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Double-Stranded Helix of Xanthan in Dilute Solution: Evidence from Light Scattering

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ABSTRACT: A sample of xanthan, a bacterial β-1,4-D-glucan with ionic side chains, and its sonicated fragments in 0.1 M aqueous NaCl and cadoxen at 25°C were studied by light scattering. Radii of gyration $\langle S^2 \rangle^{1/2}$ as a function of weight-average molecular weight M_w in these two solvents, combined with the values of about 2 for the ratio M_w (in 0.1 M NaCl)/M_w (in cadoxen), showed that xanthan dissolves as rodlike dimers in 0.1 M aqueous NaCl and as single flexible chains in cadoxen. The contour length per main chain glucose residue of the xanthan dimer was found to be 0.47 ± 0.01 nm, which agreed with the pitch (per glucose residue) of the 5_1 double-stranded helix proposed for the crystalline structure of xanthan. Thus, it was concluded that the xanthan dimer in 0.1 M aqueous NaCl has the 5_1 double-stranded helical structure.

KEY WORDS Polysaccharide / β-1,4-Glucan / Xanthan / Double Helix / Light Scattering / Radius of Gyration /

Xanthan is a water-soluble, extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. Its repeating unit[1,2] is shown in Figure 1, but, in actuality, only about one-third of the terminal D-mannose residues of the side chains is pyruvate substituted.[3] The conformation of this polymer in dilute solution has called a considerable interest of polysaccharide physical chemists, but no definite conclusion on it is as yet reached.[4,5]

Several groups[3,4,6-8] used spectroscopic methods such as optical rotatory dispersion, circular dichroism, and NMR, and concluded that xanthan takes on an "ordered" conformation in aqueous salt solution, and undergoes a thermal order-disorder conformation change when the ionic strength of the solution is lowered. Morris *et al.*[3] preferred a single-stranded helix for the ordered conformation, since it was compatible with his finding that the spectroscopically observed order–disorder change did not depend on polymer concentration. His choice was subsequently supported by the spectroscopic studies of Milas and

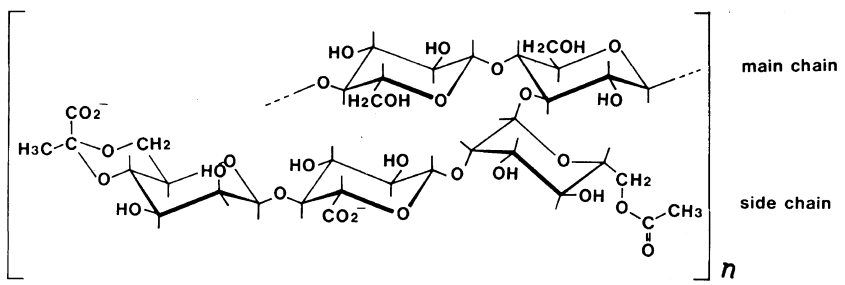

Figure 1. Repeating unit of xanthan.

Rinaudo,[9] Norton *et al.*,[10] and Frangou *et al.*[11] Another support to the single-stranded helix was given by Rinaudo and Milas[12] who found that the weight-average molecular weight M_w of a xanthan sample in 0.001—0.1 M aqueous sodium chloride (NaCl) was independent of the salt concentration.

On the other hand, Holzwarth and Prestridge[13] concluded the ordered conformation of xanthan to be a double-stranded helix of paired chains on the basis of electron microscopic observations. Subsequently, Holzwarth[14] and Paradossi and Brant[15] estimated the molar mass per unit contour length of xanthan to be 2000 nm^{-1} from hydrodynamic and light scattering data in aqueous NaCl, respectively. This value is twice that expected for a single xanthan chain. Thus, these authors argued that the ordered conformation of xanthan should consist of paired chains. However, it should be noted that Holzwarth estimated the molecular weights of his samples using the Flory–Scheraga–Mandelkern equation[16] with the familiar β parameter taken to be 2.5×10^6 (in conventional units), while Paradossi and Brant assumed xanthan in aqueous NaCl to be completely rigid, though their radius of gyration data were apparently compatible with semiflexible chains instead of rigid rods.

