

## STUDIES ON DILUTE SOLUTIONS AND DISPERSIONS OF THE POLYSACCHARIDE FROM *Xanthomonas campestris* NRRL B-1459 \*

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### ABSTRACT

Some properties of the extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* NRRL B-1459 were measured in partially purified culture fluids and in liquid systems prepared from thoroughly purified and lyophilized material. High intrinsic viscosities resulted from dispersions and solutions of polysaccharide B-1459 in 0.01M ammonium acetate and 4M urea. Heating a dispersion of B-1459 in 4M urea yielded a true macromolecular solution in which the polysaccharide had an apparent molecular weight of approximately  $2 \times 10^6$ . Light-scattering measurements on culture fluids of B-1459 made 4M in urea gave molecular weights of  $13 \times 10^6$  and  $50 \times 10^6$ . Various solution-properties and factors that affect the viscosity of dispersions prepared from lyophilized polysaccharide are presented.

### INTRODUCTION

The extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* NRRL B-1459 has gained widespread use industrially<sup>1</sup>. A useful characteristic of polysaccharide B-1459 is its ability to produce highly viscous aqueous dispersions and solutions that maintain their viscosity in the presence of many salts. Some characteristics of its dispersions and behavior under conditions of varying temperature, pH, and salt addition have been reported<sup>2</sup>. B-1459, isolated as the potassium salt, is composed<sup>3</sup> of D-glucose, D-mannose, D-glucuronic acid, acetic acid (as O-acetyl), and pyruvic acid (as an acetal substituent) in the molar proportions: 2.8:3.0:2.0:1.7:0.51–0.63. The repeating unit, composed of 16 hexose residues and their substituents<sup>4</sup>, has  $\beta$ -D-(1→2) and  $\beta$ -D-(1→4)-linkages<sup>4,5</sup>. The macromolecular properties of B-1459 were investigated in an aqueous environment because the main industrial uses of this material involve fluid systems containing water. Two aspects of some fluid systems containing B-1459 are reported here: (i) solution parameters in 4M urea, and (ii) dilute dispersion viscosities in 0.01M ammonium acetate ( $\text{NH}_4\text{OAc}$ ) and in 4M urea.

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## EXPERIMENTAL

*A. Materials.* — A lot of B-1459, designated MP-84, came from a batch-type fermentation process<sup>6</sup>. Recovery of polysaccharide involved clarification by repeated passages through a Sharples\* centrifuge, three successive precipitations from solution by ethanol in the presence of potassium chloride to remove protein, dialysis against deionized water, and adjustment of retained solution to pH 6.0–6.5 with dilute potassium hydroxide<sup>2</sup>. The resulting solution was filtered through sintered glass to remove a small amount of extraneous matter, concentrated to about 0.5% solids on a rotary evaporator under diminished pressure, and lyophilized. After the lyophilized polymer had been equilibrated in air at 20° and 50% relative humidity as previously described<sup>2</sup>, it had a moisture content of 15.6%.

A second batch of polymer, designated X-FD-1, was obtained as a culture broth sample from a continuous fermentation run in a pilot plant. This liquid was diluted with eight volumes of distilled water and then passed twice through a Sharples centrifuge to remove cells and particulate matter. The diluted and clarified culture fluid was then dialyzed against 0.01M  $\text{NH}_4\text{OAc}$  for 3 days at room temperature. The retained solution was adjusted to pH 7.3 with  $\text{NH}_4\text{OH}$  upon completion of the dialysis and was refrigerated until used. Sufficient mercuric iodide ( $\text{HgI}_2$ ) to leave a visible precipitate was added to all B-1459 systems containing 0.01M  $\text{NH}_4\text{OAc}$  to discourage microbial growth.

A portion of this  $\text{NH}_4\text{OAc}$ -dialyzed culture fluid then was dialyzed against distilled water for several days with no adjustment of pH. A fibrous mass of polysaccharide resulted by lyophilizing this portion of culture fluid. Both the culture liquid and lyophilized material retained a brownish pigment. Polysaccharide concentrations in dialyzed solutions and in culture fluid were determined by the phenol-sulfuric acid colorimetric procedure<sup>7</sup>.

