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MOLECULAR WEIGHT OF XANTHAN POLYSACCHARIDE*

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ABSTRACT

The molecular weight (M_w) and molecular-weight distribution of the extracellular polysaccharide xanthan, synthesized by the bacterium *Xanthomonas campestris*, have been determined from measurements of the sedimentation coefficient, $s_{20,w}$, and the intrinsic viscosity, $[\eta]$, with the aid of the Mandelkern–Flory–Scheraga equation. The sedimentation coefficient of native xanthan was measured by band-sedimentation of polysaccharide molecules that had been tagged with a fluorescent group; the fluorescent label permits the use of very low concentrations of polymer. A typical, native-xanthan sample has $M_w = 15 \times 10^6$; the polydispersity index M_w/M_n is 2.8. Measurement of s and $[\eta]$ for a homologous series of five xanthan samples having M_w ranging from 0.40 to 15×10^6, prepared by sonication of native xanthan, shows that, for low molecular weight, the intrinsic viscosity $[\eta]$ obeys the relation $[\eta] = KM^{1.35}$. The high value of the Staudinger exponent in this relation demonstrates that xanthan is a rod-like molecule having stiffness similar to that of native DNA, which has a Staudinger exponent of 1.32. Moreover, the absolute values of $[\eta]$ suggest that xanthan has a mass per unit length of about 1900 daltons/nm, which is twice the mass per unit length of the single-stranded structure proposed from X-ray work.

INTRODUCTION

Xanthan is an extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. The molecular structure of xanthan is of interest because of the present wide use of the polymer as a viscosity-building agent in foods[1,2], and because there is a strong possibility that large amounts of the polymer will be used in chemically enhanced oil recovery[3,4]. An understanding of xanthan's molecular structure should also help to elucidate its role in the pathogenicity of the bacterium that produces it[5].

The chemical composition and unusual rheological properties of xanthan were first examined by Jeanes, Sloneker, and their co-workers[6,7]. Recently, Lindberg

*Dedicated to Dr. Allene Jeanes on the occasion of her retirement.

et al.[8] showed that the primary structure of xanthan is comblike, with a high-molecular weight backbone of (1→4)-linked β-D-glucopyranose, as in cellulose, but with a three-sugar side-chain attached at C-3 to alternate glucose residues. The repeating unit is therefore a pentasaccharide. Previous studies in solution[6,9–11] show that xanthan possesses an ordered secondary structure. One possible model for this structure is a 5-fold single helix suggested by X-ray scattering studies of xanthan fibers[12]; electron micrographs, by contrast, suggest that the native structure is multi-stranded with either 2 or perhaps 3 strands arranged in a right-handed twist[13].

Insofar as the viscosity of this polysaccharide is the basis for its widespread use, and polymer viscosity is known to depend critically on molecular weight, it is surprising that the molecular weight (M) and molecular-weight distribution of native xanthan are not known with certainty. The only published study on the molecular weight of xanthan is that of Dintzis, Tobin, and Babcock[9], who showed that $M = 2 \times 10^6$ for xanthan solutions heated for 3 h at 90° in 4M urea. Two unheated xanthan culture-broths examined by Dintzis and co-workers by light-scattering showed $M = 13 \times 10^6$ and 50×10^5. Thus the molecular weight of native xanthan is known to lie between 2×10^6 and 50×10^6, but its precise value remained uncertain.

Electron micrographs of native xanthan show the molecule to have a contour length of 2–10 μm and to be double-stranded[13]. The observed range of contour lengths suggests that xanthan has a broad molecular-weight distribution and that native-polymer molecular weights fall between 4 and 20×10^6.

For the present study, a series of five xanthan samples spanning a 30-fold molecular-weight range was prepared by sonication and by culture selection. For each sample, measurements were made of the sedimentation coefficient s and intrinsic viscosity $[\eta]$. The molecular weight of each sample was then evaluated by use of the Mandelkern–Flory–Scheraga (MFS) equation[14,15],

$$M = \left\{ \frac{s[\eta]^{1/3} \eta_0 N_a}{\beta(1 - \bar{v}\rho)} \right\}^{3/2}, \qquad 1$$

where η_0 is the solvent viscosity, $\bar{v}$ is the partial specific-volume of the polymer, ρ is the solvent density, N_A is Avogadro's number, and β has a value near 2.5×10^6 when $[\eta]$ has the units dl/g.

Unfortunately, as Dintzis and co-workers have noted, a meaningful measurement of s cannot be made for native xanthan by standard methods in an analytical centrifuge, because xanthan possesses high viscosity and lacks a chromophore absorbing strongly at 260–280 nm. The procedure adopted here to circumvent these difficulties is to tag the xanthan with a fluorescent label and then to perform band or zonal sedimentation[16–18] of the tagged xanthan through a shallow density-gradient in a preparative ultracentrifuge. Band location after sedimentation is established by measurements of fluorescence intensity. This method allows measurement of s at xanthan concentrations of 0.01–10 μg/ml. These concentrations are so low that each xanthan molecule moves independently of the others.

