automatic capillary viscometer from Fica (France). The inner-capillary diameter was 0·5 mm. The subscript '0' is used for values in the Newtonian regime. Average molecular weights and molecular weight distributions were established by gel permeation chromatography (GPC) on 1×6000 and 1×4000 TSK PW columns (60 cm length) using a differential refractometer detector (Iota from Jobin Yvon, France) and an on-line low angle laser light scattering apparatus (Chromatix CMX 100, USA). The elution was performed at 25°C with $NaNO_3$ (0·1 M) in the presence of 0·2 g litre^{-1} NaN_3 as a bactericide and 1% ethylene glycol to prevent adsorption. The eluant was filtered using a Millipore filter (0·22 μm) before use.

Optical rotation was determined at $\lambda = 300$ nm in Spectropol 1b from Fica (France). We used a 5-cm-long quartz cell, thermostatted at 25°C. In order to follow conformational transitions, temperature was scanned from 10 to 70°C at 0·4°C min^{-1} using a temperature programmer (Haake PG 10). The change in the optical rotation is recorded continuously.

The degrees of pyruvylation and acetylation were measured by ^1H NMR. Dried xanthan was dissolved in 5×10^{-3} M sodium acetate D_2O solution at a polymer concentration of about 5 g litre^{-1}. Sodium acetate (5×10^{-3} M) is used as an internal standard. The spectra were obtained with a Brücker WP100 spectrometer at 85°C. The OH signal was suppressed by irradiation. Two-hundred scans were accumulated with a repetition time of 7 s and a sweep width of 1125 Hz. The acetate and pyruvate yields are expressed as an average number per side chain.

Sample preparation

The xanthans supplied by Rhône-Poulenc (Melle, France) (A) and Shell (Sittingbourne, UK) (D) have a weight average molecular weight around $3·3 \times 10^6$ and 7×10^6, respectively. Each side chain contains on average one acetate and one pyruvate group for sample A and 0·75 and 0·40, respectively, for sample D. The xanthan is purified as described previously (Rinaudo & Milas, 1978), dried under reduced pressure at 30°C for 48 h and stored under atmospheric conditions. Before use, the moisture content, typically from 10 to 12%, was determined by thermogravimetry with a Setaram balance (model G 70). All

samples, even those prepared by enzymic hydrolysis, had a N_2 content less than 0·5%.

Enzymic hydrolysis was performed using a crude basidiomycete cellulase obtained from Rapidase (France). The enzyme concentration was 0·2 g litre^{-1}, the polymer concentration 1 g litre^{-1} and the temperature 35°C. Enzymic attack was carried out at different salt concentrations (NaCl from 0 to 2×10^{-2} M for 12 h) in order to get samples with different molecular weights (Rinaudo & Milas, 1980) (Table 1). These samples were precipitated twice by ethanol in excess NaCl, washed successively by ethanol–water mixtures containing decreasing quantities of water and dried.

The samples were used either directly or after fractionation based on liquid crystalline phase separation. Xanthan is a stiff molecule which forms a cholesteric phase at a given critical concentration (C^*) controlled by the molecular weight (Maret *et al.*, 1981; Milas & Rinaudo, 1983). This effect was also recently found with schizophyllan (Itou & Teramoto, 1984). This effect is at least in qualitative agreement with the Flory's theory for rigid molecules (Flory, 1956, 1961). For xanthan C^* varies as $\overline{M}_w^{-1}$ (Milas & Rinaudo, 1981). An example of such a fractionation is given in Table 2. A xanthan sample partially degraded by enzyme is fractionated by progressive increase of the polymer concentration by solvent evaporation in a rotary evaporator