Another portion, 900 ml, of X-FD-1 culture fluid was taken from the refrigerated stock bottle after 7 months' storage. (Microorganism growth was inhibited, but not completely stopped during storage.) This portion was dialyzed overnight at 5° against distilled water and then centrifuged at  $40 \times 10^3 \times g$  for 1 h. The resulting, clarified liquid (light yellow) was divided into two equal volumes. One volume was lyophilized directly; the other was made 1% in potassium chloride, an equal volume of ethanol was added, and the system was stirred for 1 h. The gel of B-1459 that formed upon addition of alcohol was gathered by decanting the alcohol-water solution. The gel was then redispersed into distilled water to its original concentration of 0.195%. After dialysis against distilled water for 60 h at 5°, the alcohol-potassium chloride-treated B-1459 system was lyophilized.

A third batch of polymer came from two frozen samples of culture fluid taken from batch fermentation runs. The samples<sup>8</sup>, designated X-C-9(68) Fertrol No. 2

\*Mention of firm names or trade products does not imply an endorsement or recommendation by the Department of Agriculture over other firms or similar products not mentioned.

and X-L-10(68) Fertrol No. 1, were each thawed, then combined, and diluted with distilled water to a concentration of 0.4 to 0.5%. The diluted culture fluid was centrifuged at  $40 \times 10^3 \times g$  for 1.5 h to remove particulate matter and some polymer, which appeared as a gel on the pad of debris in the centrifuge tube. The light yellow, clarified liquid was dialyzed in a refrigerator for 1 week against 0.01M  $\text{NH}_4\text{OAc}$  and then part of it was lyophilized. Another portion of this culture fluid was then made 4M in urea and dialyzed against 4M urea at 5° before use.

Dispersions were prepared by weighing lyophilized samples of B-1459 into 25-ml volumetric flasks. About 10 to 15 ml of the desired solvent was added, and the flasks were shaken overnight. More solvent was added the next day, and shaking was continued for a few h. The proper amount of solvent was then added to bring the liquid to volume. If the polymer was not dispersed well, a stirring bar was placed in the flask and the contents were stirred, with occasional shaking, to ensure proper mixing. Sometimes, it was sufficient to shake the stoppered sample vigorously to obtain an even dispersion. Concentrations up to about 0.25% were dispersed evenly by these methods. The 25 ml of dispersed material was used as stock from which dilutions were made. Dispersion in 4M urea sometimes required 2 to 7 days' storage at room temperature before an apparently stable viscosity resulted.

Many samples of B-1459 were vacuum-dried before being dispersed. One technique (No. 1) used was to place the lyophilized samples in a vacuum oven at room temperature, turn on both vacuum and heat, and allow the samples dry overnight (ca. 17 h). The vacuum oven reached the desired drying temperature of 70° to 75° in about 1 h. Another method (No. 2) was to vacuum-dry the samples overnight at room temperature (ca. 23°) and then heat under vacuum the next day for 5 to 6 h. Dispersion viscosity depended upon which of these two methods was used to dry the lyophilized sample from which the dispersion was prepared.

Solutions in 4M urea were prepared from batch MP-84. A lightly stoppered, 25-ml volumetric flask containing lyophilized sample and about 15 ml of 4M urea was placed into a water bath for 3 h at 90°. The flask was shaken occasionally to dissolve the polysaccharide during heating. After the flask had cooled, solvent was added to the volumetric mark and the flask was shaken vigorously to ensure even mixing of contents.