It is important to note that the samples studied are probably composed of two

or more noncovalently linked strands, as in native xanthan[13]. The transition in optical rotation at elevated temperature, which in native xanthan signals the disassembly of the subunits, also occurs at elevated temperatures for the sonicated samples studied here.

EXPERIMENTAL

Materials. — Polysaccharides were obtained from two sources. Sample E was prepared from commercial "Keltrol" powder produced by the Kelco Co. Sample F was from a culture broth grown in this laboratory by C. J. McCoy. The *X. campestris* strain used by McCoy was kindly supplied by M. C. Cadmus, and the organism was grown according to protocols described by Jeanes *et al.*[19]. All polymer samples were freed of cells, protein, and other debris by centrifugation and two precipitations by ethanol, as described previously[10]. This procedure is essentially that of Jeanes *et al.*[6].

Samples A–D were prepared from purified Keltrol (Sample E) by sonication of 200-ml batches for 60, 12, 8, and 4 min with a 350 watt Heat Systems–Ultrasonics Co. ultrasonic apparatus. Acetone (1%) was added to solutions before sonication, and samples were maintained below 25° during sonication. Sonicated samples were repurified by centrifugation, reprecipitation from sodium chloride-EDTA solutions, and dialysis to remove metal eroded from the sonicator probe.

Fluorescent derivatives of xanthan. — These derivatives were prepared by isocyanide coupling[20,21] of fluoresceinamine to the carboxyl groups of xanthan. 5-Aminofluorescein, acetaldehyde, and cyclohexyl isocyanide (Sigma Chemical Co., MCB Co., and Aldrich Chemical Co., respectively), were used as reagents, as follows: xanthan (5–25 mg) was dissolved in 100 ml of 2mM sodium chloride, pH 7. To this solution was added 100 ml of solution containing 1 part of dimethyl sulfoxide and 2 parts of water. Acetaldehyde (30 μl) and dye (3 mg in 3 ml of dimethyl sulfoxide) were then added and allowed to react for 3 h at room temperature. The labelled polysaccharide was then purified by several cycles of precipitation with ethanol and finally dialyzed against 0.5% sodium chloride to remove the last traces of reactants. The extent of dye labelling, determined by visible absorption spectrophotometry, was found to be 0.1–0.8% by weight.

Sedimentation velocity. — Measurements were made by band sedimentation in 14-ml tubes spun in a Beckman L2-65B preparative ultracentrifuge equipped with an SW-40 rotor. Samples (0.5 ml) contained 1–10 μg/ml of fluorescently labelled polymer, or 50–150 μg/ml of unlabelled xanthan, dissolved in buffer containing 70mM sodium chloride and 4mM sodium phosphate, pH 7. The samples were layered onto shallow, linear density-gradients containing 0.7M sodium chloride, 0.04M phosphate buffer at the top and 1.4M sodium chloride, 0.04M phosphate buffer at the bottom. The resultant densities at the top and bottom of the gradient were 1.03 and 1.05 g/ml. The density gradient functioned to stabilize the liquid against convection. The tubes were spun for 2–13 h, generally at 40,000 r.p.m. (198,000*g* average force), and band location of the fluorescent samples was established with the aid of an ISCO

density-gradient fractionator and a Perkin–Elmer 204 A fluorescence spectrophotometer fitted with a flow cell. Non-fluorescent samples were collected in 1-ml fractions with a fraction collector and then analyzed for carbohydrate.

Capillary viscosity. — Measurments were made with a no. 75 Cannon–Ubbelohde, 4-bulb, shear-dilution viscometer having shear stresses at the wall equal to 8.23, 5.15, 3.10, and 1.79 dyne.cm^{-2} in a bath regulated to $\pm 0.005°$ at 30.6°. Samples were dissolved in 0.75M sodium chloride, 0.04M phosphate, pH 7, and were diluted in the viscometer with the buffer. The buffer concentration approximated the average environment experienced by xanthan molecules in the sedimentation experiment. The measured viscosities were corrected for non-Newtonian character to the wall shear-stress by the method of Rabinowitch[22].

Low-shear-stress viscosity. — A Couette rotating-cylinder viscometer, constructed in this laboratory by using principles described by Gill and Thompson[23] and by Zimm and others[24–26], was used. The inner cylinder (rotor) was suspended in the test solution by a feedback-controlled pressure regulator (Cartesian diver principle). This type of suspension eliminates all mechanical bearings except the solution in question and eliminates the possibility of interference by films at solution–air interfaces. The outer, stationary cylinder is made of precision glass-tubing. The rotor contains a lacquered aluminum ring to which a torque is applied by a rotating magnetic field. The rotating magnetic field is provided by the stator of a commercial electric motor. The driving torque on the aluminum ring in the rotor is varied by adjusting the electrical voltage applied to the motor windings. Shear stresses of 0.0025–0.01 dyne.cm^{-2} were employed; the corresponding shear rates were approximately 0.05–1 sec^{-1}. These shear stresses are so low that dilute xanthan solutions behave as Newtonian fluids. The sample temperature was 20°.