In this paper, we present light scattering data leading to a clear-cut answer to the question of whether the ordered conformation of xanthan in aqueous salt solution is a single-stranded or double-stranded helix. The conclusion given below was made possible by our finding that xanthan can be dissolved in cadoxen tris(ethylenediamine) cadmium dihydroxide) as "disordered," *i.e.*, flexible, single chains.

EXPERIMENTAL

Samples

Aqueous solutions (1 or 0.5% polymer) of a commercial xanthan sample (Kelco Keltrol) were treated with activated charcoal, and exposed to 10 or 19.5 kHz sonic irradiation (Cho-onpa Kogyo Model USV-150N10 or Kaijo Denki Model TA-6280 N) for 0.5 to 168 h. The jacket of the sonication vessel was maintained below 25°C by circulating water of about 8°C. Each of the sonicated solutions was centrifuged in a Sorvall RC2-B centrifuge at 14000 *g* for 1 h, and the supernatant was poured into a large quantity of acetone to reprecipitate the polymer. In this way, four samples of different molecular weights, designated below as X3, X6, X7, and X8, were prepared. The original unsonicated sample was similarly purified and designated as X4.

Each of these five samples was divided into 4 to 6 parts by repeating fractional precipitation with 0.5 M aqueous NaCl as the solvent and acetone as the precipitant. Appropriate middle fractions X3-5, X4-5, and X7-3b from samples X3, X4, and X7 were chosen for the present study. Fractions X8-3 and X6-4 from samples X-8 and X-6, respectively, were further fractionated by gel filtration with 0.05 M aqueous NaCl as the eluant. Two columns (5 cm in inner diameter and 25 cm in length) connected in series were packed with Sepharose CL-6B gel, and loaded with 50 cm^3 of an 0.05 M aqueous NaCl solution of either sample; the initial polymer concentration was about 1%. Ten fractions were separated from either sample, and three middle fractions X8-3-8, X8-3-5, and X6-4-4 were chosen.

The six fractions so selected for the present work were reprecipitated from aqueous NaCl (0.05—0.5 M) solutions into acetone, washed with acetone, dried *in vacuo*, and stored at −20°C until use.

Preparation of 0.1 M NaCl Solutions

Light scattering measurements were made after the samples had been converted to Na salts to enhance their solubility in water. The conversion was effected as follows.

A given xanthan sample was dissolved in pure water at about 5°C, and the solution was passed through an ion exchanger [Amberlite

IR-120+IRA-400 or 410 (1 : 2)], with the temperature of the solution kept at about 4°C (for lower molecular weight samples X8-3-8, X8-3-5, and X6-4-4) or below 15°C (for other samples). The acid-form polymer was neutralized by addition of 0.1 *N* sodium hydroxide (NaOH) at 10°C; the three lower molecular weight samples were neutralized at room temperature, with 0.05 M NaCl added. The change in pH of the solution was monitored using a Beckman pHI 70 pH meter.

An aliquot of each neutralized solution was lyophilized to determine the polymer mass concentration c. The rest was used for light scattering measurement, with NaCl added to a concentration of 0.1 M in order to suppress polyelectrolytic effects of the polymer. When freeze-dried, none of our xanthan samples completely dissolved in 0.1 M NaCl.

The amount of 0.1 *N* NaOH consumed for neutralization and the known value of c were used to evaluate the degree of pyruvation (DS_{pyr}) for each sample. The values obtained for our samples ranged from 0.32 to 0.37.