**B. Methods.** — A model A Zimm viscometer<sup>9</sup> was used to measure viscosities at shear rates that varied from about 0.5 to 2.0  $\text{sec}^{-1}$ . Sedimentation velocity and synthetic-boundary measurements were made in a Spinco ultracentrifuge in double sector, 30-mm cells. From the sedimentation velocity measurements, a sedimentation coefficient (*s*) was calculated by the standard formula<sup>10</sup>. Partial specific volumes of B-1459 in solution were calculated from density measurements made at  $25.00 \pm 0.05^\circ$  in 35-ml double-column pycnometers. Diffusion was measured at 0.9° in a Tiselius cell in a Spinco Model H electrophoresis instrument. Density, diffusion, molecular weight, and sedimentation measurements were made on solutions prepared from batch MP-84. Estimated errors are indicated in values presented in Tables I and II.

Molecular weight determined by light scattering,  $\bar{M}_{\text{LS}}$ , was measured in a

modified Brice Phoenix instrument, which was calibrated on the basis of a value of  $16.3 \times 10^{-6}$  for the Rayleigh ratio of benzene at 546 nm at 25.0°. Measurements were made at 546 nm and 436 nm. Samples used for light-scattering measurements were dialyzed for at least 2 weeks before measurements were made. These samples were centrifuged in a Spinco preparative centrifuge as follows:

| <i>Sample</i>                               | <i>Preparative centrifugation treatment</i> |
|---------------------------------------------|---------------------------------------------|
| B-1459 solution in 4M urea                  | 1 h at $105 \times 10^3 \times g$           |
| X-FD-1 culture fluid                        | 3 h at $78 \times 10^3 \times g$            |
| XC-XL culture fluid                         | 1.5 h at $78 \times 10^3 \times g$          |
| XC-XL culture fluid heated for 3.5 h at 85° | 20 min at $35 \times 10^3 \times g$         |

Two problems encountered were those of achieving a true macromolecular solution and deciding when the polymer was in a true state of solution. A solution of B-1459 in 4M urea could be produced by heating the system for 1 to 4 h at 90°. Major proportions of gel component in unheated or incompletely solubilized B-1459-4M urea systems were detected by measurements in the ultracentrifuge. Sometimes, gel or high-molecular-weight aggregates were detected by the rapid appearance of material at the bottom of a cell during a sedimentation run. Measurement of area in a synthetic-boundary pattern served as the criterion of concentration in solution when the presence was suspected of gel that would not be exhibited clearly in a sedimentation run. The high concentration-dependence shown by B-1459 required use of low concentrations to observe solution properties best.

## RESULTS

*Molecular weight studies.* — The molecular weight of B-1459 was studied in 4M urea because initially it was considered to be a better solvent than 0.01M  $\text{NH}_4\text{OAc}$ . Solutions of B-1459 prepared from lyophilized samples of batch MP-84 were used in determining molecular weights,  $\bar{M}_{\text{SD}}$ , from sedimentation and diffusion measurements. A molecular weight was calculated according to the Svedberg equation:

$$\bar{M}_{\text{SD}} = \frac{RTs^\circ}{D^\circ(1 - \bar{v}\rho)} \quad (1)$$

where  $R$  is the gas constant,  $T$  is temperature ( $^\circ\text{K}$ ),  $\bar{v}$  is the partial specific volume,  $\rho$  is the density of the solvent, and  $s^\circ$  and  $D^\circ$  are the sedimentation and diffusion constants extrapolated to zero concentration. Fig. 1 displays the sedimentation behavior of B-1459 in 4M urea and in 0.01M  $\text{NH}_4\text{OAc}$ . There is a distinct difference in concentration dependence between the week-old and 2-month-old samples in 4M urea as indicated by the different slopes of lines A and B. A portion of the sample in 4M urea was dialyzed to 0.01M  $\text{NH}_4\text{OAc}$ . Some  $1/s$  values of this sample are indicated in Fig. 1.