Partial specific volume ($\bar{v}$) *and density* ρ. — These values were evaluated from density measurements made with a Mettler–Parr Model DMA-50 density meter. Solutions containing 1.5 g of xanthan/l were dialyzed against 0.75M sodium chloride, 0.04M phosphate, pH 7. The density ρ and carbohydrate content c_2 of the dialyzed solution, and the solvent density ρ_0, were then measured. The partial specific-volume ($\bar{v}_2$) was determined from the relationship:

$$\bar{v}_2 = [1 - (\rho - \rho_0)/c_2]/\rho_0 ,$$

which is valid at low concentrations (c_2).

Carbohydrate determinations. — These analyses were effected by the phenol–sulfuric acid method of Smith *et al.*[27] with redistilled phenol. Glucose was used routinely to check the procedure; it was established that 64.4 μg of xanthan/ml (sodium salt) produces the same absorbance as 50 μg of glucose/ml in this test.

RESULTS

The movement of xanthan molecules from the top to the bottom of the centrifuge tube is illustrated by the data of Fig. 1, which shows the position of a band

of fluorescently tagged, native xanthan molecules after 0, 2, 4, and 6 h of sedimentation at 38,000 r.p.m. Because of the method used to analyze the band position, it was necessary to conduct four separate experiments to obtain these results. Two points are noteworthy in Fig. 1. First, the band broadens as it proceeds down the tube. This broadening is attributable to heterogeneity of the sedimentation coefficient, that is, to the distribution of molecular weight, rather than to diffusion of molecules from a sharp starting-band. This was proved by resedimenting two fractions, one taken from the fast-moving edge of the band after 4 h of sedimentation, the other taken from the slow-moving edge of the 4-h band. The "fast" fraction remained faster than the "slow" one on resedimentation. Moreover, an unrealistically large diffusion-coefficient, $4\times10^{-5}\ \mathrm{cm^2\ sec^{-1}}$, would be required to generate the observed broadening.

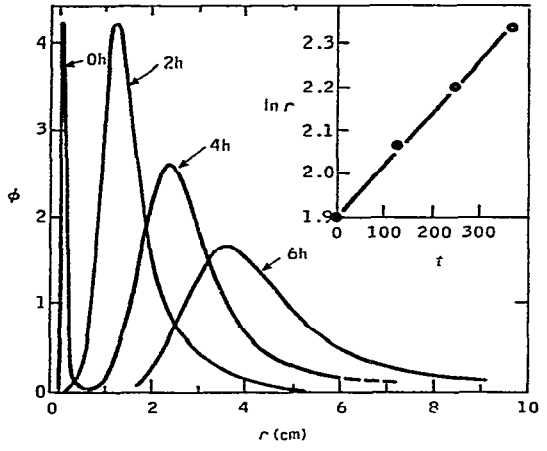

Fig. 1. Position of native xanthan band after 0, 2, 4, and 6 h of sedimentation at 38,000 r.p.m. (approximately 190,000*g*). The ordinate is fluorescence intensity, ϕ; the abscissa is distance r from the meniscus of the tube. The areas of the curves are normalized to the same value, except for the 0-h band, which should be ten times as high as is shown. The inset shows ln r, where r is the position of the band maximum, plotted versus sedimentation time in min, after a small correction for solvent-density variation with r.

The second point to note in Fig. 1 is that the band moves in a controlled manner and broadens as one would expect for an ideal but heterogeneous sample. Specifically, as the particle velocity dr/dt equals $s\omega^2 r$, where r is the distance of the molecule from the rotor center and ω is the rotor speed, it may be expected that

$$\ln r - \ln r_0 = s\omega^2(t-t_0)\,.$$

Thus, in the absence of concentration effects and other complications, a plot of ln r *vs* t should be linear with slope $s\omega^2$. For the band maxima in Fig. 1, this linear relationship is demonstrated in the inset in Fig. 1. In addition, at different points in the band, for example, along the leading and trailing edges, each has well-defined

sedimentation coefficients. This behavior shows that polymer entanglement, gelation, and other non-ideal effects seen at high concentration are absent here.

For the remaining xanthan samples, the sedimentation coefficient was obtained in a similar but abbreviated way, namely, from the distance travelled by the fluorescent band during a single 2–13-h sedimentation at 31,000–40,000 r.p.m. The speed and time were chosen to cause a 2.5–3-cm movement of the band peak. The bandshapes after sedimentation are given in Fig. 2 for the lowest and highest molecular-weight samples and for the unsonicated, commercial sample. In each case, measurements