TABLE 1
Samples Obtained by Enzymic Degradation

Sample (*A*)	$\overline{M}_w$	*I*	%[*Ac*][a]	%[*Py*][a]	[η] (*ml g*$^{-1}$)	[α][b]
1	$4{\cdot}2 \times 10^4$	1·63	100	63	30	−14·2 (+16·8)[c]
2	$6{\cdot}5 \times 10^4$	1·5	100	—	36	−20 (+19·2)[c]
3	$8{\cdot}5 \times 10^4$	1·59	100	81	48	−52·8 (−14·8)[c]
4	$1{\cdot}75 \times 10^5$	1·56	100	—	84	−49·6 (−9·6)[c]
5	$2{\cdot}5 \times 10^5$	1·54	100	86	137	−76 (−30·4)[c]
6	$3{\cdot}8 \times 10^5$	1·50	100	96	257	−58·8 (−24)[c]
7	$7{\cdot}5 \times 10^5$	1·73	100	96	690	−94 (−48·8)[c]
8	$3{\cdot}3 \times 10^6$	—	100	100	6200	−117 (−52)[c]

[a] Yield of substituent expressed in number percentage per side chain.
[b] [α] measured at 25°C and 300 nm.
[c] Values of optical rotation for the disordered conformation.

TABLE 2
Fractionation of a Depolymerized Xanthan Sample by Liquid Crystalline Phase Separation

Sample (A)	$\overline{M_w}$	*I*	*Polymer concentration in the liquid crystalline phase (g litre^{-1})*	*Concentration ratios between anisotropic and isotropic phase*	%[*Ac*]	%[*Py*]
Initial	260000	2·5	—	—	100	90
F1	437000	2·1	38	1·046	100	81
F2	287000	1·43	43·7	1·066	100	86
F3	235000	1·35	49·6	1·053	100	89
F4	222000	1·28	70·8	1·15	100	89
F5	148000	1·33	—	—	100	90

at 30°C. Each time, 20% (weight per cent) of the initial xanthan is recovered in the liquid crystalline phase sedimented in the biphasic domain. For each stage of the fractionation the polymer concentrations were determined in both phases. The polymers recovered were characterized by GPC and NMR.

Ultrasonication was carried out using a Branson apparatus (Model B 12, 150 W–20 kHz) equipped with a standard microsonde, 3 mm in diameter. Forty millilitres of a purified xanthan solution (2 g litre^{-1}) in the ordered conformation in 0·1 M NaCl is sonicated at 0°C for various intensities and times. Molecular weight and viscosity measurements are determined directly on these solutions (Table 3). Solutions for optical rotation measurements were sonicated in 0·01 M NaCl as the conformational transition is then located around 50°C and is easy to determine.

RESULTS AND DISCUSSION

Influence of the depolymerization process on the conformational transition and chemical composition

Enzymic degradation causes a reduction in pyruvate as shown in Tables 1 and 2. This would be expected to cause both a decrease in

TABLE 3
Samples Obtained by Sonication[a]

Samples (*A*)	$\overline{M_w}$	*I*	%[*Ac*]	%[*Py*]	$[\eta]_0$ ($ml\,g^{-1}$)
1′	$3{\cdot}3\times10^5$	1·40	100	100	292
2′	$4{\cdot}1\times10^5$	1·18	100	100	428
3′	5×10^5	1·44	100	100	578
4′	$6{\cdot}2\times10^5$	—	100	100	666
5′	1×10^6	1·50	100	100	1010
6′	$2{\cdot}1\times10^6$	—	100	100	4000
7′	$3{\cdot}3\times10^6$	—	100	100	6200

[a] $[\alpha]$ in ordered conformation $= -116 \pm 2°$. $[\alpha]$ in disordered conformation $= -52 \pm 1°$.

the specific optical rotation due to the reduction in chromophore yield and an increase in the melting temperature due to a decrease in the number of charged groups (Holzwarth & Ogletree, 1979; Rinaudo *et al.*, 1983). Experimentally the optical rotation values for the ordered conformation depend strongly on the degree of degradation even though the difference ($\Delta[\alpha]$) between the ordered and disordered states is only slightly changed by degradation (Table 1). The temperature of the conformational transition (T_m) varies with molecular weight in the opposite direction to that expected from the decrease in pyruvate content on degradation (Fig. 2). This result suggests that the enzyme could degrade preferentially some irregularities in the molecule as suggested by Sutherland (1984), resulting in modifications of the conformation. Even though the N_2 content does not change significantly on degradation, it is possible that proteins are associated with the xanthan that affect enzymic degradation. In contrast, the samples obtained by sonication have their substituent yields unchanged (Table 3) and the T_m values are independent of the molecular weight in the range investigated as would be expected for stereoregular polymers.