Preparation of Cadoxen Solutions

The polymer was reprecipitated from an 0.05—0.1 M NaCl solution of Na salt xanthan into acetone containing 5% water, washed with acetone, and dried *in vacuo* for 4 days. Cadoxen solutions were prepared by mixing weighed amounts of the Na salt xanthan so prepared with the solvent. In calculating c in these solutions, the amount of NaCl remaining in the polymer was neglected, since it was found by potentiometry to be less than 0.3 wt% of the sample.

Vacuum-dried Na salt xanthan samples in pure water were titrated with aqueous NaOH. The results showed that more than 95% carboxylic groups of their side chains carried Na. Thus, in molar mass per cellobiose unit of xanthan, the vacuum-dried Na salt samples differed no more than 0.1% from complete neutralization.

The solvent cadoxen was prepared at one time. A 28 wt% aqueous solution (2000 cm^3) of ethylene diamine was saturated with cadmium oxide at about 0°C under vigorous stirring, and kept at 0°C for 10 h. The solution was twice warmed to 30°C and cooled to 5°C, centrifuged at 9000 g for 0.5 h, and the supernatant was filtered through a glass filter. The cadoxen so prepared, which was transparent and stable at 25°C, was kept stored at about 5°C in a refrigerator until use. Its composition was 4.8 wt% in cadmium and 26.7 wt% in ethylenediamine, and its density and refractive indices n_0 for 436 and 546 nm wavelengths at 25°C were 1.059 $g\,cm^{-3}$ and 1.398 and 1.392, respectively.

Our cadoxen differed from that of Henley[17] in regard to the NaOH content; Henley's cadoxen contained about 0.5 *N* NaOH, but ours none. Our preliminary tests showed that addition of NaOH hardly enhanced the solubility of xanthan in cadoxen.

Light Scattering

Intensities of light scattered from 0.1 M aqueous NaCl and cadoxen solutions of Na salt xanthan at 25°C were measured on a Fica 50 light scattering photometer in an angular range from 22.5 to 150°. Vertically polarized incident light of 436 or 546 nm was used. The instrument was calibrated with benzene at 25°C as the reference liquid. The Rayleigh ratio of this liquid was taken to be 46.5×10^{-6} and 16.1×10^{-6} cm^{-1} at 436 and 546 nm,[18] respectively; and its depolarization ratio was estimated to be 0.41_1 for 436 nm and 0.40_6 for 546 nm by the method of Rubingh and Yu.[19]

Optical clarification of 0.1 M aqueous NaCl solutions of Na salt xanthan was made by the method[20] established for schizophyllan. For cadoxen solutions, this was done by filtration through a Millipore filter (Type FH or FG).

When the intensity was measured with an analyzer set in the horizontal direction, Na salt xanthan in 0.1 M aqueous NaCl exhibited a certain degree of depolarization. Thus, the measured reduced-scattering intensity $R_{Hv}(\theta)$

at a scattering angle θ in this solvent was combined with R_θ [$\equiv R_{Uv}(\theta)$] measured in the same solvent with no analyzer to estimate the optical anisotropy parameter δ^2 using the general relation[21]

$$\lim_{\substack{\theta\to 0\\ c\to 0}} [R_{Hv}(\theta)/Kc]/\lim_{\substack{\theta\to 0\\ c\to 0}} (R_\theta/Kc)=3\delta^2/(1+7\delta^2) \tag{1}$$

where K is an optical constant. The parameter δ is proportional to the optical anisotropy ε defined by

$$\varepsilon=\frac{3(\alpha_1-\alpha_2)}{\alpha_1+2\alpha_2} \tag{2}$$

with α_1 and α_2, respectively, the longitudinal and transverse polarizabilities of a cylindrically symmetric scattering element. For rigid rods, δ is equal to $\varepsilon/\sqrt{45}$.[21]

The δ^2 was estimated to be 2.2×10^{-3}, 1.9×10^{-3}, and 1.7×10^{-3} for samples X8-3-8, X8-3-5, and X6-4-4, respectively, and was less than 10^{-3} for other higher molecular weight samples. These δ^2 values for the three lower molecular weight samples were substituted into the well-known relations[21,22]

$$M^*=M_w(1+7\delta^2) \tag{3}$$

$$A_2^*=A_2/(1+7\delta^2)^2 \tag{4}$$

to evaluate the desired (true) M_w and second virial coefficients A_2 from the apparent molecular weights M^* and the apparent second virial coefficients A_2^* determined from Kc/R_θ data by the conventional method. No correction for optical anisotropy was made for other samples.