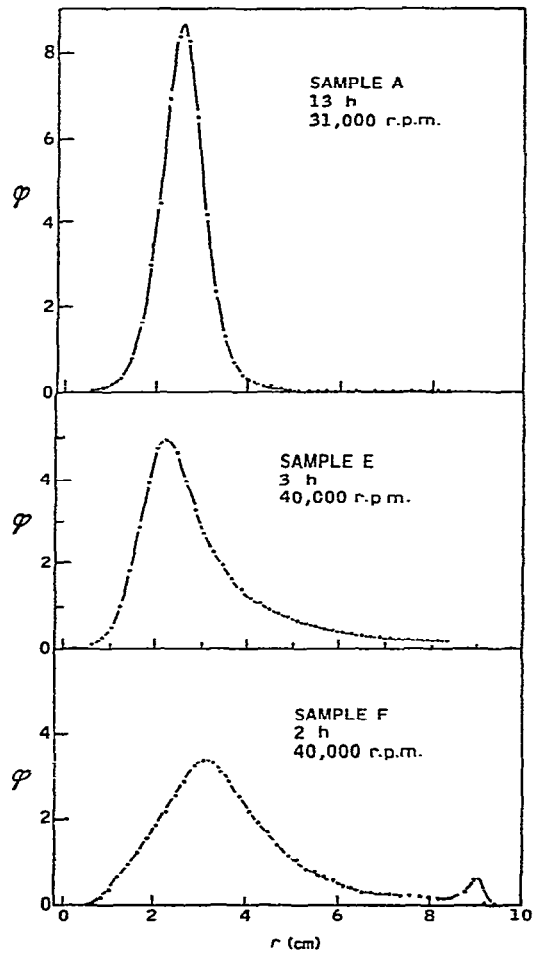

Fig. 2. Band sedimentation of Samples A, E, and F. The ordinate in each instance is fluorescence intensity; the abscissa is distance r from the meniscus. The small peak at 9.2 cm is material collected in the bottom of the tube. Sample A (sonicated 60 min), sedimented for 13.2 h at 31,000 r.p.m. Sample E (native, Kelco), sedimented for 3.0 h at 40,000 r.p.m. Sample F (selected high M culture broth) after 2 h of sedimentation at 40,000 r.p.m. All experiments performed at 20° in 4–8% sodium chloride gradients containing 0.04M sodium phosphate buffer, pH 7.

were made at two concentrations, typically 5 and 10 μg/ml, to allow evaluation of s at zero concentration. The observed variation in s with concentration between 5 and 10 μg/ml was less than 2%, which is comparable to the experimental error.

The evaluation of s_w, the weight-average value of s, was carried out by reading off the fluorescence intensity ϕ_i at 50 points i from the top to the bottom of the tube. For each point, two quantities, Δr_i and $\bar{r}_i$, were evaluated. The quantity $\Delta r_i \equiv r_i - r_0$ is the distance moved by the polymer to reach point r_i; the starting value r_0 is the position of the band maximum at zero time. The quantity $\bar{r}_i \equiv r_0 + (\Delta r_i/2)$ is the average distance from the rotor center of molecules that end up at point r_i. The sedimentation coefficient s_i for material at point r_i is approximated by the relation

$$s_i = \Delta r_i/\omega^2 \bar{r}_i \Delta t, \qquad 2$$

where ω is the rotor speed and Δt is the time at speed plus a small correction for time spent starting and stopping. This expression is exact except for neglect of the slight density-gradient in the tube, a point to be discussed later.

The weight average, s_w, is then given by:

$$s_w = (1/\omega^2 \Delta t)\left[\sum_{i=1}^{50} \Delta r_i \phi_i/\bar{r}_i\right] \Big/ \sum_{i=1}^{50} \phi_i. \qquad 3$$

The value of s_w is then corrected by the usual method[28] to standard conditions (water, 20°) to obtain a weight-average, standardized value $s_{20,w}$.

In order to be certain that the binding of dye to xanthan did not alter its molecular weight significantly, we measured the sedimentation coefficients of unlabelled samples A–D. In this experiment, band location after sedimentation was established by fractionating the 14-ml contents of the centrifuge tube into 1-ml fractions and then analyzing each fraction for its carbohydrate content. This gave 14 data points to define the band position. The starting xanthan concentration was of necessity 50–100 μg/ml, which is 10 times that used with the fluorescent samples. The values of $s_{20,w}$ obtained by this method, and the value of $s_{20,w}$ obtained from the fluorescently labelled derivatives of the same samples, both extrapolated to $c = 0$, are as follows: A, 5.8 and 6.1; B, 7.6 and 7.6; C, 9.4 and 8.9; and D, 10.6 and 11.1 S, respectively. The relatively close agreement in these numbers is evidence that fluorescent labelling had not changed the molecular weights of the polymer significantly.

Measurements of the intrinsic viscosity $[\eta]$ of samples A–E were performed on the same fluorescently tagged samples examined in the sedimentation studies, by using a four-bulb shear-dilution capillary viscometer for samples A–C and the low-shear Couette instrument for samples D and E. The viscosity was determined at several polymer concentrations and shear stresses, so that the data could be extrapolated to zero shear-stress and zero concentration. The determination at zero concentration was carried out by plotting $(1/c) \ln \eta/\eta_0$ *versus* c and extrapolating to $c = 0$; the extrapolated value, called $[\eta]_\tau$, is the limiting viscosity number at a finite shear-stress τ. The values of $[\eta]_\tau$ for several values of τ was then extrapolated to $\tau = 0$.