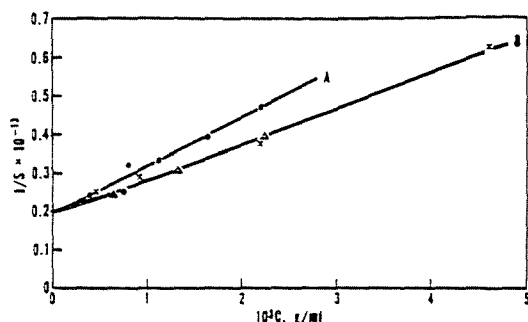


Fig. 1. Sedimentation behavior of polysaccharide B-1459 from *Xanthomonas campestris* (MP-84). ● = Sample in 4M urea for 1 week, line A. × = Sample in 4M urea for 2 months, line B. △ = Sample in 0.01M NH<sub>4</sub>OAc for 1 month, line B. All samples stored at 5°. All  $1/s$  values measured at or corrected to 25°. Values of  $1/s$  for 0.01M NH<sub>4</sub>OAc solutions corrected to 4M urea solution. All concentrations corrected for radial dilution.

Results of diffusion measurements are shown in Fig. 2. Solutions were stored at 5°, and it was necessary to dialyze them for 2 to 3 weeks to obtain meaningful

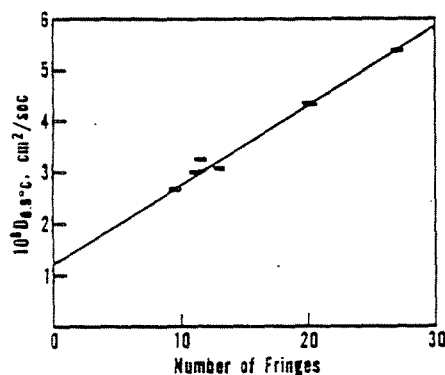


Fig. 2. Diffusion behavior of B-1459 (MP-84) in 4M urea. Concentration expressed in number of Rayleigh fringes (27 fringes = 0.21%).

diffusion patterns. Diffusion coefficients were determined by the height-area method using the equation:

$$A/\lambda_{\max} = (4\pi Dt)^{1/2} \quad (2)$$

where  $A$  is the area under and  $\lambda_{\max}$  is the maximum height of the refractive index gradient curve, and  $t$  is the time from the start of the diffusion process. It was extremely difficult to obtain sharp, zero-time boundaries in the diffusion cell with B-1459. Zero-time corrections of the order of 20 to 40 h were encountered. A photograph of a typical asymmetric schlieren pattern of diffusing B-1459 polymer is shown in Fig. 3. The diffusion constant measured at 0.9° was corrected to 25° by the usual formula<sup>11</sup> to yield a value of  $D_{25} = (2.36 \pm 0.40) \times 10^{-8}$  cm<sup>2</sup>/sec. Uncertainties encountered in extrapolation of diffusion and sedimentation constants to zero concentration led to an estimated possible error of about 22% in  $\bar{M}_{SD}$ .



Fig. 3. Diffusion pattern of B-1459 (MP-84) in 4M urea.  $c = 0.1\%$ ,  $T = 0.9$ ; bar angle =  $35^\circ$ . Time from formation of boundary = 290 h. Solvent region is on the left, solution on the right.

Light-scattering measurements were made in 4M urea on a solution prepared from batch MP-84 and on culture fluids from X-FD-1 and the combined XC-XL samples. Preparative centrifugation, as previously described, was used in an attempt to rid these systems of a possible "micro-gel" component. Systems from MP-84 and X-FD-1 were first centrifuged until their original concentration was reduced by a factor of at least one-third. The XC-XL culture fluid sample was of low concentration,  $c \sim 0.060\%$ , and it lost a negligible amount of polymer during preparative centrifugation. The Zimm plot of the MP-84 solution (Fig. 4) showed curvature in