Apparent radii of gyration $\langle S^2\rangle^{*1/2}$ for the three lower molecular weight samples were corrected for optical anisotropy, assuming that the equation for rigid rods[23]

$$\langle S^2\rangle^*=\langle S^2\rangle\left(1+\frac{47}{7}\delta^2-\frac{4}{\sqrt{5}}\delta\right)\Big/(1+7\delta^2) \tag{5}$$

is applicable to these samples in 0.1 M aqueous NaCl (see below). It was found that δ was positive, since $R_{Hh}(\pi/2+\Delta\theta)-R_{Hh}(\pi/2-\Delta\theta)$ was positive for $\pi/8\gtrsim\Delta\theta>0$.[23,24] Here, R_{Hh} denotes the reduced-scattering intensity measured with the polarizer and analyzer both oriented in the horizontal direction.

Differential Refractometry

Specific refractive index increments $(\partial n/\partial c)_\mu$ for dialyzed 0.1 M NaCl solutions of Na salt xanthan at 25°C were determined using a differential refractometer of the modified Schulz–Cantow type. Here, the subscript μ denotes the chemical potentials of the diffusible components in the solution. The dialysis was effected using Visking gel-cellophane membranes and the dialyzer described elsewhere.[25] The values of $(\partial n/\partial c)_\mu$ obtained were 0.144 and 0.141 $cm^3\,g^{-1}$ for 436 and 546 nm, respectively. The former agreed with Paradossi–Brant's value[15] of 0.144 $cm^3\,g^{-1}$ for the same system at 27°C.

The values of $(\partial n/\partial c)_\mu$ for cadoxen solutions of Na salt xanthan were estimated by the following indirect method, since no semipermeable membrane resistant to the solvent was available.

Visking gel-cellophane membranes were soaked in cadoxen diluted with water to different compositions. They were stable up to a w (wt% of cadoxen) of 85%. Hence, water-diluted cadoxen solutions of sample X7-3b were dialyzed at w from 50 to 85%. For any w, the equilibrium was attained in 2 days. The values of $(\partial n/\partial c)_\mu$ determined for the dialyzed solutions are plotted against w in Figure 2. It can be seen that $(\partial n/\partial c)_\mu$ is almost independent of w in the range studied. This behavior may be attributed to the accident that the decrease in excess refractive index Δn with an increase in n_0 happened to be offset by the increase in Δn due to a preferential adsorption of cadmium onto the polymer chain. Extrapolation of the indicated lines to $w=100\%$ yields values of 0.167 and 0.164 $cm^3\,g^{-1}$ for $(\partial n/\partial c)_\mu$ in cadoxen at 436 and 546 nm,

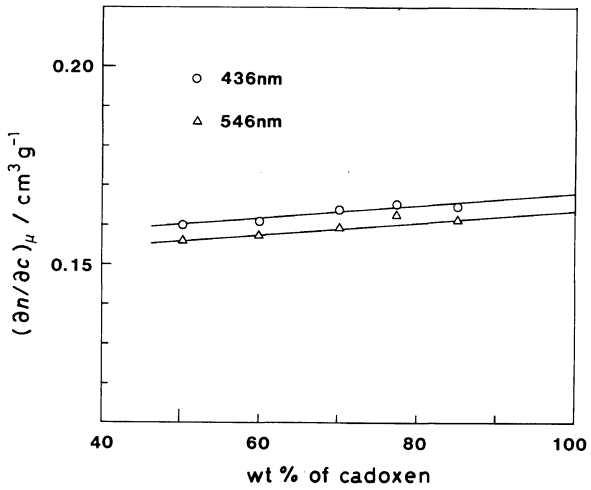

Figure 2. Composition dependence of $(\partial n/\partial c)_\mu$ for Na salt xanthan in mixtures of water and cadoxen at 25°C.

respectively.