Extrapolation to $c=0$ may be carried out confidently for all molecular weights. For low molecular-weight (Samples A–C), extrapolation of capillary data to $\tau=0$ is also straightforward, because the non-Newtonian character of these samples is weak. However, xanthan of high molecular-weight (Samples D–F) shows non-linear dependence of $[\eta]_\tau$ on τ for the shear stresses (1.8–8.2 dyne/cm^2) provided by the no. 75 capillary, shear-dilution viscometer used. Accurate extrapolation of capillary data to $\tau=0$ is therefore not possible with these samples; the extrapolation usually gives too low a value of $[\eta]$.

In order to compare capillary viscosity-data obtained at 30.6° to sedimentation data determined at 20°, a correction was applied to the viscosity data, based on measurements of the temperature dependence of viscosity for samples A and E. The values of $[\eta]$ obtained at 30.6° have been multiplied by the factor 1.0, 0.98, and 0.96 for samples A, B, and C, respectively, to obtain $[\eta]$ at 20°.

The intrinsic viscosity of samples D and E was measured at shear stresses of 0.0025–0.01 dyne.cm^{-2} with the low-shear-stress, Couette viscometer. At these shear stresses, xanthan solutions containing 20–100 μg polymer/ml and 0.5M sodium chloride were Newtonian. The intrinsic viscosity of sample E, the unsonicated commercial polymer, was 12,300 ml/g at 20°. The intrinsic viscosity of the very-high-molecular-weight sample F, derived from a selected culture-broth, was not measured because of accidental loss. It will be shown later that the intrinsic viscosity of this sample in 0.5M sodium chloride may be estimated from its sedimentation coefficient to be 30,000–35,000 ml/g.

The determination of M from $s_{20,w}$, $[\eta]$, ρ_0, and v_2 is then straightforward (Eq. *1*). The value of ρ_0 is 1.033 g/ml for 0.75M sodium chloride, 0.04M sodium phosphate buffer, pH 7, the approximate average buffer experienced by the xanthan molecules during sedimentation. The value of $\bar{v}_2$ for xanthan in this buffer was found to be 0.593 ml/g, which agrees well with the value of 0.59 obtained previously by Dintzis for xanthan in 4M urea[9].

It is important to note that the value of M, which we designate $M_{s\eta}$, obtained via the MFS equation from weight-average values of s and $[\eta]$, is not in general equal to M_w, the weight-average molecular weight[29]. However, M_w can be calculated from $M_{s\eta}$ if the molecular-weight distribution is known. This calculation will be performed here; it is found that M_w is 5–21% greater than $M_{s\eta}$ for xanthan samples A–E.

In Table I are listed the values of $[\eta]$, $s_{20,w}$ and $M_{s\eta}$ for the five samples A–E. Native xanthan (E) was found to have $M_{s\eta}=12.2\times10^6$. Extensive sonication (Sample A) decreases $M_{s\eta}$ to 380,000.

It is useful to plot log $s_{20,w}$ and log $[\eta]$ *vs* log $M_{s\eta}$ to obtain a measure of chain stiffness in xanthan. Fig. 3 shows the results. The plot for $s_{20,w}$ has slope 0.22 at low M and 0.34 at high M. Similarly, the plot for $[\eta]$ has slope 1.35 at low M and a lower slope, 0.96 at high M. The fact that the exponent b in the relation $[\eta]=K_\eta M^b$ equals 1.35 for low M demonstrates that xanthan occurs as a very stiff chain. For comparison, according to Eigner and Doty[30], the value of b for native, double-stranded DNA of low molecular weight is 1.32.

TABLE I

SEDIMENTATION COEFFICIENT, INTRINSIC VISCOSITY, AND MOLECULAR WEIGHT OF XANTHAN SAMPLES

Sample	*Description*	$S_{20,w} \times 10^{13}$, *sec*	$[\eta]$ *ml/g*	$M_{s\eta} \times 10^{-6}$	$M_n \times 10^{-6}$	$M_w \times 10^{-6}$	M_w/M_n
A	Sonicated for 60 min	6.1	270	.38	.18	.40	2.3
B	Sonicated for 12 min	7.6	1100	1.08	.58	1.1	1.9
C	Sonicated for 8 min	8.9	1900	1.83	1.1	2.0	1.8
D	Sonicated for 4 min	11.1	3900	3.67	2.0	4.2	2.1
E	Native, commercial	16.9	12,300	12.2	5.3	14.8	2.8
F	Native, culture broth[a]	28.2	(35,000)	(52)	(25)	(62)	(2.4)

[a]Values in parentheses for Sample F are based upon an estimated value of $M_{s\eta}$ deduced from an extrapolation of the S–M curve of Fig. 3.

For sample F, which had an average $s_{20,w}$ value of 28.2 *S*, low-shear-stress viscosity data were unfortunately not obtained. The molecular weight of this sample cannot therefore be obtained from the MFS equation. However, the curve of log *s* versus log *M* (Fig. 3) provides an estimate of *M* if the measured curve in Fig. 3 is extrapolated to larger values of *s*. The value of 28.2 *S* corresponds to $M_{s\eta} = 52 \times 10^6$ and $[\eta] = 30{,}000$–$35{,}000$ ml/g.