Influence of the depolymerization process on the molecular weight distribution

Enzymatic degradation leads to samples with a relatively high polydispersity index (*I*). Fractionation by liquid crystalline phase separa-

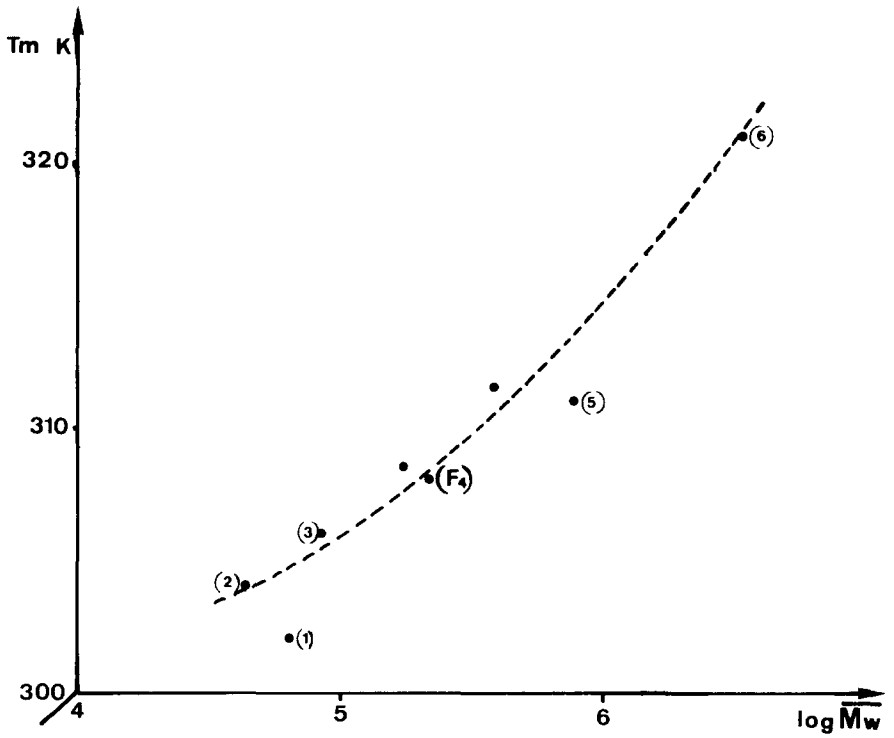

Fig. 2. Dependence of the conformational melting temperature (T_m) on the molecular weight ($\overline{M_w}$) for fractions obtained by enzymatic degradation of xanthan (A). Numbers in parentheses refer to Table 1.

tion gives a lower I value, but it was demonstrated by GPC that low molecular weight molecules were dragged along by the anisotropic phase even if the largest molecular weight species are concentrated in this phase.

In contrast, degradation by sonication gives a polydispersity index (I) of around 1·4. So, we consider that these samples can be used without further fractionation. In Fig. 3, the dependence of the weight average molecular weight on the time of sonication at a given power but for different polymer concentrations is shown. From the molecular weight distributions obtained by GPC,the number average molecular weight ($\overline{M_n}$) is calculated. For random scission of a polymeric chain, it is predicted that:

$$1/\overline{M_n} = 1/(\overline{M_n})_0 + kt$$

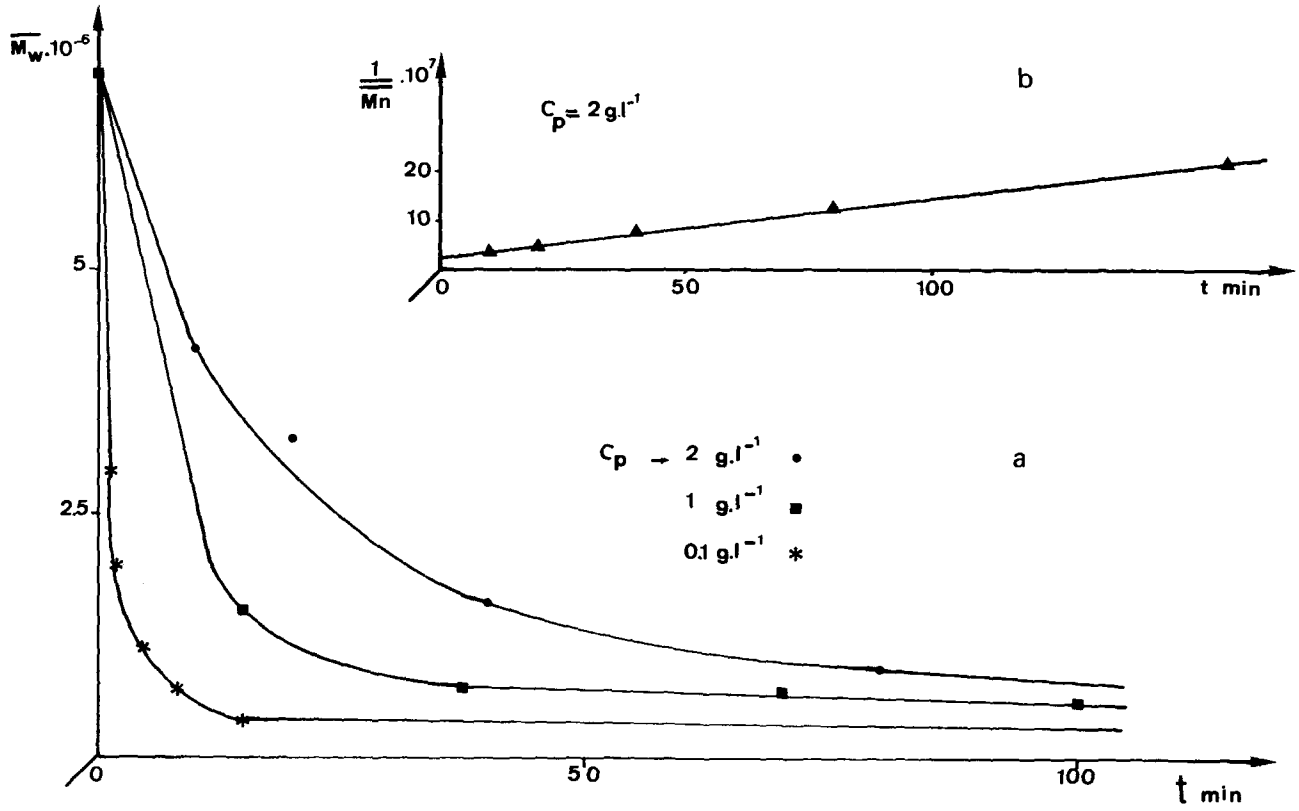

Fig. 3. (a) Variation of the molecular weight ($\overline{M_w}$) as a function of the sonication time for xanthan (D); (b) dependence of $1/\overline{M_n}$ on sonication time.

in which $(\overline{M}_n)_0$ is the initial value, t the time and k the rate constant. Figure 3(b) indicates, at least in the range tested, a random process of depolymerization is probable for short sonication times (Milas & Rinaudo, 1981).