Sedimentation Equilibrium

Samples X8-3-8, X8-3-5, and X6-4-4 in 0.1 M aqueous NaCl at 25°C were investigated by sedimentation equilibrium, using a Beckman Model E ultracentrifuge equipped with an electronic speed control system. A Kel-F 12-mm double sector cell was used for samples X8-3-8 and X8-3-5, and a filled-Epon 30-mm double sector cell for sample X6-4-4. The liquid column was adjusted to about 1.5 mm, and the rotor speed was chosen as 8000, 6700, and 4800 rpm for samples X8-3-8, X8-3-5, and X6-4-4, respectively. The buoyancy factor $1-\bar{v}\rho_0$ for Na salt xanthan in 0.1 M aqueous NaCl at constant μ and 25°C was determined to be 0.401. Here, $\bar{v}$ is the partial specific volume of the polymer and ρ_0, the solvent density.

RESULTS AND DISCUSSION

Molecular Weights and Radii of Gyration

Figure 3 illustrates the light scattering envelopes for sample X6-4-4 in 0.1 M aqueous NaCl at 25°C. Panels A and B show, respectively, the concentration dependence of $(Kc/R_\theta)^{1/2}$ for fixed θ and the angular dependence of Kc/R_θ for fixed c. The data points for each θ in panel A can be linearly extrapolated to infinite dilution. The infinite-dilution values of Kc/R_θ so obtained are shown by filled circles in panel B. The filled circles in panel A indicate the zero-angle values of $(Kc/R_\theta)^{1/2}$ obtained by extrapolating the data points in panel B. The light scattering envelopes for sample X7-3b in cadoxen are displayed in Figure 4.

The numerical results obtained for M_w, A_2, and $\langle S^2\rangle^{1/2}$ by light scattering are all summarized in Table I, along with those for M_w, A_2, and M_z/M_w (M_z is the z-average molecular weight) by sedimentation equilibrium. What is most striking is the finding that the values of M_w (in 0.1 M NaCl)/M_w (in cadoxen) given in the ninth column of the table are close to 2. This figure indicates that if Na salt xanthan dissolves in cadoxen as single chains, it should exist as dimers in 0.1 M aqueous NaCl.

The value of M_w for sample X8-3-8 in the water–cadoxen mixture of $w=85\%$, also listed in Table I, is quite close to that for the same sample in pure cadoxen, suggesting that Na salt xanthan in these two solvents exist as essentially identical species.

