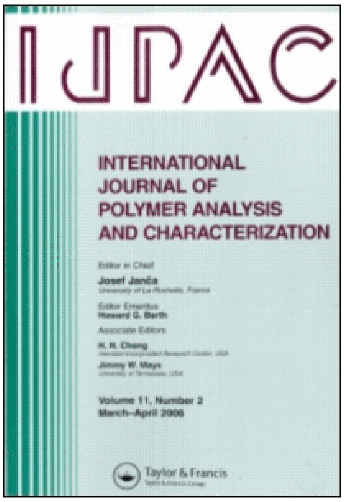

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Conformations of Xanthan in Solution: Analysis by Steric Exclusion Chromatography

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Conformations of Xanthan in Solution: Analysis by Steric Exclusion Chromatography

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The determination of the radius of gyration and intrinsic viscosity as function of molecular weight for xanthan polysaccharide allows for a better understanding of the conformation of this polymer in solution. The native ordered conformation of xanthan, which corresponds to that obtained during its biosynthesis appears to be a single chain characterized by a persistence length of approximately 400 Å. In contrast, the renatured ordered conformation restored after the first denaturation of the native conformation exhibits behavior suggesting double-stranded conformation as described previously in the literature. In fact, this conformation seems to correspond to a folded xanthan chain.

KEY WORDS Polysaccharide, xanthan, conformation, radius of gyration, intrinsic viscosity, molecular weight

INTRODUCTION

The aim of this work is to show that the determination of the variation of the radius of gyration $\langle S^2\rangle^{1/2}$ and the intrinsic viscosity $[\eta]$ of ionic polysaccharides as a function of the molecular weight, obtained by steric exclusion chromatography (SEC), can give information on structure and conformation. In view of this, experimental data are compared with theoretical data using wormlike-chain models characterized by a persistence length which contains an electrostatic contribution and an expansion coefficient dominated by electrostatic interactions. This method is used in this work to try to elucidate the nature of ordered conformations of the bacterial polysaccharide xanthan.

EXPERIMENTAL

Two different samples of xanthan were used: The first one is "P100" from Rhône-Poulenc (Melle, France), which is extracted from an unpasteurized fermentation broth. The native conformation was preserved during purification and sonication steps by using a salt con-

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tent (NaCl) equal to at least 0.1 M. The second sample is an industrial powder from Kelco "Keltrol RD" (San Diego, Calif. USA) for which the isolation process is not given. Both samples were purified after chain degradation by sonication in order to obtain an average molecular weight in the range of 10^6 g/mol [1].

According to the melting temperature of the ordered conformation [1], the denatured conformation is obtained by heating the xanthan solution in 0.01 M NaCl at 80°C (1 min). The renatured conformation is stabilized after cooling at ambient temperature [1].

SEC experiments with three detectors online were performed on these samples. We have used a Waters 150C apparatus (Milford, Mass.) with two Shodex OH pak B-805 and SB-804 MQ columns (Showa Denko, Tokyo, Japan) with 0.1M NH_4NO_3 as the eluent. A Hewlett-Packard 1050 pump (Avondale, Penn.), a home-made viscometer, and a multiangle laser light scattering (MALLS) Dawn DSP-F from Wyatt Technology (Santa Barbara, Calif.), were added to the Waters 150C [2]. All measurements were performed at 30°C. The dn/dc value used was 0.155 mL/g [1].

The $\langle S^2\rangle^{1/2}$ (M) curves were obtained using ASTRA 3.02 and EASI 7.02 software from Wyatt Technology, with the scattering angles from 20 to 90°, Zimm calculation were performed using a second-order least-square fit. Intrinsic viscosity curves $[\eta]$ (M) and fits with the theoretical models were achieved using inhouse software.

