# Some Physicochemical Properties of a Wheat Flour Hemicellulose in Solution[1]

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**ABSTRACT**

A hemicellulose that did not complex with borate was isolated from wheat flour "tailing." Electrophoretically, it migrated as a single component but ultracentrifuge patterns showed some polydispersity. The ratio $(M_{SD})/(Mn)$ was equal to 118,000. In agreement with other studies this polysaccharide was found to be basically an arabinoxylan with short, labile arabinose side branches. It is proposed that in solution the molecule has a random coil configuration that is somewhat rigid and extended owing to these branches.

The wheat flour hemicelluloses or "water-insoluble pentosans" constitute a part of the endosperm polysaccharides that occur in the "tailings" fraction of wheat flour. The composition of these hemicelluloses has been studied by numerous workers and a review on this subject has been published by Bechtel et al. (1). Fewer studies, however, have been concerned with the chemical structure of these hemicelluloses. Montgomery and Smith extracted a hemicellulose fraction from the starch-free "tailings" and fractionated the acetylated derivative (2). From fractional precipitation experiments they concluded that their derivative was essentially homogeneous and that the polysaccharide consisted of a linear polymer of 1,4-linked D-xylopyranose units with L-arabofuranose side-chain residues attached to certain D-xylose units of the framework, principally through position 3. Recently Upton and Hester isolated a carbohydrate-protein complex from the "tailings" fraction (3). When they subjected the complex to starch-gel electrophoresis, little migration was observed. They attributed the inability of the complex to migrate to incomplete solubilization of the complex in the buffer used in the experiment. Recently Perlin and Suzuki (4) also made an examination of the entire hemicellulose fraction; their studies dealt principally with the composition of the alkali insoluble portion.

In all of these investigations, very little use was made of hydrodynamic measurements, such as ultracentrifugation, viscosity, and diffusion, as well as free-boundary electrophoresis, to assess the homogeneity or study the physica properties of these hemicelluloses. In the present work some of these physica measurements were carried out on a wheat flour hemicellulose isolated by a procedure that was devised by the author (5). This procedure involved the chromatography of a neutral aqueous extract of crude hemicellulose on a diethylaminoethyl cellulose column (borate form). Five fractions were obtained, each differing in percent sugar composition. One of these fractions contained an appreciable amount of hemicellulose that did not complex with borate on the column and was eluted with water. This fraction was analyzed by free-boundary electrophoresis and ultracentrifugation and shown to behave essentially as a single component. Also from sedimentation, diffusion, and viscosity measurements calculations of approximate molecular weight of the pentosan molecule were made. The results are discussed in this paper.

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## MATERIALS AND METHODS

### Preparation of Hemicellulose

The hemicellulose was prepared from a hard red winter wheat flour by a procedure described in a previous paper (5), with one exception. The temperature of the amylase digestion was raised to 37°C. The preparation used in these studies corresponded to fraction 1 (5). Moisture content was 9.9%, anhydrosugar composition, based on dry weight of hemicellulose, was: arabinose, 26%; xylose, 72%; and glucose, 1%.

### Electrophoresis Studies

Free-boundary electrophoresis of the hemicellulose was carried out at 4°C. in a Perkin Elmer Model 38A electrophoresis apparatus, equipped with a cylindrical schlieren lens system. Solutions of concentrations of 0.5 to 1.0% (w./v.) were prepared by stirring the lyophilized polysaccharide in the appropriate buffer. Conductivity measurements of the solutions were made with a Radiometer conductivity meter at 4°C. Runs were generally made with a current of 0.01 amperes. Mobility values were calculated by the method of Alberty (6).

### Chemical Analyses

Esterification of the hemicellulose (30 mg.) and colorimetric analyses for total ester formed were performed according to the procedure of Kaye and Kent (7). Time of esterification was 2 hr. at 100°C. A standard curve was prepared using ethyl acetate dissolved in methyl alcohol, and the amount of ester present in the esterified polysaccharide was read from this curve.