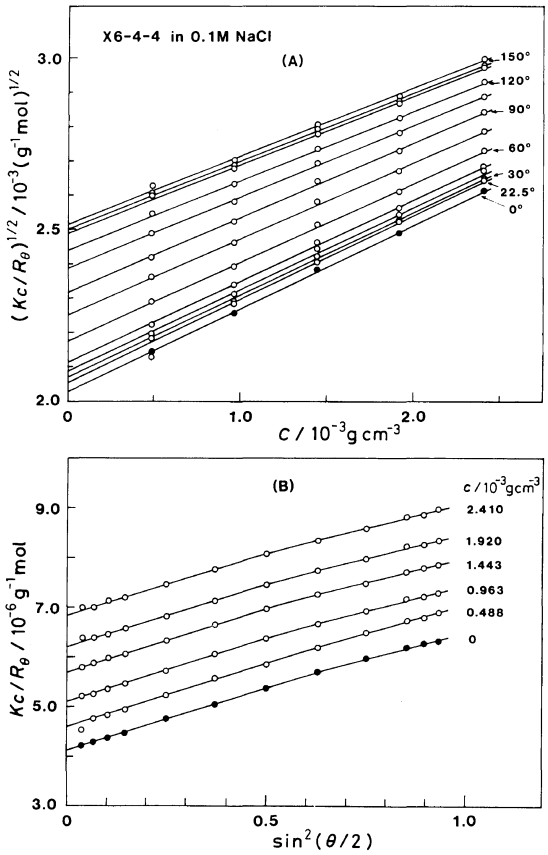

Figure 3. Light scattering envelopes for sample X6-4-4 in 0.1 M aqueous NaCl at 25°C.

Figure 5 shows the molecular weight dependence of $\langle S^2\rangle^{1/2}$ for Na salt xanthan in 0.1 M aqueous NaCl and cadoxen at 25°C. The two curves fitting the data points for these solvents intersect at an M_w of about 2×10^5, indicating that the conformation of the polymer in 0.1 M aqueous NaCl is different from that in cadoxen. The slope of the curve for 0.1 M NaCl is about unity in the region of M_w below 2.5×10^5 and gradually decreases with increasing M_w in the region of higher M_w. This behavior indicates that Na salt xanthan in 0.1 M aqueous NaCl is almost completely rigid and somewhat flexible below and above $M_w\sim2.5\times10^5$, respectively. On the other hand, the cadoxen curve is straight with a slope of 0.55 over the entire range of M_w studied, indicating that xanthan behaves like a flexible coil in cadoxen.

The smaller unfilled circles in Figure 5 represent the $\langle S^2\rangle^{1/2}$ values of Paradossi and Brant.[15] In the region of M_w below 10^6, these circles appear distinctly above our data points. The reason for this discrepancy remains to be seen.

Single Chain in Cadoxen

Our $\langle S^2\rangle^{1/2}$ data for xanthan in cadoxen are compared in Figure 6 with the reported data for similar β-1,4-D-glucans, cellulose[17] and Na salt carboxymethyl cellulose (CMC),[26] in 1 : 1 water–diluted cadoxen. Here, N_w denotes the number of main chain glucose residues; the molar mass per glucose residue of Na salt

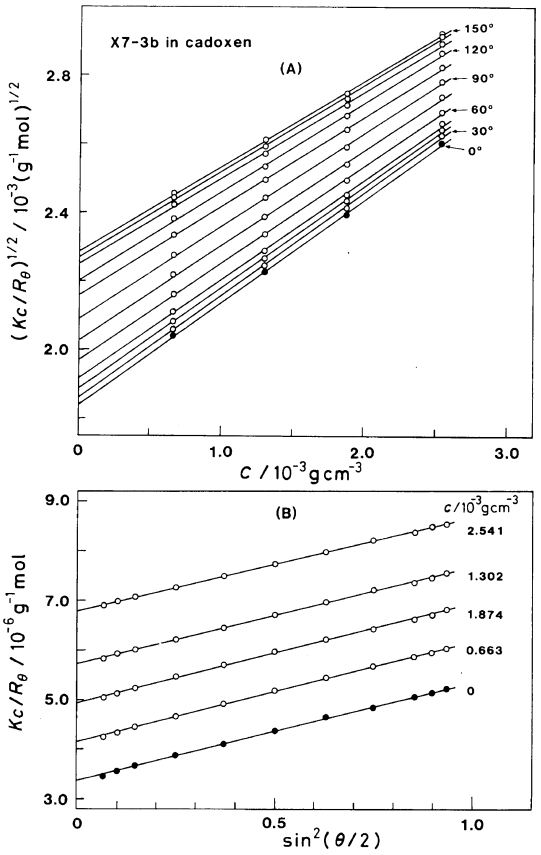

Figure 4. Light scattering envelopes for sample X7-3b in cadoxen at 25°C.