THEORETICAL APPROACHES [3,4]

Persistence Length

The Odijk's model introduces [5]

$$L_t = L_p + L_e \tag{1}$$

where L_t, L_p, and L_e are the total, intrinsic and electrostatic persistence lengths, respectively, in which

$$L_e(\text{Å}) = \frac{\lambda^2}{12Q\kappa^2}\left[3 - \frac{8}{Y} + \exp(-Y)\cdot\left(Y + 5 + \frac{8}{Y}\right)\right] \tag{2}$$

where Q is the Bjerrum length $Q = e^2/DkT \sim 7$ Å at 25°C, kT is the Boltzmann term, D is the dielectric constant, and λ is the polyelectrolyte charge parameter equal to, Q/A with A being the average distance between the projection of the charges onto the chain axis. When $\lambda > 1$, we assume it is equal to 1 because of ionic condensation. $Y = L\kappa$ where L is the contour length and κ is the Debye-Huckel parameter: κ^{-1} (Å) = $3.03\ c^{-1/2}$; $c = c_s + c_p/\lambda$; c_p and c_s are respectively the polyelectrolyte and salt concentrations (equiv/L)).

Radius of Gyration

From Benoit and Doty relation [6] and the Odijk approach [4], we can write:

$$\langle s^2\rangle = \frac{LL_t}{3} - L_t^2 + 2\frac{L_t^3}{L} - 2\frac{L_t^4}{L^2}\left[1 - \exp\left(-\frac{L}{L_t}\right)\right]\alpha^2_{s,el} \tag{3}$$

where L is the contour length of the polymer, and $\alpha_{s,el}$ the expansion factor owing to long-range electrostatic interaction:

$$\alpha_{s,el}^{5} - \alpha_{s,el}^{3} \approx \frac{134}{105}\left(1 - 0.885\left(\frac{L}{2L_t}\right) - 0.462\right)z_{el} \tag{4}$$

with

$$z_{el} = \left(\frac{3}{8\pi}\right)^{3/2} \beta_{el}\,(L/2)^{1/2} L_t^{-7/2} \tag{5}$$

and

$$\beta \approx \beta_{el} = \frac{8L_t^2}{\kappa}\int_0^{\frac{\pi}{2}} \sin^2\theta \int_0^{\frac{w}{\sin\theta}} x^{-1}(1-\exp(-x))\,dx\,d\theta \tag{6}$$

with

$$w = 2\pi A^{-2}\,Q\kappa^{-1}\,\exp(-\kappa d) \tag{7}$$

where d is the rod diameter.

These relations are deduced from the works of Odijk and Houwaart [7], Yamakawa and Tanaka [8], and Fixman and Skolnick [9].

Intrinsic Viscosity

From Yamakawa-Fujii theory [10] and Odijk approach [11], the intrinsic viscosity, assuming wormlike-chain behavior, is given by:

$$[\eta] = \phi(L_r, d_r)\left(\frac{M_L}{2L_t}\right)^{-3/2} M^{1/2}\alpha_\eta^3 \tag{8}$$

Where $L_r = (L/2L_p)$ and $d_r = (d/2L_p)$; M_L is the molar mass per unit length, d the chain diameter, and ϕ is a parameter depending on draining effects [10]. According to the Weill and des Cloizeaux method [12], α_η, the viscometric expansion factor, is deduced from the calculated value of $\alpha_{s,el}$.

RESULTS AND DISCUSSION

Xanthan in solution undergoes a conformational transition that can be monitored by temperature and ionic strength changes. Controversy still exists about the exact nature of the ordered conformation of xanthan which is in most cases considered as a double helix [13]. Nevertheless some behaviors are compatible with single-stranded chains [14]. In fact, at least two different ordered conformations exist. The first one (native conformation, N) corresponds to the conformation obtained during the fermentation process, and the second one (renatured conformation, R) is stabilized after denaturation (D) of the N conformation as follows [1]:

Little information is available on the denatured D and native N conformations.

$$\begin{tikzcd}
N \arrow[r, "T\uparrow", "\mu\downarrow"'] & D \arrow[r, shift left, "T\downarrow\mu\uparrow"] & R \arrow[l, shift left, "T\uparrow\mu\downarrow"] \\
\text{irreversible} & \text{reversible} &
\end{tikzcd}$$

The characteristics of the samples used in this work are given in Table I, before and after denaturation, the differences between the results obtained from static and SEC experiments are caused by a decrease of xanthan concentration by retention during filtration through a 0.2-μm membrane. The concentration decrease is usually not taken into account in static light scattering measurements in contrast to SEC experiments.