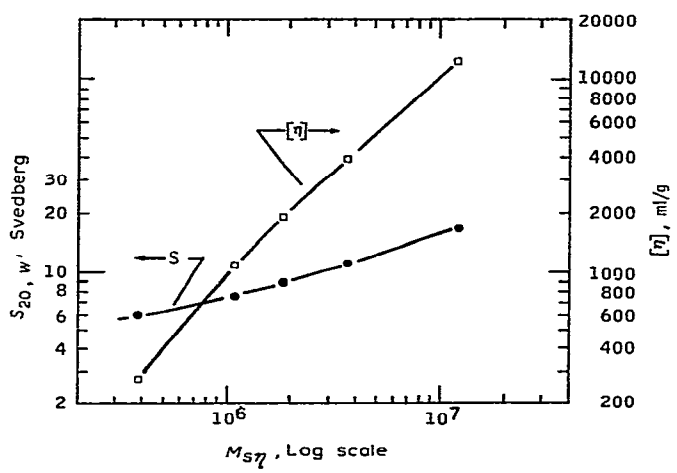

Fig. 3. Sedimentation coefficient $s_{20,w}$ (●) and intrinsic viscosity $[\eta]$ (□) for xanthan samples of different molecular weight, $M_{s\eta}$.

The data in Fig. 3, giving the dependence of $s_{20,w}$ on $M_{s\eta}$ over a broad molecular-weight range, allow ready conversion of the observed shape of the fluorescent band after sedimentation (Fig. 2) into a molecular-weight distribution, for that sample, as follows. First to be evaluated is the parameter *a* in the relation $s = K_s M^a$ appropriate to that sample from the slope of the curve for log *s* *vs* log $M_{s\eta}$

(Fig. 3); as already noted, the value of a ranges from 0.22 to 0.34 for Samples A–E. Once a is known, K_s is evaluated, so that $s_{20,w} = K_s M_{s\eta}^a$. The value of $M_{s\eta}$ used to obtain K_s is that given by Gibbons[29]:

$$M_{s\eta} = \{\sum_i M_i c_i / \sum_i c_i\}^{3/2} \{\sum_i M_i^{(2-3a)} c_i / \sum_i c_i\}^{1/2}. \qquad 4$$

This relation is based on the MFS equation and assigns to $[\eta]$ a dependence on M^{2-3a}. This is preferable to using $M_{s\eta}$ from Eq. *1* if the values of M_n, M_w, and $M_{s\eta}$ are to be compared for each sample. The value of M_i at each point i on the band is found from the value of s_i at that point:

$$M_i = (s_i/K_s)^{1/a}. \qquad 5$$

The value of s_i is given by Eq. *2*. As the amount of polymer having molecular weight M_i is known from the fluorescence intensity, ϕ_i, the molecular weight distribution is then known.

The molecular-weight distributions obtained in this way from the distributions in s are shown in Fig. 4 for samples A, B, D, and E. The distribution function for sample C is deleted from the figure for simplicity. Inspection of Fig. 4 shows that, for highly sonicated Sample A, more than half of the polymer (by weight) falls between 1.9×10^5 and 4.6×10^5 daltons. For unsonicated, commercial xanthan (Sample E), a relatively broad molecular-weight distribution is observed, with 50% of the polymer showing $3.6 \times 10^6 < M < 12 \times 10^6$. About 1% of this sample and about 10% of sample F possess $M > 10^8$.

Once the molecular-weight distribution for each sample is in hand, the number and weight-average molecular weights, M_n and M_w, and the ratio M_w/M_n, are readily calculated. These are given in Table I for the six samples. The ratio of M_w/M_n varies

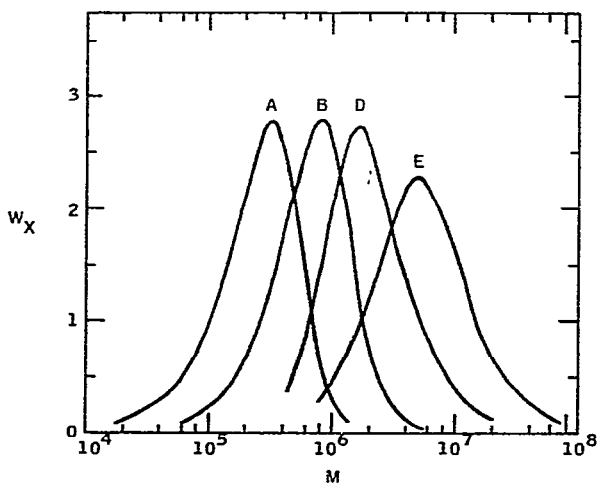

Fig. 4. Molecular-weight distribution of Samples A, B, E, and F. The ordinate W_x is the relative weight-fraction. The curves are separately normalized to have the same area.

from a high of 2.8 for Sample E to a low of 1.8 for Sample C. This ratio is rather sensitive to the low-molecular-weight cutoff used in the calculations.