Table I. Results from light scattering and sedimentation equilibrium measurements on Na salt xanthan samples in 0.1 M aqueous NaCl and cadoxen at 25°C

Sample	In 0.1 M NaCl				In cadoxen			
	$M_w \times 10^{-4}$	$A_2 \times 10^4$ / cm^3 mol g^{-2}	$\langle S^2 \rangle^{1/2}$ / nm	M_z / M_w	$M_w \times 10^{-4}$	$A_2 \times 10^4$ / cm^3 mol g^{-2}	$\langle S^2 \rangle^{1/2}$ / nm	M_w (in 0.1 M NaCl) / M_w (in cadoxen)
X4-5	740	3.17	378		356	4.60	144	2.08
X3-5	142	3.99	142		74.3	4.30	60.9	1.91
X7-3b	60.3	4.35	74.8		29.5	5.52	32.7	2.04
X6-4-4	24.0	5.02	36.3		12.2	6.48	21.1	1.97
	23.3[a]	5.21[a]		1.1_5[a]				
X8-3-5	11.2	6.18	16.6		4.53	11.2	12.3	2.47
	11.2[a]	6.65[a]		1.1_4[a]				
X8-3-8	7.40	6.80	$10._8$		3.48	10.1	$10._8$	2.13
	7.28[a]	7.56[a]		1.1_6[a]	3.56[b]	9.68[b]	$11._0$[b]	

[a] From sedimentation equilibrium.
[b] In the water–cadoxen mixture of $w = 85\%$.

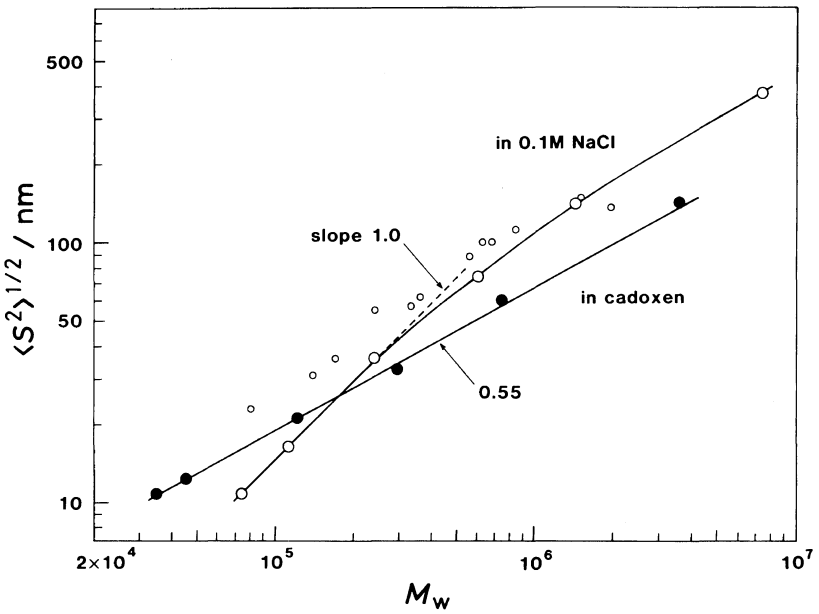

Figure 5. Double-logarithmic plots of $\langle S^2\rangle^{1/2}$ *vs.* M_w for Na salt xanthan in 0.1 M aqueous NaCl and cadoxen at 25°C. Smaller unfilled circles, data of Paradossi and Brant[15] for Na salt xanthan in 0.1 M aqueous NaCl at 27°C.