Figure 1 represents the variation of $\langle S^2\rangle^{1/2}$ for the N and R conformations of the P100 sample with molecular weight. The filled symbols correspond to the theoretical fits, Equation (3), using M_L ($M_L = M/L$) and L_p ($L_t = L_p + L_e$) as variables. L_e is calculated from Equation (2) and $\alpha_{s,el}$ from Equations (4)–(7). Figure 2 gives the variation of $[\eta]$ (M) for the two conformations and the comparison with theoretical model, Equation (8) using M_L and L_p as variables. The best values obtained are listed in Table II. (According to the literature [5,16] and the results obtained, we used d = 16 and 24 Å, respectively for the N and R conformations. Nevertheless the d value is not of primary importance for interpreting the results).

From $\langle S^2\rangle^{1/2}$ (M) variations it is clear that the P100 "N" conformation is well represented by a single-chain conformation (theoretical M_L = 98 g/Å) with a persistence length equal to 460 Å. In contrast, the P100 "R" conformation is more rigid and is well described by a double-chain model (theoretical M_L = 196 g/Å) and a persistence length equal to 1600 Å. The variations of $[\eta]$ (M) confirm this change but the modified Yamakawa-Fujii model, which takes into account the polyelectrolyte effects (Equation (8), leads to lower persistence lengths as already reported for hyaluronate [17].

Since the molecular weight between the N and R samples does not increase (the small decrease observed may be caused to small aggregates), the R conformation appears as an intramolecular modification of the N conformation.

To confirm these results, we renatured a purified but unsonicated xanthane P100 by heating. Afterwards, we sonicated it under its renatured conformation, and the mass distribution was measured before and after heating over the melting temperature of the ordered conformation. The results are given in Figure 3 with the mass distribution of a xanthan sonicated in the native state, before and after renaturation. These data confirm that the renatured xanthan is well described by the double-chain model and that the renaturation is an intra-molecular mechanism: the sonication of the renatured xanthan broke the double chains in several pieces. The denaturation by heating caused the dissociation of these double-chain pieces to single chains halving the molar mass. The renaturation leads to folding of these single chains forming new double chains.

The commercial Kelco sample, for which the isolation process is not given, has an intermediate conformation and exhibits higher intrinsic viscosity and radius of gyration. The

TABLE 1

Characteristics of the different xanthan samples and conformations.

Sample N = Native R = Renatured	$M_w \times 10^{-6}$[a] ± 0.2	$M_w \times 10^{-6}$[b] ± 0.05	$[\eta]$ (mL/g)[a] ± 100	k'[c] ± 0.05	$[\eta]$ (mL/g)[b] ± 50	$\langle S^2\rangle_z^{1/2}$ (Å)[a] ± 150	$\langle S^2\rangle_z^{1/2}$ (Å)[b] ± 50
P100 N	1.55	1.8	1410	0.49	1400	1500	2000
P100 R	1.3	1.6	1650	0.82	1700	1700	2100
Kelco [d]	1.3	1.2	1410	0.49	1470	1600	1650
Kelco R	1.1	1.1	1030	0.66	1150	1550	1500

[a] static measurements; [b] from SEC experiment; [c] k': Huggins coefficient; [d] the exact conformation is unknown.