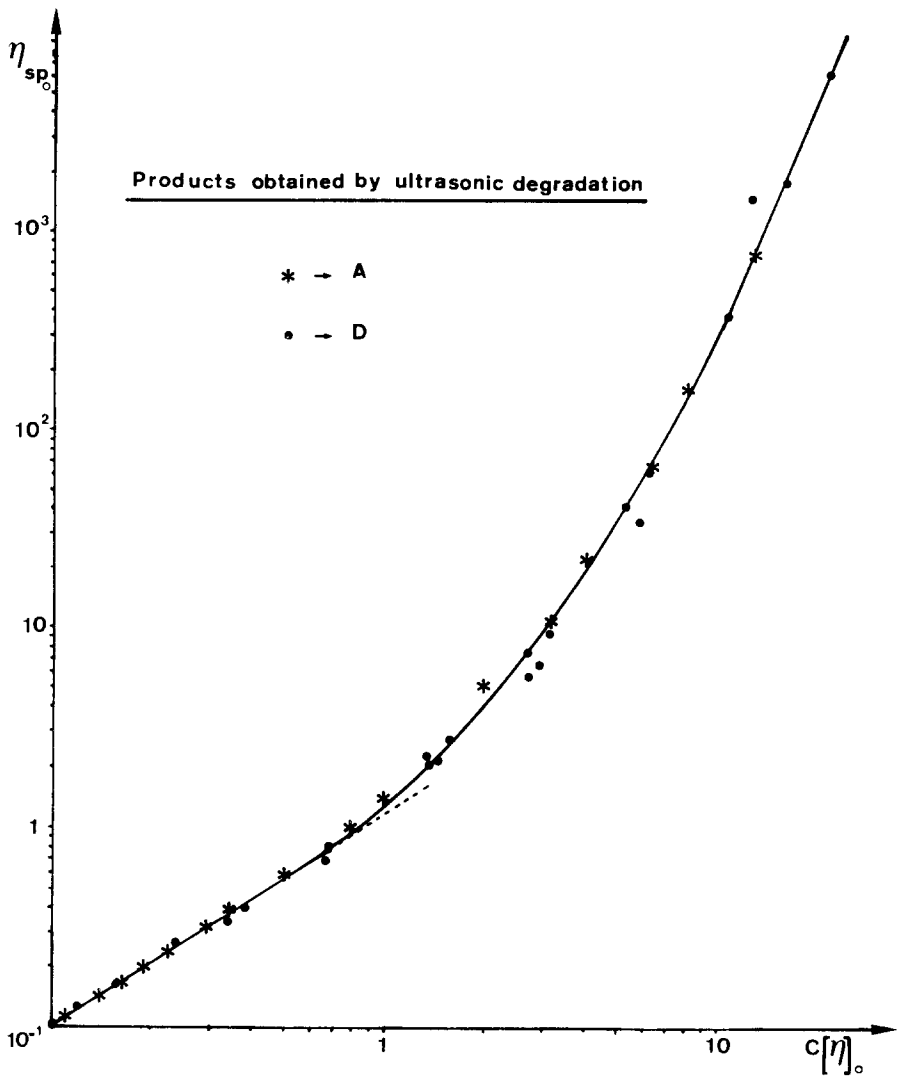

Fig. 4. Specific viscosity as a function of the overlap parameter $C[\eta]_0$ for various xanthan molecular weights obtained by sonication of (A) and (D) xanthans.

Influence of the depolymerization process on the viscosity

When we use the xanthan samples obtained by sonication, the specific viscosity can be uniquely related to the overlap parameter $C[\eta]_0$ as shown in Fig. 4. However, in Fig. 5 we observe that for xanthan degraded by enzymes the η_{sp} versus $C[\eta]_0$ relationship depends on the molecular weight of the sample considered. Figure 6 shows a double-