For titration experiments, solutions of the hemicellulose (0.8%) were prepared by dissolving the lyophilized material in carbon dioxide-free glass-distilled water and titrated under nitrogen with 0.1N sodium hydroxide with an automatic titration apparatus (Radiometer). The titrations were performed over 1-hr. periods to allow equilibrium to be attained after addition of alkali.

Partial hydrolysis of the hemicellulose was performed as follows: 100 mg. of the lyophilized preparation was dissolved in 10 ml. 1N hydrochloric acid and allowed to stand at room temperature for 72 hr. At the end of this time the white precipitate that had formed was centrifuged down, washed several times with the 1N acid, and lyophilized. The supernatant plus washings were dialyzed against distilled water until no chloride ion was detected in the dialysate, the dialysate was evaporated to a small volume, deionized with Dowex 2 ($HCO_3$-form), and lyophilized. Finally all of the solids above were weighed, hydrolyzed, and analyzed quantitatively for monosaccharides by a procedure described previously (5).

### Sedimentation Studies

Runs were made on solutions having 0.5 and 0.25% hemicellulose in 0.1N sodium chloride, pH 7.4, in a Spinco Model E analytical ultracentrifuge at 19.8°C. A double-sector epon cell was used at the speed of 926.6 rev. per sec. Measurement of the rates of sedimentation were made at the different concentrations, the sedimentation values were computed (8) and corrected in the usual manner to sedimentation values corresponding to water at 20°C., i.e., $s_{20,w}$. Values of $^1/s_{20,w}$ were then plotted against percent concentration of solute, and the limiting value ($s^\circ_{20,w} = 1.99 \times 10^{-13}$) was obtained.

**Determination of Diffusion Coefficient**

The diffusion coefficients of solutions of hemicellulose (0.2 to 0.5% in 0.1N sodium chloride) were determined with the Perkin Elmer Model 38A electrophoresis apparatus equipped with a Brinkman temperature regulator that controlled the bath temperature at 20.00° ± 0.02° C. A standard Tiselius electrophoresis cell equipped for schlieren optics was used as the diffusion cell, and diffusion coefficients were calculated by the maximum ordinate and area method (8). These values were corrected to water at 20°C. and extrapolated to zero solute concentration giving $D^{\circ}_{20,w}$ equal to $1.26 \times 10^{-7}$ sec.$^{-1}$

**Determination of Intrinsic Viscosity**

A glass rotating cylinder viscometer with a rotor having a radius of 0.5 cm., operating at a shear gradient of 1.5 sec.$^{-1}$ was used (9).

The temperature for all runs was 20.00° ± 0.02°C. Solvent and solutions were filtered through sintered-glass funnels (medium porosity) before each run. The ratios of $(\eta_{sp})/c$ and $(1\ n\eta_{rel})/c$ were plotted against c (concentration of solute in g. per deciliter) and extrapolated to zero solute concentration to obtain the intrinsic viscosity $[\eta]$ equal to 3.90 dl. per g.

**Determination of Partial Specific Volume**

The densities of three different concentrations of the hemicellulose in 0.1N sodium chloride were determined at 20.00° ± 0.02°C. with a Lipkin pycnometer of 2-ml. capacity (10) and a value of 0.674 ml. per g. for the apparent partial specific volume (V) was obtained by the tangent method (8).

**Determination of Molecular Weight**

The molecular weight of the hemicellulose as determined from the sedimentation and diffusion data was estimated by the Svedberg equation (8) $\overline{M}_{s,D} = (RT_s)/((1-\overline{V}\rho)D))$ where T is the absolute temperature, R the gas constant, $\rho$ the density of the solvent, s the sedimentation coefficient ($s^{\circ}_{20,w}$), D is the diffusion coefficient ($D^{\circ}_{20,w}$) and $\overline{V}$ is the partial specific volume. An average value of $\overline{M}_{s,D}$ equal to 118,000 was obtained.