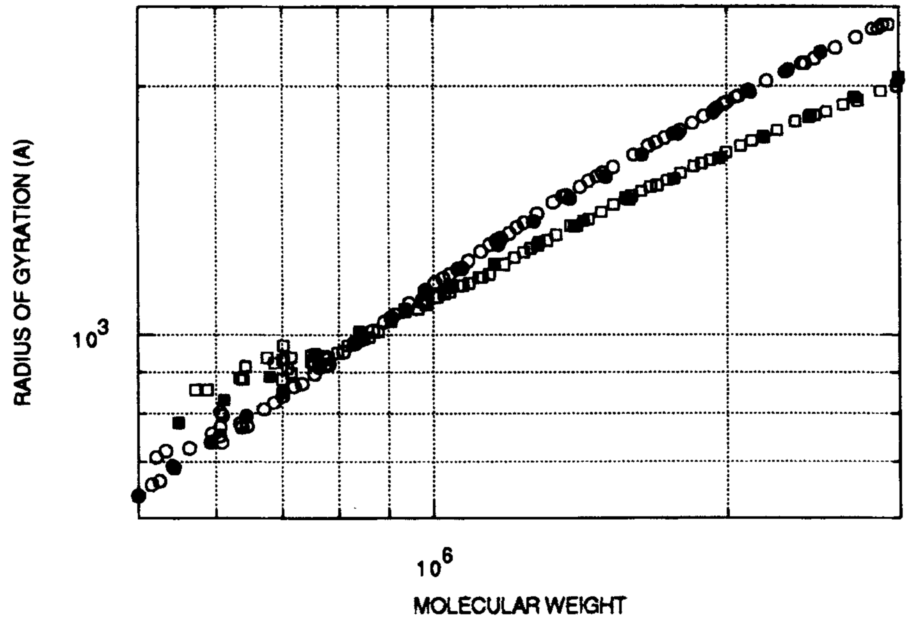

FIGURE 1 Radius of gyration as a function of molecular weight for the P100 sample. □, ○, for the N and R conformations, respectively; filled symbols: theoretical values from Equation (3) with M_L and L_p given in Table II.

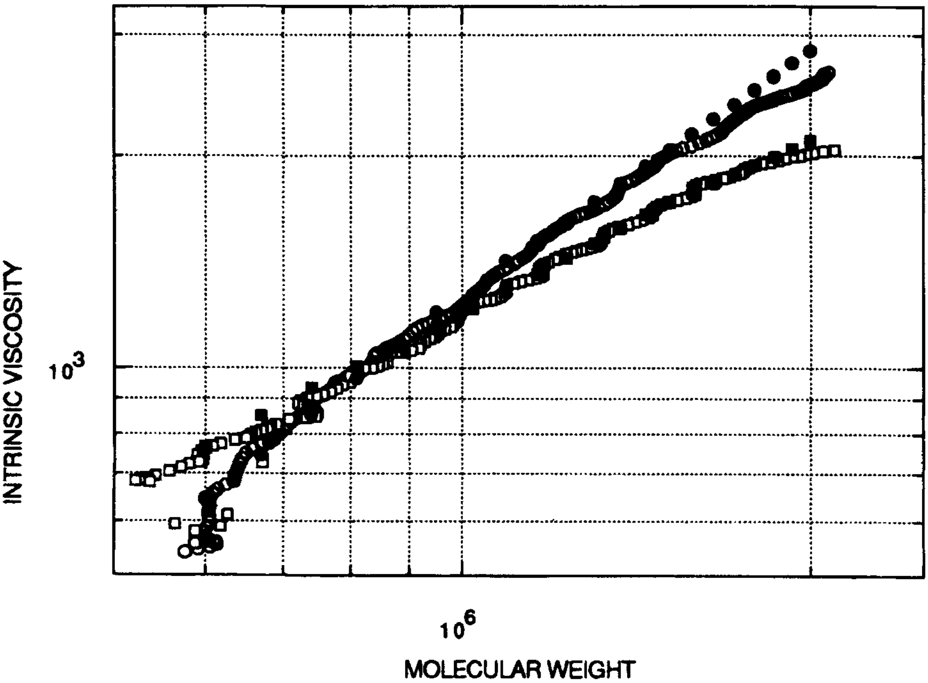

FIGURE 2 Intrinsic viscosity as a function of molecular weight for the P100 sample. Symbols: see Figure 1. Theoretical values were obtained from equation 8 with M_L and L_p given in Table II.

use of the theoretical models is not well adapted. In contrast, after heating, the R conformation is similar to that of the renatured P100 (Figures 4 and 5, Table II) and the characteristics found are similar to those given in the literature [16].