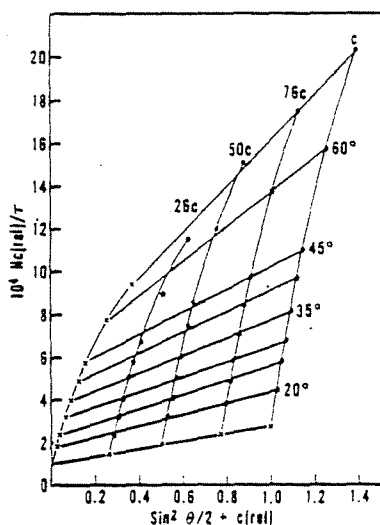


Fig. 4. Zimm plot of B-1459 (MP-84) solution in 4M urea prepared by heating a lyophilized sample for 3 h at  $90^\circ$ . Initial concentration,  $c = 3.4 \times 10^{-3}$  g/ml. Angles of  $20^\circ$  to  $45^\circ$  from the incident beam are indicated in  $5^\circ$  increments. The two highest angles are  $60^\circ$  and  $75^\circ$  from the incident beam.  $\bullet$  = Measured values.  $\times$  = Extrapolated values at zero angle and zero concentration.

the lines of constant concentration, as did the Zimm plot (not shown) of culture fluid X-FD-1. Molecular-weight ranges in MP-84 solution were estimated by extrapolating to zero angle those measurements that were  $45^\circ$  and less from the incident beam. Values of molecular weight determined by light scattering,  $\bar{M}_{LS}$ , of  $13 \times 10^6$

and  $50 \times 10^6$  were obtained from the low angle extrapolations of Zimm plots from culture fluid batches X-FD-1 and XC-XL, respectively. (Since batches X-FD-1 and XC-XL were not identically grown or treated, one should not expect identical  $\bar{M}_{LS}$  values.) The solution prepared from batch MP-84 had a range of  $\bar{M}_{SD}$  that was lower than its range of  $\bar{M}_{LS}$ , which was lower than values of  $\bar{M}_{LS}$  determined on culture fluid samples from other batches. Some parameters of B-1459 solution in 4M urea are shown in Table I.

TABLE I

B-1459 (MP-84) IN 4M UREA

|                 |                                                                  |
|-----------------|------------------------------------------------------------------|
| $(dn/dc)_{546}$ | 0.135 ml/g                                                       |
| $[\eta]_G = 0$  | $29 \times 10^2$ ml/g                                            |
| $\bar{V}_{25}$  | 0.59 ml/g                                                        |
| $D_{0.09}^0$    | $(1.2 \pm 0.2) \times 10^{-8}$ cm <sup>2</sup> sec <sup>-1</sup> |
| $s_{0.25}^0$    | $(5.0 \pm 0.2) \times 10^{-13}$                                  |
| $\bar{M}_{SD}$  | $(1.4 \pm 0.3) \times 10^6$                                      |
| $\bar{M}_{LS}$  | $(3.6 \pm 0.7) \times 10^6$ (Fig. 4)                             |

Heating either the lyophilized B-1459 or the culture fluid decreases molecular size. This observation is supported by two measurements: (i) The intrinsic viscosity of an aged dispersion in 4M urea is about twice as great as the intrinsic viscosity of a solution prepared by heating a portion of the dispersion for 3 h at 90° and (ii) the  $\bar{M}_{LS}$  value of the XC-XL culture fluid decreased from  $50 \times 10^6$  to  $11 \times 10^6$  after the culture fluid was heated for 3.5 h at 85°.

*Viscosity studies.* — Changes in viscosity values were the basis for judging the approximate stability of dispersions and solutions. Dispersions of B-1459 in redistilled water and in 0.01M NH<sub>4</sub>OAc were prepared from samples of batch MP-84 and were stored at room temperature and at 5°. Storage at 5° helped maintain viscosity in both solvent systems. The dispersion in water suffered a 50% decrease in relative viscosity within 3 weeks at room temperature (*ca.* 25°). The dispersion in 0.01M NH<sub>4</sub>OAc was stable for 2 months at room temperature and was stable for about 8 months when stored at 5°. Solutions in 4M urea, prepared from batch MP-84, yielded stable viscosity values for at least 4 months when stored at 5°.