**Osmotic Pressure Measurements**

Osmotic pressure determinations on the wheat flour hemicellulose dissolved in 0.1N sodium chloride were made with a Melab recording osmometer. The osmotic height at 18°C. was determined at concentrations of solute ranging from 5.4 to 1.3 g. per liter and the data plotted according to $(\Pi/c) = (RT)/(\overline{M}_n)$, where $\Pi$ is the osmotic pressure in cm. of solvent. The number average molecular weight $\overline{M}_n$ was calculated from the intercept of the $\Pi/c$ vs. c graph (see Fig. 4). The value obtained for $\overline{M}_n$ at zero concentration was 52,000.

## RESULTS AND DISCUSSION

When the wheat flour hemicellulose was examined electrophoretically in three different buffers (phosphate, pH 7.6, borate, pH 9.3, and sodium hydroxide-glycine, pH 10.1) it migrated as a single symmetrical boundary in both limbs of the cell. A typical pattern is shown in Fig. 1. Such behavior indicated that this polysaccharide was homogeneous with respect to charge density. In these buffers the hemicellulose always migrated as an anion. In the presence of boric acid or borate ion, polysaccharides generally form complexes and in this way the

carbohydrate is provided witha negative charge (11). However, this hemicellulose did not complex with borate because it was not adsorbed in the diethylaminoethyl cellulose (borate form) (5). Since it migrated in buffers other than sodium borate, it was assumed that the borate ion was not necessary for charge production and the charge on the polysaccharide was due to the ionization of acidic groups inherent to the molecule itself. The increase in mobility of the carbohydrate with increase in pH also indicated that acidic groups are present (see Table I). Other polysaccharides, know to contain acid groups, were studied by free-boundary electrophoresis by Larsen and Haug (12), and by Colvin et al. (13) and were shown to behave similarly. The quantities of acid groups in the hemicellulose were estimated by esterification (7), and on the basis of the hydroxamic acid determination they exist to the extent of 8 meq. per 100 g. of carbohydrate.Titration of the hemicellulose with base gave a value of 7 meq. per 100 g. The identity of these acids was not determined; however, they are probably uronic acids. According to Whistler and Smart (14), this type is found in practically all hemicellulose preparations. Calculated on the basis of glucuronic acid, the hemicellulose studied here contained about 1% sugar acid.When hemicellulose was sedimented in 0.1N sodium chloride, the presence of one main component with some polydispersed material was apparent from photographs of ultracentrifuge patterns taken over a period of 7 hr. (see Fig. 2). Polydispersity was especially noticeable at the lower concentration of 0.25%. There was also a marked dependence of the sedimentation coefficient on solute concentration.

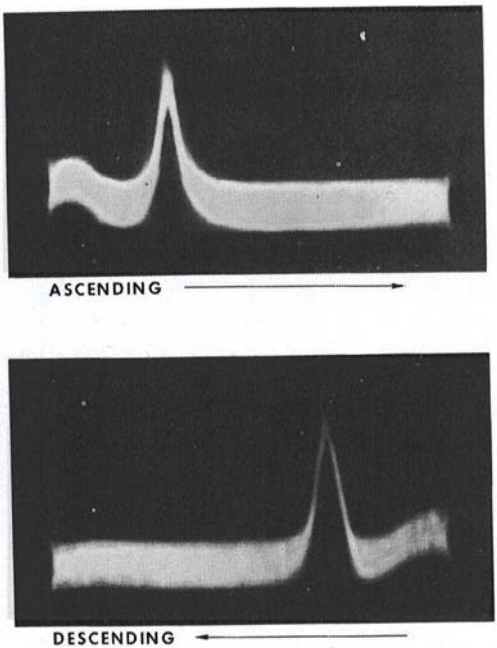


**Fig. 1. Moving boundary electrophoresis pattern of wheat flour hemicellulose (9.5 mg./ml.) in sodium borate buffer, 0.05M, pH 9.3; 170 v., 9.2 ma. Time of run: 198 min. (The salt boundary anomalies indicated in the figure were pronounced.)**