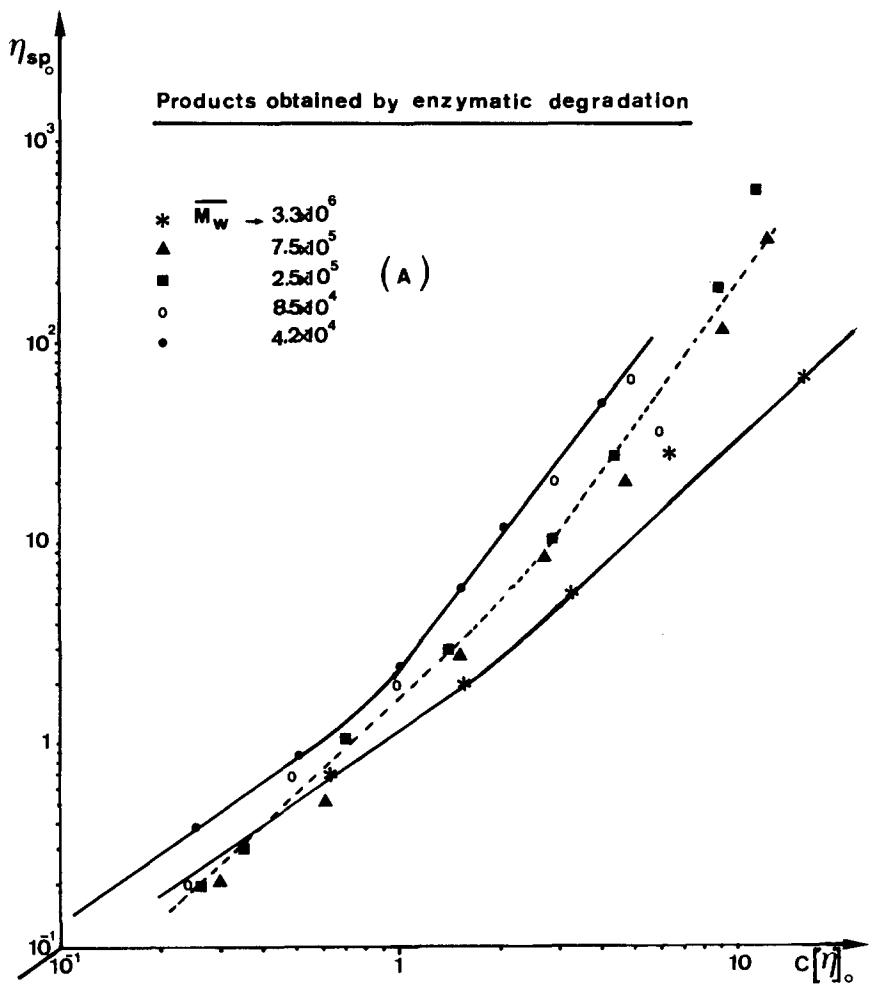

Fig. 5. Specific viscosity as a function of the overlap parameter $C[\eta]_0$ for various xanthan molecular weights obtained by enzymatic degradation of xanthan (A).