Some representative viscosity characteristics of dispersions prepared from lyophilized and vacuum oven dried B-1459 obtained from culture fluid batch X-FD-1 are displayed in Fig. 5. The polyelectrolytic nature of the polymer is clearly indicated by the thixotropic behavior and higher viscosity of the dispersion in distilled water (curve A). The behavior of a dispersion in 4M urea, that was aged 7 days at 5°, is shown by line B<sub>1</sub>. After 2 months' storage at 5°, the viscosity of this system, represented by line B<sub>2</sub>, had markedly increased. The viscosity of a dispersion aged 7 days at 5° in 0.01M NH<sub>4</sub>OAc is shown by line D. Even after 2 months' storage at 5°, the viscosity of this system remained constant.

Viscosity behavior of B-1459 polymer not isolated from the culture fluid was different from that of lyophilized and dried material. Line C (Fig. 5) represents the

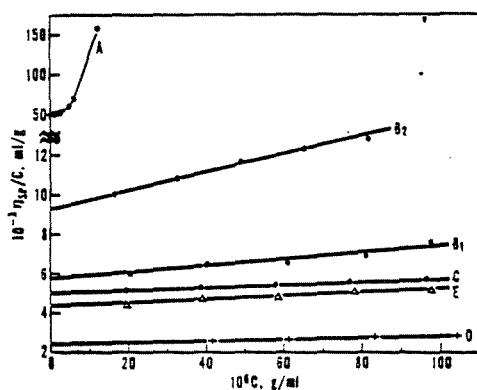


Fig. 5. Viscosity behavior of B-1459 (from X-FD-1). (A) Lyophilized, not dried, polymer dispersed overnight in redistilled water. Note scale change. (B<sub>1</sub>) Lyophilized and dried polymer, dispersed 7 days in 4M urea at 5°. (B<sub>2</sub>) Sample B<sub>1</sub> aged 2 months at 5°. (C) Clarified culture fluid dialyzed against 0.01M NH<sub>4</sub>OAc and stored 4 weeks at 5°. (D) Lyophilized and dried polymer, dispersed 7 days in 0.01M NH<sub>4</sub>OAc at 5°. (E) Sample C made up to 4M urea for 4 days at 5°. Lyophilized samples dried by technique 1, but not equilibrated in constant temperature-humidity room before drying. Ethanol-KCl precipitation not used on these samples.

behavior of clarified culture fluid that had been dialyzed against 0.01M NH<sub>4</sub>OAc for several days. The dialyzed system had been stored for 4 weeks at 5°. Curve E represents the viscosity of the dialyzed culture fluid that has been made about 4M in urea. Five milliliters of 0.01M NH<sub>4</sub>OAc dialyzed culture fluid was diluted to 100 ml with 4M urea, and the fluid was aged 5 days at 5° before measurements were taken. Dilutions were made with 4M urea.

Some effects upon the intrinsic viscosity,  $[\eta]$ , of dispersions prepared from a lyophilized batch of B-1459 from X-FD-1 culture fluid are summarized in Table II. In this table are compared the effects of (i) dehydrating versus not dehydrating the sample, (ii) 0.01M NH<sub>4</sub>OAc versus 4M urea as a solvent, (iii) dehydrating the samples by methods 1 or 2, and (iv) ethanol-potassium chloride precipitation from the culture fluid versus direct lyophilization.

TABLE II

INTRINSIC VISCOSITY OF B-1459 DISPERSIONS

| Sample                                         | $[\eta]$ ml/g $\times 10^{-3}$ |                               |
|------------------------------------------------|--------------------------------|-------------------------------|
|                                                | 0.01M NH <sub>4</sub> OAc      | 4M Urea                       |
| (A) Culture fluid not treated with ethanol-KCl |                                |                               |
| Dehydrated                                     | 1.7 $\pm$ 0.1 (9.0 $\pm$ 0.2)  | 8.1 $\pm$ 0.3 (8.1 $\pm$ 0.3) |
| Not dehydrated                                 | 5.8 $\pm$ 0.1                  | 5.9 $\pm$ 0.1                 |
| (B) Culture fluid treated with ethanol-KCl     |                                |                               |
| Dehydrated                                     | 9.2 $\pm$ 0.2 (9.0 $\pm$ 0.2)  | 8.2 $\pm$ 0.2 (8.1 $\pm$ 0.2) |
| Not dehydrated                                 | 7.7 $\pm$ 0.1                  | 7.0 $\pm$ 0.1                 |

Lyophilized polymer obtained from culture fluid batch X-FD-1 stock solution that was stored for 7 months at 5°. Values for samples dehydrated by drying method 2 are indicated in parentheses.