TABLE I. ELECTROPHORETIC MOBILITIES OF WHEAT FLOUR HEMICELLULOSE

| Buffer | pH | Mobility (volts$^2$cm.$^{-1}$sec.$^{-1}$ X $10^5$ |
|---|---|---|
| $NaH_2PO_4$ (0.008M)-$NaHPO_4$ (0.06M) | 7.6 | −0.9 |
| Sodium borate (0.05M) | 9.3 | −1.6 |
| Sodium hydroxide (1M) Glycine (2M) | 10.1 | −7.3 |

The reduced viscosity $(\eta_{sp})/c$ was also strongly dependent on the concentration (see Fig. 3), and the high value obtained for $[\eta]$ suggested that the hemicellulose molecules were very asymmetrical and/or highly solvated. It was not determined if the hemicellulose molecules were shear-dependent, however, if such a dependence existed it was virtually eliminated in the cylinder viscometer that was used in these studies and operated at a very low shear rate. The pronounced decrease in the sedimentation rate with increased solute concentration was probably due to the high viscosity of the hemicellulose solution. The frictional ratio $f/f_o$, of this molecule was calculated from the following relationship (8):

$$f/f_o = 1.00 \times 10^{-8} \left[1 - 0.9982\,\overline{V}/(D^\circ_{20,w})^2(s_{20,w})\,\overline{V}\right]^{1/3}$$

where $f/f_o$ is the ratio of the resistance to motion (such as that experienced in sedimentation or diffusion) of the molecule to the resistance of a spherical anhydrous molecule of the same molecular weight. If one assumes that

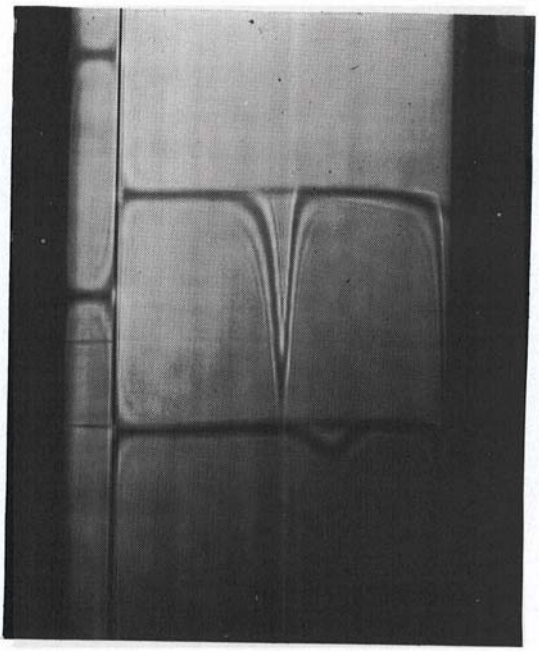

**Fig. 2. Sedimentation pattern of wheat flour hemicellulose dissolved in 0.1N NaCl, pH 7.4. Double sector cell, phase plate angle: 50°, concentration above photo: 0.25%, below: 0.5%, average speed: 926.6 r.p.s. Sedimentation is from right to left. Photograph was taken 352 min. after start of run.**