DISCUSSION

The use of the Mandelkern–Flory–Scheraga equation to obtain M for rod-like molecules has occasionally been questioned. In particular, it has been argued on theoretical grounds[15,31] that the value of β in Eq. *1* should be $3.0–3.5 \times 10^6$ for rod-like molecules, rather than 2.5×10^6. However, the thorough experimental study of DNA viscosity–molecular-weight relations carried out by Eigner and Doty[30] strongly supports $\beta = (2.5 \pm 0.3) \times 10^6$ over the molecular-weight range 2×10^5 to 2×10^8, which spans the xanthan range examined here.

The only previous measurements of s for xanthan were reported by Dintzis *et al.*[9], who worked with xanthan that had been heated in 4M urea for 3 h at 90°. Such samples exhibited $s_{25} = 5 \times 10^{-13}$ sec, $\bar{M}_w = 1.8–3.6 \times 10^6$. This value of s is smaller than that of highly sonicated Sample A, for which $M_w = 0.36 \times 10^6$. It is possible that Dintzis' samples exhibited a smaller value of s for $M = 1.8 \times 10^6$ because of a larger friction factor arising from polyelectrolyte expansion at the low ionic strengths used.

The measurement of s in swinging-bucket rotors is not as precise as measurements in an analytical rotor, because the sample cannot be examined during the run. In addition, the shape of the preparative tube does not have the preferred sector-shape, which prevents sedimenting molecules from striking the container walls. Band sedimentation engenders further complications, inasmuch as the sample moves in a density gradient. In the present case, the densities at the top and bottom of the tube were 1.03 and 1.05 g/ml. As a consequence, $(1 - \bar{v}_2 \rho)$ ranges between 0.389 and 0.377. This gradient, as well as the complications introduced by the solvent in the 0.5-ml sample (which has density 1.00) were neglected. Instead, a density of 1.033 g/ml was used in computing s. This corresponds approximately to the average density experienced by molecules under the experimental conditions, in which the band maximum moved only 2.5–3 cm down from the top of the tube during the experiment.

The intrinsic viscosity of native xanthan, which is 12,300 ml/g for Sample E, has been measured previously by several groups. Dintzis and his co-workers[9], using an ultra-low-shear, Couette viscometer and extrapolating to $c = 0$, observed $[\eta] = 1–9 \times 10^3$ ml/g, according to the method of preparation and the solvent (0.01M ammonium acetate or 4M urea). The higher values agree reasonably well with the results for sample E. They also observed slow changes in viscosity over periods of several weeks. Intrinsic viscosities of 5000–7000 ml/g were measured by Holzwarth[10] in 0.27M sodium chloride–0.014M calcium chloride, pH 7, using a capillary viscometer and a moderately low-shear Couette instrument; the data were extrapolated to zero concentration and zero shear-stress, but it is now apparent that the extrapolation to zero shear-stress was faulty. From a study of the rheology of xanthan over a broad range of shear stresses[32,33], Whitcomb and Macosko estimated that $[\eta] = 22{,}000–25{,}000$ ml/g at zero shear-stress in distilled water.

Examination of the plot of log $[\eta]$ *vs* log $M_{s\eta}$ (Fig. 3) shows a slope of 1.35 at low M and a slope of 0.96 at high M. This behavior is entirely consistent with a locally stiff polymer chain. As already noted, the stiffness is quite comparable to that of DNA.

The molecular weights for native xanthan in this paper may be compared with estimates determined from contour-length measurements in electron micrographs[13]. The samples used in obtaining the micrographs were similar to E. The micrographs gave a length of 2–10 μm, which gives $M = 4\text{–}20 \times 10^6$ for a double-stranded molecule. Sample E agrees well with these values. The weight-average molecular weights of the unsonicated samples E and F, 14.8 and 62×10^6, agree well with molecular weights 13 and 50×10^6 obtained by light-scattering studies for two untreated culture broths by Dintzis and co-workers[9]. Dintzis and co-workers considered these samples to be suspensions. The values are well outside the range measured by Dintzis for solutions of samples heated for 3 h at 90° in 4M area. The present study suggests that the high molecular-weights noted by Dintzis for unheated samples were correct solution-values; samples having these molecular weights may be sedimented like true solutions if sufficiently dilute.

It might be supposed that M and its distribution for such commercial samples as E are affected by the rough handling in the commercial process. However, similar sedimentation behavior is observed for many xanthan samples gently isolated from broths grown in laboratory shake-flasks under standard conditions. Under other conditions, much larger molecular-weights are obtained, as in Sample F. This indicates that the molecular weights of native xanthan depends strongly on fermentation conditions. These differences are obscured in viscosity measurements made at high shear-stresses and at concentrations of 0.1 mg/ml or greater.