Precipitation of polysaccharide from the culture fluid with alcohol-potassium chloride removes protein. Lyophilized material (X-FD-1) after alcohol-potassium chloride precipitation contained 0.24 weight percent nitrogen; that prepared directly from the clarified culture fluid contained 1.71 weight percent nitrogen. (Batch MP-84, which is highly purified, contained 0.06% nitrogen.) Estimates of 10% protein in directly lyophilized X-FD-1 samples and 1.4% in ethanol-potassium chloride precipitated X-FD-1 samples are made by using a conversion factor of 6.0 between weight of nitrogen and weight of protein. Values of  $[\eta]$  in part (A) of Table II have been calculated by using concentrations 0.91 times those measured on a weight basis. This procedure is used to correct for the greater amount of protein in the directly lyophilized samples. Values of  $[\eta]$  in part (B) of Table II are calculated on a direct concentration by weight basis.

## DISCUSSION

The highly concentration-dependent behavior of B-1459 (MP-84) solution in 4M urea is shown in several ways. One may use the data in Fig. 1 to calculate a concentration dependence coefficient,  $K_s$ , from the equation:

$$1/s = 1/s^0[1 + K_s c] \quad (3)$$

where  $c$  is the concentration in g/ml. Values of  $K_s$  in 4M urea equal to 614 ml/g and 435 ml/g are calculated for the week-old solution and the 2-month-old solution, respectively. The asymmetry shown by the diffusion photograph (Fig. 3) is a reflection of concentration dependence or polydispersity, or both of these properties. Sedimentation patterns (not shown) of a fresh B-1459 solution in 4M urea and culture fluids in 0.01M  $\text{NH}_4\text{OAc}$  appear to have infinite boundaries at polymer concentrations above 0.2%. The schlieren patterns of a fresh solution in 4M urea appear as sharp spikes at concentrations of about 0.15 to 0.1%; at a 0.05% concentration the pattern opens to give the appearance of a sawtooth. This non-Gaussian character of the sedimentation pattern is another indication of concentration dependence.

The difference in molecular-weight values calculated for the 4M urea solution of B-1459 (MP-84) is possibly caused by a mode of high-molecular-weight particles, or micro-gel, which would be heavily weighted in the  $\bar{M}_{LS}$  average. A few percent of micro-gel could remain in the solutions despite preparative ultracentrifugation and could also remain undetected in the synthetic boundary measurements. Light scattering becomes less concentration dependent at angles closer to the incident beam as is indicated by decreasing slopes of lines at constant angle (Fig. 4). Because of this, and the fact that lines at constant concentration have less curvature at lower scattering angles, we chose measurements between  $45^\circ$  and  $20^\circ$  from the incident beam as being most meaningful. Of course, a weight average molecular weight would be obtained from the  $\bar{M}_{LS}$  value in the absence of micro-gel. The  $\bar{M}_{SD}$  value does not represent a simple average. This is particularly true for B-1459 solutions in which asymmetric schlieren patterns are obtained in measurements of  $s$  and  $D$ . Therefore,

our molecular-weight determinations should be considered as qualitative indications of the molecular system in solution.

Light-scattering measurements on both the solution and the culture fluids indicate polydisperse systems of high molecular weight. We cannot say whether the observed  $\bar{M}_{LS}$  values are representative of B-1459 polymer molecules or aggregates. Despite the probability that some micro-gel contributes to the  $\bar{M}_{LS}$  values measured on the culture fluids, molecular weights in the culture fluids are well above those in the 4M urea solution.