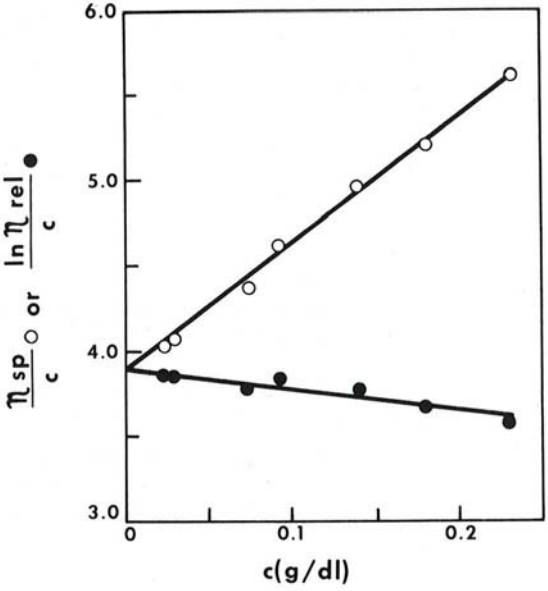


**Fig. 3. The relationship between viscosity of wheat flour hemicellulose and concentration, solvent: 0.1N sodium chloride, temp. 20°C.**

hemicellulose is a rigid particle, for example a prolate ellipsoid in solution, a value of $f/f_0 > 1$ indicates that the hemicellulose is hydrated or that its particle shape deviates from spherical. Both of these factors may be of influence. A value of $f/f_0 = 5.3$ was obtained from this calculation and, if the effect of hydration is ignored, further evidence of high asymmetry is indicated. However it was felt that the assumption of a rigid, unsolvated ellipsoid for the shape of this hemicellulose was not valid, for reasons discussed later in this paper.

Previous structural work by Montgomery and Smith (2) and Marchessault et al. (15) has shown that this hemicellulose is a polymer chain of β-1, 4-xylopyranose units with short side branches of single arabinofuranose groups, most of which are linked to the 2-OH group of the xylose unit in the chain. In a few places along the chain, some of these xylose units are appended by two arabinose residues. Whether these residues are regularly or randomly spaced has not been determined; however if this hemicellulose has a structure similar to, the soluble wheat flour arabinoxylan studied by Goldschmid and Perlin (16), its arabinose branches are probably unevenly spaced. These arabinose side chains were labile under acidic conditions. The results of partial hydrolysis experiments (Table II) show that with prolonged exposure to 1N hydrochloric acid at room temperature, most of the arabinose was split off from the xylan backbone and is found in the dialysate of the hydrolysis supernatant. Upon release of the arabinose, the xylan portion becomes insoluble and precipitates from solution. These results are in agreement essentially with those obtained by Upton and Hester (3) on the graded hydrolysis of this hemicellulose.

The hemicelluloses prepared in this laboratory always contained, in addition to arabinose and xylose, small amounts of glucose that could not be removed despite thorough treatment with alpha-amylase and chromatographic separation. The

TABLE II. ANALYSES OF HYDROLYSATE OF WHEAT FLOUR HEMICELLULOSE

| Fraction | Percent of Solids Recovered | XYL[a] | ARA[a] | GLU[a] |
|---|---|---|---|---|
| Precipitate | 60 | 95 | 5 | ... |
| Dialysate of supernatant | 25 | ... | 98 | 2 |
| Residue of supernatant | 3 | 50 | ... | 50 |

[a]Abbreviations for sugars are as follows: XYL, xylose; ARA, arabinose; GLU, glucose. Units are reported in percent sugar present.

purified preparation of Montgomery and Smith (2) also contained 2% glucose. In their work, however, no comment was made about the structural role of this sugar. The results in Table II of this paper show that upon partial hydrolysis of hemicellulose, the glucose remained in solution and that after centrifugation and dialysis of this solution almost all of this glucose was found in the dialysis residue along with xylose.

Thus, after partial acid hydrolysis most of the glucose was still in some polymeric form either as glucose polymer or as part of the hemicellulose molecule. Also there is the possibility that this polymeric glucose may exist as an impurity in the hemicellulose preparation but in too low a concentration to be detected by the ultracentrifugation and electrophoretic techniques used in this work.