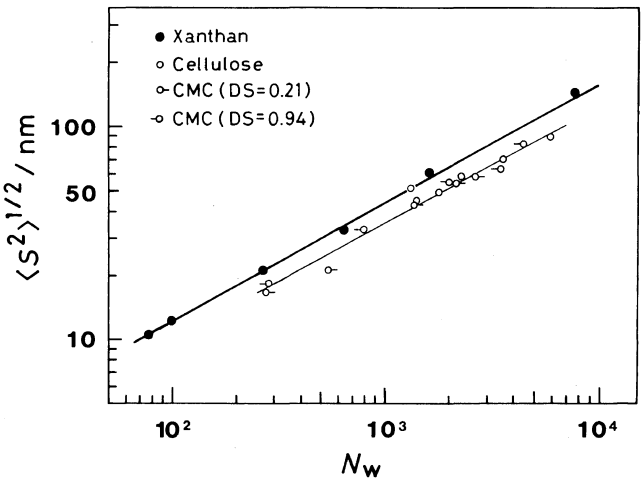

Figure 6. Comparison of $\langle S^2\rangle^{1/2}$ for Na salt xanthan in cadoxen with those for cellulose[17] and Na salt carboxymethyl cellulose[26] with the indicated degrees of substitution (DS) in 1 : 1 water–diluted cadoxen.

xanthan with DS_{pyr} ranging from 0.32 to 0.37 is (460 ± 1) g mol^{-1}. The curve for xanthan is almost parallel and close to that for these other glucans. This finding allows us to conclude that Na salt xanthan dissolves in cadoxen as single chains and has an effective length per main chain residue comparable with those of cellulose and CMC in 1 : 1 water–diluted cadoxen.

Double-Stranded Helix in 0.1 M NaCl

With this evidence of single chain xanthan in cadoxen and the above-mentioned M_w (in 0.1 M NaCl)/M_w (in cadoxen) values of about 2, we arrive at the important finding that the predominant species of Na salt xanthan in 0.1 M aqueous NaCl is a dimer. Furthermore, this dimer should be rodlike for molecular weights lower than about 2.5×10^5, as can be deduced from the behavior of $\langle S^2 \rangle^{1/2}$ shown in Figure 5.

The well-known expression for $\langle S^2 \rangle^{1/2}$ of a rigid rod is

$$\langle S^2 \rangle^{1/2} = \frac{1}{\sqrt{12}} \left(\frac{M}{M_L} \right) \tag{6}$$

where M is the molecular weight of the polymer and M_L, the molar mass per unit contour length of the rod. If our $\langle S^2 \rangle$ data for M_w below 3×10^5 in 0.1 M aqueous NaCl are substituted into this equation, M_L of the xanthan dimer is found to be $1940 \pm 40\,\mathrm{nm}^{-1}$. This value happens to be very close to $2000\,\mathrm{nm}^{-1}$ obtained by Paradossi and Brant,[15] who used the asymptotic particle scattering function for completely rigid, long rods. Our M_L value yields 0.47 ± 0.01 nm for the length h per main chain glucose residue of the xanthan dimer.

Moorhouse *et al.*[27] found from an X-ray diffraction study that the crystalline structure of xanthan is a 5_1 single-stranded helix, but did not rule out the possibility of a double-stranded helix. Later, Okuyama *et al.*,[28] reinvestigating the fiber density and possible packing arrangements in detail, concluded that crystalline xanthan assumes a 5_1 double-stranded helix, in which each chain contains five cellobioses in a pitch of 4.7 nm, *i.e.*, 0.47 nm per glucose residue. Our h of 0.47 ± 0.01 nm estimated above from light scattering data perfectly agrees with this pitch per glucose residue. Thus, our final conclusion is that the paired chains of the xanthan dimer in 0.1 M aqueous NaCl arrange themselves in the 5_1 double-stranded helical structure.

As can be seen from the $\langle S^2 \rangle^{1/2}$ data shown in Figure 5, the xanthan dimers with molecular weights above about 3×10^5 in 0.1 M aqueous NaCl are not completely rigid. Application of Benoit–Doty's theory[29] for wormlike chains to these data gives a persistence length of about 120 nm for the xanthan double helix. The rigidity of this order is intermediate between those of double-stranded DNA (60 nm)[30] and the triple helix of schizophyllan (150—200 nm).[20,31]

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