It was noted earlier that xanthan and DNA are equally stiff chains, that is, the Staudinger exponent b in the relation $[\eta] = K_\eta M^b$ is almost identical for these two molecules. The similarity extends also to the coefficient K_η or to $[\eta]$ itself. Table II compares the intrinsic viscosity of xanthan samples A, B, and C with the intrinsic viscosity of DNA of molecular weight identical to that of the xanthan samples. The intrinsic viscosities agree to within 15%. As the molecules are similarly rodlike, their

TABLE II

COMPARISON OF INTRINSIC VISCOSITY OF XANTHAN WITH THAT OF DNA OF THE SAME MOLECULAR WEIGHT

Sample	M	$[\eta]$, *ml/g*	
		Xanthan	*DNA*[a]
A	0.40×10^6	270	261
B	1.1×10^6	1100	990
C	2.0×10^6	1900	2180

[a] Using the relation $[\eta] = 1.05 \times 10^{-5} M^{1.32}$ given by Eigner and Doty[30] for DNA having $M < 3 \times 10^6$.

mass per unit length must be similar. The mass per unit length of the single-stranded model of xanthan (sodium salt) that is favored by X-ray work[12] is 996 daltons/nm. The mass per unit length of B-DNA (sodium salt), 1891 daltons/nm, is almost exactly twice this value. This suggests that native xanthan in solution may be made up of two of the single-stranded 5-fold helices proposed by Moorhouse *et al.*[12] to exist in xanthan fibers. This observation provides completely independent support for EM evidence[13] that native xanthan is made up of two or perhaps three intertwined strands.

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REFERENCES

1 W. H. McNeely and K. S. Kang, in R. L. Whistler Ed., *Industrial Gums*, Academic Press, New York, 1973 pp. 476–497.
2 T. R. Andrew, in P. A. Sandford and A. I. Laskin Eds., *Extracellular Microbial Polysaccharides, ACS Symp. Ser.*, 45 (1977) 231–241.
3 G. J. Hirasaki and G. A. Pope, *Soc. Petr. Eng. J.* 14 (1974) 337–346, and references therein.
4 E. I. Sandvik and J. Maerker, in ref. 2, pp. 242–264.
5 J. C. Sutton and P. H. Williams, *Can. J. Bot.*, 48 (1970) 645–651.
6 A. Jeanes, J. E. Pittsley, and F. R. Senti, *J. Appl. Polym. Sci.*, 5 (1961) 519–526.
7 J. H. Sloneker and A. Jeanes, *Can. J. Chem.*, 40 (1962) 2066–2071.
8 P. E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.
9 F. R. Dintzis, G. E. Babcock, and R. Tobin, *Carbohydr. Res.*, 13 (1970) 257–267.
10 G. Holzwarth, *Biochemistry*, 15 (1976) 4333–4339.
11 E. R. Morris, D. A. Rees, G. Young, M. D. Walkinshaw, and A. Drake, *J. Mol. Biol.*, 110 (1977) 1–16.
12 R. Moorhouse, M. D. Walkinshaw, and S. Arnott, in ref. 2, pp. 90–102.
13 G. Holzwarth and F. G. Prestridge, *Science*, 197 (1977) 757–759.
14 L. Mandelkern and P. J. Flory, *J. Chem. Phys.*, 20 (1952) 212–215.
15 H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, 75 (1953) 179–184.
16 M. K. Brakke, *J. Am. Chem. Soc.*, 73 (1951) 1847–1848.
17 D. Freifelder, *Methods Enzymol.*, 27D (1973) 140–150.
18 R. Hinton and M. Dobrota, *Density Gradient Centrifugation*, Elsevier–North Holland, Amsterdam, 1976, 97–113.
19 A. Jeanes, P. Rogovin, M. C. Cadmus, R. W. Silman, and C. A. Knutsen, *U.S. Dep. Agric. Agric. Res. Serv. Rep.*, ARS-NC-51 (1976).
20 P. Vretblad and R. Axen, *Acta Chem. Scand.*, 27 (1973) 2769–2780.
21 A. N. de Belder and K. O. Wik, *Carbohydr. Res.*, 44 (1975) 251–257.
22 J. R. Van Wazer, J. W. Lyons, K. Y. Kim, and R. E. Colwell, *Viscosity and Flow Measurement*, Wiley, New York, 1963.
23 S. J. Gill and D. S. Thompson, *Proc. Natl. Acad. Sci. USA*, 57 (1967) 562–566.
24 L. C. Klotz and B. H. Zimm, *Macromolecules*, 5 (1972) 471–481.

25 J. MELSHEIMER, *Kolloid Z. Z. Polym.*, 250 (1972) 97–101.
26 E. L. UHLENHOPP AND B. H. ZIMM, *Methods Enzymol.*, 27D (1973) 483–491.
27 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH *Anal. Chem.*, 28 (1956) 350–356.
28 C. TANFORD, *Physical Chemistry of Macromolecules*, Wiley, New York. 1961.
29 R. A. GIBBONS, *Biopolymers*, 10 (1971) 411–416.
30 J. EIGNER AND P. DOTY, *J. Mol. Biol.*, 12 (1965) 549.
31 J. E. HEARST, E. BEALS, AND R. A. HARRIS, *J. Chem. Phys.*, 48 (1968) 5371–5377.
32 P. J. WHITCOMB, B. J. EK, AND C. W. MACOSKO, in ref. 2, pp. 160–173.
33 P. J. WHITCOMB AND C. W. MACOSKO, personal communication.