From the structural studies just mentioned (2,15,16) this hemicellulose can be considered as basically an arabinoxylan with all of its xylose in the linear chain part of the molecule and the arabinose appended in short side branches from this chain. Because of these branches this molecule probably has a somewhat "stiffer" form than that of a xylose chain with no side branches and will not coil up or contract to as great an extent as the latter. At the same time, this hemicellulose molecule would not be classified as a rigid particle, but would tend to have a configuration of a polymer approximating a random coil in solution. Somewhat analogous are polysaccharides such as alginic acid (17) and arabic acid (18). In their uncharged forms these molecules are proposed to have random coil configurations that are somewhat rigid and extended owing to molecular side branches.

If the random coil configuration is adopted in preference to that of the rigid particle, the equivalent sphere model of Mandelkern et al. (19) can be used to calculate the molecular weight of this hemicellulose in solution. The following equation has been derived for the molecular weight of a linear polymer that forms a random coil.

$$M^{2/3} = \frac{Ns^{\circ}_{20,w}[\eta]^{1/3}\eta_0}{(2.5 \times 10^6)(1 - V\rho)}$$

where N is the Avogadro number and $\eta_0$ is the viscosity of the solvent in poises. The coefficient ($2.5 \times 10^6$) has been confirmed experimentally and is said to be constant for flexible linear polymers. It is the value of a ratio of the constants of

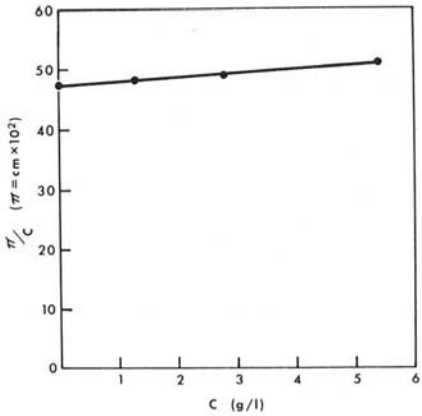


**Fig. 4. The relationship between osmotic pressure and concentration of hemicellulose solutions. Solvent: 0.1N sodium chloride, temperature of run: 18°C.**

proportionality that relate the viscosity coefficient and frictional coefficient to the root mean square distance between chain ends of a linear molecule. Substitution of the experimental values into the above equation gives M=126,000, a value that is fairly close to the one obtained from sedimentation and diffusion data, i.e., $M_{s,D}$ = 118,000.

The number average molecular weight ($\overline{M}_n$ = 52,000) of this hemicellulose obtained by osmotic pressure (see Fig. 4) was lower than those weights reported above. This result was expected, in view of the polydispersity observed in the ultracentrifuge patterns. If the polysaccharide is polydispersed, a small amount of small molecules has a large effect on lower $\overline{M}_n$ (14). In Fig. 2 on the solvent side of the boundaries there is a noticeable amount of polydispersed material with sedimentation coefficients smaller than that of the main component. No molecular weight values obtained on similar preparations of the wheat flour hemicellulose could be found in the literature and no comparison could be made with the average values obtained here. The weight average obtained from sedimentation and diffusion for polymers having some polydispersity can be considered only an approximation of the true weight average, and only a rough idea of the extent of polydispersity can be gained from the ratio $(M_{s,D})/(M_n)$. For this hemicellulose preparation a value of 2.3 was obtained. For comparison with unfractionated synthetic polymers a value of 2 is to be expected for normal polymer distribution (20). Therefore polydispersity of this hemicellulose is in the range of that of synthetic polymers. In general most laboratory preparations of polysaccharides show an appreciable difference between $\overline{M}_n$ and $\overline{M}_w$ (21), in contrast to smaller differences found for proteins. Further fractionation of this hemicellulose should yield less polydispersed preparations that could be studied with physicochemical methods in order to acquire a clearer picture of the dimension of this polysaccharide in solution.