CONCLUSION

From these results it appears that the xanthan is most probably synthetized as a single-ordered chain, but after the first denaturation of the native ordered structure, a double-chain conformation is stabilized. Since the molecular weight does not increase, chain folding is most probable, resulting in a partially antiparallel double-chain conformation as described in the literature [15]. Furthermore, the values of L_P, M_L and **a** (exponent of the Mark-Houwink equation) obtained in this work from viscometric data for the renatured conformation are in agreement with those given by Sato [16].

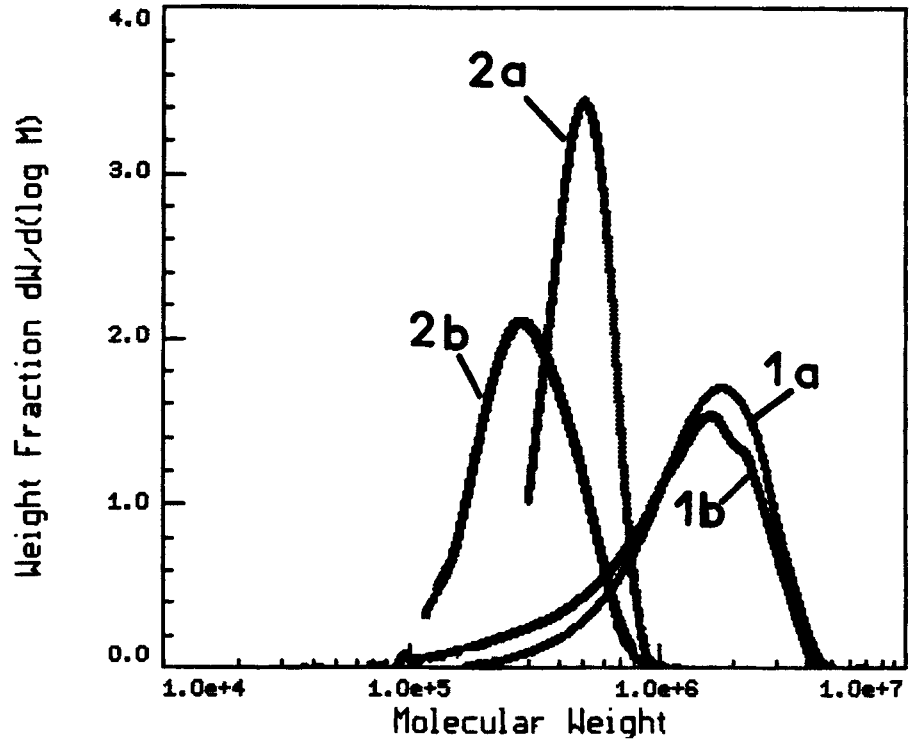

FIGURE 3 Weight fraction as a function of molecular weight of two samples of xanthan P 100 sonicated in the native state, 1, and in the renatured state, 2, before, 1a and 2a, and after, 1b and 2b, heating over the melting temperature of the ordered conformation (2a, 1b and 2b are renatured forms).