Attempts to detect measurable amounts of gel by ultracentrifuge synthetic-boundary measurements yielded negative results in both dilute 4M urea solutions or dialyzed culture fluid samples in 0.01M  $\text{NH}_4\text{OAc}$ . Therefore, under these conditions of low gravity and concentration,  $c < 0.2\%$ , we conclude that B-1459 is capable of existing in a solution state. Sedimentation measurements made on 0.1% dispersions in 0.01M  $\text{NH}_4\text{OAc}$  prepared from lyophilized material show gel to be present. Since the dialyzed culture fluid in  $\text{NH}_4\text{OAc}$  and 4M urea behaves as a truly solvated system, we conclude the process of lyophilization alters the solubility of B-1459, as well as its viscosity properties. Perhaps lyophilization in some manner "denatures" the polysaccharide.

Our studies show that a variety of factors may affect dispersion viscosity. We noted an "aging" effect of B-1459 systems in 4M urea. The viscosity of B-1459 initially placed in 4M urea is lower than its viscosity later. This behavior, illustrated in Fig. 5 by lines  $B_1$  and  $B_2$ , is most easily observed in lyophilized samples although it sometimes may be seen in culture-fluid samples. The rate of viscosity change increases when the sample is exposed to higher temperatures. A lyophilized sample stored at room temperature may reach higher viscosities within 24 to 48 h. Sedimentation patterns of B-1459 samples that have been in 4M urea at 5° for 6 months or longer show peaks that have a more normal appearance than those seen in nonaged samples. The change in concentration dependence illustrated by lines A and B in Fig. 1 is another example of B-1459 undergoing change in 4M urea. The changes in dispersion viscosity and solution properties of an already solvated entity suggest to us that B-1459 could be reacting slowly with 4M urea. These changes might involve a disaggregation of molecular aggregates or a chemical reaction between urea and polysaccharide. (Formation of carbohydrate carbamates prepared by heating starch with urea has been reported by Paschall<sup>1,2</sup>.)

Our experience with dispersion viscosity measurements has shown that extreme care must be used to ensure that time-dependent behavior does not make comparisons invalid. All of the values in Table II were measured after dispersions had aged at room temperature for at least 5 days. Values of  $\eta_{sp}/c$  were then checked to ensure that no changes occurred during the time span of these measurements. After an initial period of instability, viscosities of dispersions in 0.01M  $\text{NH}_4\text{OAc}$  and 4M urea at room temperature decrease slowly with time.

Some general conclusions about dispersions prepared from lyophilized polysaccharide (X-FD-1) are indicated in Table II. The only extreme viscosity difference

occurred in 0.01M  $\text{NH}_4\text{OAc}$  dispersions of high-protein content because drying method 1 lowered dispersion viscosity of the samples. Provided drying method 2 is used, dehydration of sample increased viscosity in all situations investigated. Samples precipitated with ethanol-potassium chloride, but not dried, yielded higher viscosities than samples neither ethanol-potassium chloride precipitated nor dried. Perhaps the polysaccharide is "denatured" in some manner by the precipitation and drying process. A final comment concerns  $[\eta]$  values in Table II, part A, Dehydrated, as compared with  $[\eta]$  values illustrated by lines B<sub>2</sub> and D (Fig. 5), which would be even higher had a correction for protein content been made. Measurements listed in Table II were made on material stored for 7 months at 5° as partially clarified culture fluid. During this time the polysaccharide had altered, as indicated by lower  $[\eta]$  values, and hence identical materials were not used for measurements in Table II and Fig. 5.

The purpose of this study has been to determine some molecular parameters and viscosity characteristics of polysaccharide B-1459 in dilute solution and dispersion. The dilute solution systems consist of high-molecular-weight entities that interact strongly. High intrinsic viscosity is maintained under the conditions of our experiments. Dispersion viscosity may be enhanced by appropriate treatment of the polymer before dispersion. The most interesting question remains to be answered. What specific subunits and structures of the polysaccharide chain cause the property of high viscosity observed in fluid systems of B-1459?

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