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### Literature Cited

1. BECHTEL, W. G., GEDDES, W. F., and GILLES, K. A. Carbohydrates. In: Wheat: Chemistry and technology, ed. by I. Hlynka; Monograph Series, vol. III, pp. 277-352. American Association of Cereal Chemists: St. Paul, Minn. (1964).
2. MONTGOMERY, R., and SMITH, F. The carbohydrates of Gramineae. V. The constitution of a hemicellulose of the endosperm of wheat (*Triticum vulgare*). J. Am. Chem. Soc. 77: 2834-2837 (1955).
3. UPTON, ELIZABETH M., and HESTER, E. ELIZABETH. Nonstarchy polysaccharides and proteins of soft wheat flour tailings. Cereal Chem. 43: 156-168 (1966).
4. PERLIN, A. S., and SUZUKI, S. A note on the polysaccharides of wheat flour squeegee. Cereal Chem. 42: 199-201 (1965).
5. COLE, E. W. Isolation and chromatographic fractionation of hemicelluloses from wheat flour. Cereal Chem. 44: 411-416 (1967).
6. ALBERTY, R. A. An introduction to electrophoresis. I. Methods and calculations. J. Chem. Educ. 25: 426 (1948).
7. KAYE, M. A. G., and KENT, P. W. Ester and lactone linkage in acidic polysaccharides. Part I, pp. 79-83. J. Chem. Soc. (1953).
8. LUNDGREN, H. P., and WARD, W. H. Molecular size of proteins in amino acids and proteins, ed. by D. M. Greenberg. Charles H. Thomas: Springfield, Ill. (1951).
9. ZIMM, B. H., and CROTHERS, D. M. Simplified rotating cylinder viscometer for DNA. Proc. Natl. Acad. Sci. 48: 905-911 (1962).
10. LIPKIN, M. R., DAVISON, J. A., HARVEY, W. T., and KURTZ, S. S., Jr. Pycnometer for volatile liquids, control of diffusion as an aid in precision pycnometry. Ind. Eng. Chem. Anal. Ed. 16: 55-58 (1944).
11. NORTHCOTE, D. H. Electrophoresis of some neutral polysaccharides. Biochem. J. 58: 353-358 (1954).
12. LARSEN, D., and HAUG, A. Free-boundary electrophoresis of acidic polysaccharides from the marine alga, *Ascophyllum nodosum* (L.) LeJol. Acta Chem. Scand. 17: 1646-1652 (1963).
13. COLVIN, J. R., COOK, W. H., and ADAMS, G. A. Electrophoretic homogeneity of polysaccharides in molar alkali. Can. J. Chem. 30: 603-607 (1952).
14. WHISTLER, R. L., and SMART, C. L. Polysaccharide chemistry. Academic Press: New York (1953).
15. MARCHESSAULT, R. H., HOLAVA, H., and TIMELL, T. E. Correlation of chemical composition and optical rotation of native xylans. Can. J. Chem. 41: 1612-1618 (1963).
16. GOLDSCHMID, H. R., and PERLIN, A. S. Interbranch sequences in the wheat arabino-xylan selective enzymolysis studies. Can. J. Chem. 41: 2272-2277 (1963).
17. COOK, W. H., and SMITH, D. B. Molecular weight and hydrodynamic properties of sodium alginate. Can. J. Biochem. Physiol. 32: 227-239 (1953).
18. VEIS, A., and EGGENBERGER, D. N. Light scattering in solutions of a linear polyelectrolyte. J. Am. Chem. Soc. 76: 1560-1563 (1954).
19. MANDELKERN, L., KRIGBAUM, W. R., SCHERAGA, H. A., and FLORY, P. J. Sedimentation behavior of flexible chain molecules: Polyisobutylene. J. Chem. Phys. 20: 1392-1397 (1952).
20. TANFORD, C. Physical chemistry of macromolecules. Wiley: New York (1961).
21. BANKS, W., and GREENWOOD, C. T. Physical properties of solutions of polysaccharides. Advances in carbohydrate chemistry, chap. 18, pp. 357-398. Academic Press: New York (1963).

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