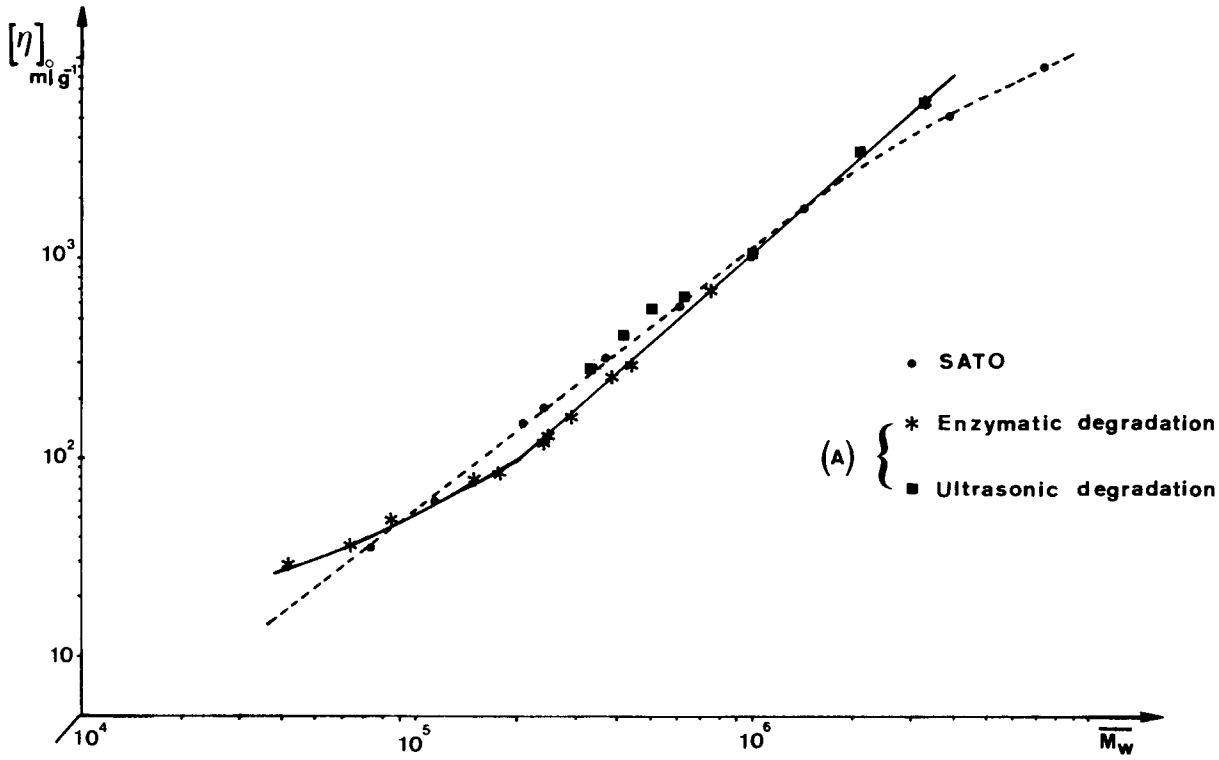

Fig. 6. Double-logarithmic plots of $[\eta]_0$ vs. $\overline{M_w}$ for Na xanthan (A) in 0·1 M aqueous NaCl at 25°C: comparison with Sato's results.

logarithmic plots of $[\eta]_0$ against $\bar{M}_w$ for the xanthans degraded by the two processes. Also included in this figure are the results of Sato *et al.* (1984). Our values are in good agreement with those of Sato *et al.* if we take into account only the sonicated samples, i.e. the same method used by Sato *et al.* Enzymically degraded samples show unexpected behaviour with a marked change in the slope of the plot at intermediate molecular weights. This could also be explained by protein–xanthan associations or selective degradation which would, respectively, change the apparent molecular weight and the stiffness of the xanthan molecules.

CONCLUSION

All these results show that enzymic degradation results in fractions that behave anomalously whereas ultrasonic degradation gave fractions with the expected rheological behaviour.

Degradation by enzymatic attack leads to xanthan samples with different contents of pyruvate and to peculiar hydrodynamic and structural behaviour. We think that these anomalies are due to the existence of a complex between proteins and xanthan which could form during the degradation. Another hypothesis could be a selective enzymic degradation or a conformational modification. The polydispersity index of the samples is relatively high and further fractionation, like the liquid crystal phase separation as described in this paper, is required. The selectivity of this process must be related to the number of fractions separated.

In contrast, ultrasonic degradation leads to samples with a low polydispersity index which can be used without fractionation and may be assumed as representative of a given molecular weight sample. There are no changes in the substituent contents in this case. The melting temperature of the ordered conformation is independent of molecular weight. The relationship between $[\eta]_0$ and $\bar{M}_w$ is identical to that described by Sato, even if we do not agree with its interpretation in terms of a double helix. A master curve of η_{sp} as a function of the overlap parameter $C[\eta]_0$ is only obtained with sonicated xanthan samples. This curve is discussed elsewhere by Milas *et al.* (1985).

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