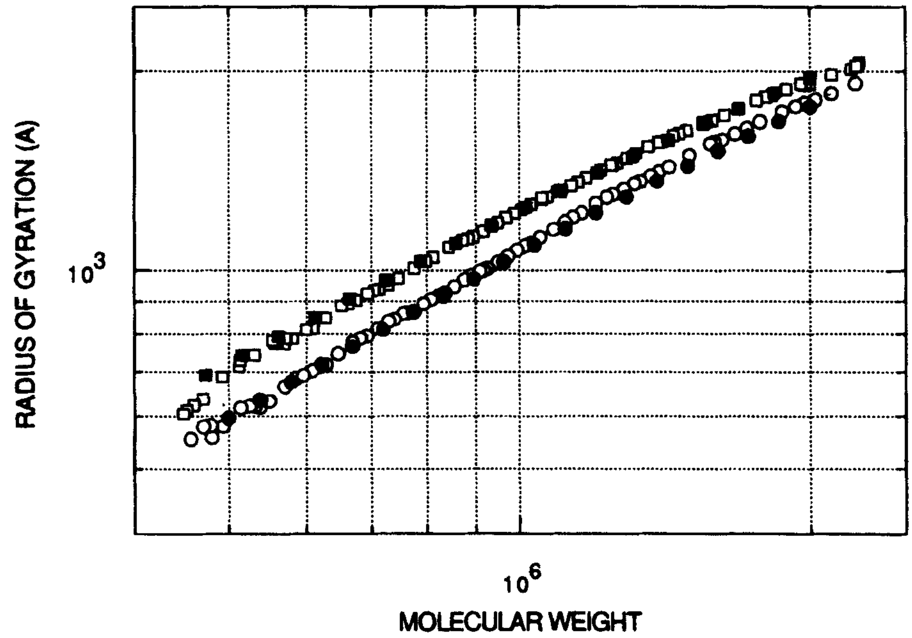

FIGURE 4 Radius of gyration as a function of molecular weight for the Kelco sample. Symbols: see Figure 1. Theoretical values were obtained from Equation (3) with M_L and L_p given in Table II.

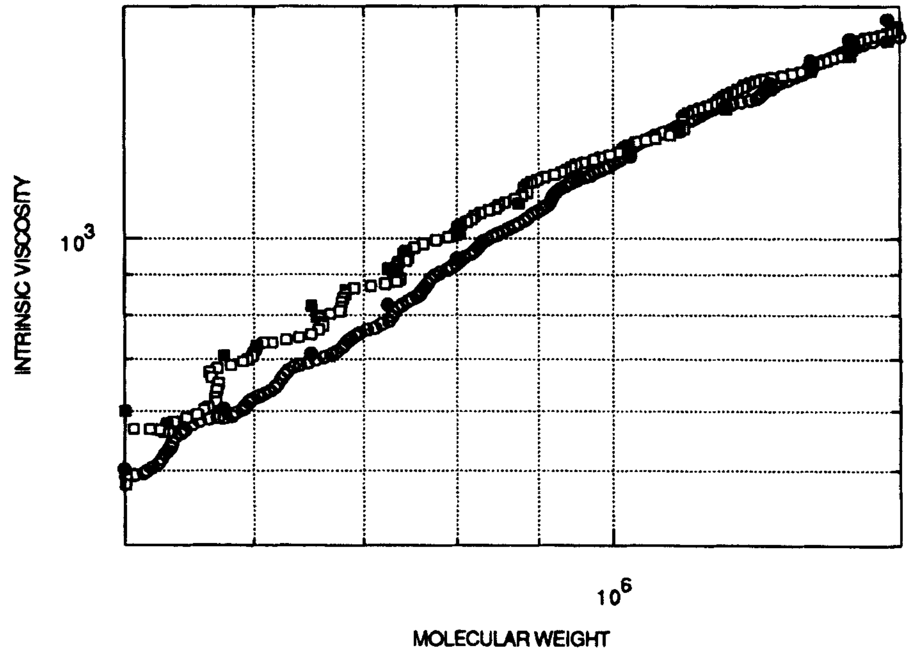

FIGURE 5 Intrinsic viscosity as a function of molecular weight for the Kelco sample. Symbols: see Figure 1. Theoretical values were obtained from Equation (8) with M_L and L_p given in Table II.

TABLE II

Mass per unit length M_L and persistence length L_p used to fit the experimental data from theoretical models.

conformation N = native R = renatured	from $[\eta](M)$ and $[\eta] = KM^a$			from $\langle S^2\rangle 1/2$ (M) and $\langle S^2\rangle 1/2$ $*K''M^v$		
	M_L (g/Å)	L_p (Å)	$a^{(b)}$	M_L (g/Å)	L_p (Å)	$v^{(b)}$
N P100	119	310	0.9	109	460	0.54
R P100	198	1200	1.25	196	1600	0.75
(a) Kelco[a]	140	465	1.14	160	1200	0.66
R Kelco	195	1090	1.3	210	1500	0.75

[a] The exact conformation is unknown.

[b] Average value in the range of molecular weight between $5 \cdot 10^5$ and $2 \cdot 10^6$ g/mol.

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